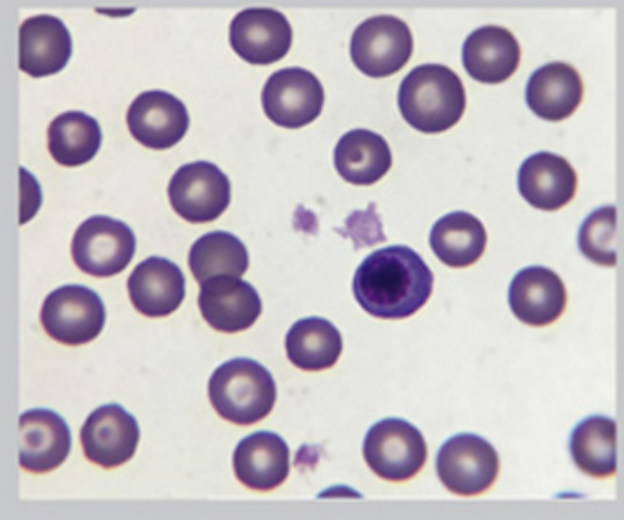
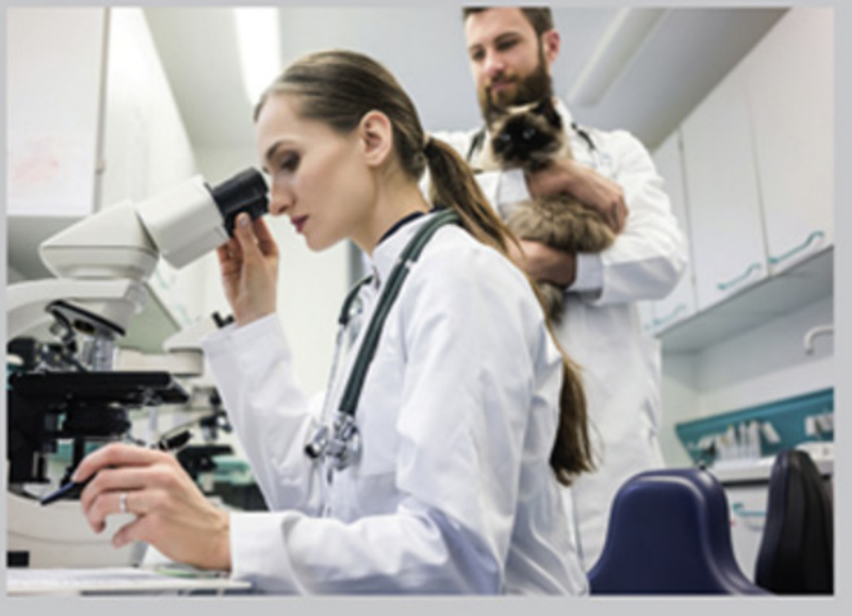
Margi Sirois

Laboratory Procedures for Veterinary Technicians

Seventh Edition





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CLINICAL CHEMISTRY

Plasma Sample Preparation,
Serum Sample Preparation,
ACTH Stimulation Test,
Dexamethasone Suppression Tests,
Dexamethasone Suppression and ACTH Corticotropin Stimulation Test, Combined,
Glucose Tolerance Test, Intravenous,

Compression Smear Technique,
Compression Smear Technique, Modified,
Fine Needle Biopsy Aspiration Technique,
Fine Needle Biopsy Nonaspiration Technique,
Imprint Sample Collection,
Line Smear Technique,
Punch Biopsy Sample Collection,
Scraping Sample Collection,
Starfish Smear Technique,
Swab Sample Collection,
Tzanck Sample Collection,
Wedge Biopsy Sample Collection,

GENERAL LABORATORY

Microscope Calibration,
Microscope Operation,
Microhematocrit Centrifuge Calibration,
Refractometer Use and Care,

Avian Manual White Blood Counts,
Bone Marrow Aspirate Evaluation,
Buccal Mucosa Bleeding Time Test,
Coverslip Blood Smear Preparation,
Crossmatching,
Wedge Film Blood Smear Preparation,

Gram Stain Procedure,
Inoculation of Agar Slant and Butt,
Quadrant Streak Method for Isolating Bacteria,
Typical Sequence of Testing of Microbiology Specimens,

Baermann Technique,
Fecal Examination, Direct Smear,
Buffy Coat Smear,
Cellophane Tape Preparation,
Fecal Centrifugal Flotation,
Fecal Sedimentation,
Fecal Flotation, Simple,
Fecal Culture,
Knott's Technique, Modified,
McMaster Quantitative Egg-Counting Technique, Modified,
Millipore Filtration Procedure,

Urinalysis, Routine,
Urinary Catheterization: Male Cat,
Urinary Catheterization: Male Dog,
Urinary Catheterization: Female Dog,
Urine Collection by Cystocentesis,
Urine Sediment for Microscopic Examination, Preparation,

Laboratory Procedures for Veterinary Technicians

ELSEVIER

LABORATORY PROCEDURES FOR VETERINARY TECHNICIANS, ISBN: 978-0-323-59538-4

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This edition is dedicated to my family, especially Dan-the-wonder-husband, whose constant support is always the wind beneath my wings. To my children, Jen and Daniel, I am so proud to be your mom—you will always be my favorite son and daughter. To Tally, Delta, and Belle … woof woof.

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PREFACE

In recent years, there has been tremendous growth in the number and types of laboratory data that can be obtained in the in-house veterinary practice laboratory. Laboratory procedures remain an important aspect of most veterinary practices, both diagnostically and financially, and a major responsibility of the technician. Performing the tests in the in-house veterinary practice laboratory also provides improved service to both the patient and client and an additional revenue source for the clinic.

This edition is an effort to collect the relevant clinical laboratory information needed by the practicing veterinary technician. Veterinary assistant and veterinary technology students will also find this a valuable everyday reference. Principles and procedures for laboratory diagnostics in clinical chemistry, microbiology, hematology, hemostasis, parasitology, urinalysis, immunology, and cytology are all presented. Information on commonly performed tests done in referral laboratories is also described to allow greater understanding of the clinical relevance of these tests. Reviews of anatomy and physiology topics are included in many sections to aid in developing an understanding of the rationale for performance of specific tests.

This new edition has been significantly updated with information on new technology and expanded information that reflects the latest developments in the veterinary clinical laboratory. Technician tips are interspersed throughout the text to highlight important points. Additional full-color illustrations have been added, including photomicrographs of blood cells, cytology, and microbiology samples, and urine sediment. Expanded information on new clinical analyzers is also included. Key points and recommended readings are included in each chapter.

Step-by-step procedure boxes for all commonly performed hematology, cytology, and parasitology laboratory tests are included in this new edition. The procedure boxes represent those skills that veterinary technician students must perform during their educational program, as well as additional procedures that are commonly performed by veterinary technicians in private veterinary practice.

ACKNOWLEDGMENTS

This volume would not have been possible without the hard work of all the contributors to the first six editions. I sincerely thank them for their efforts. I am grateful to the editors of the earlier additions, especially Teri Merchant, now retired from Elsevier and greatly missed, and Shelly Stringer, who effortlessly filled Teri's very large shoes. Brandi Graham, Maria Broeker, and Carol O'Connell have been invaluable in providing expert assistance in finalizing this new edition. I am grateful to Tim Baum and Katie Foust for their dedication to continuous improvement for this edition and the many veterinary technicians and veterinarians that provided assistance in obtaining many of the new illustrations in this edition.

To my friends, family, colleagues, current and former students, thank you all for your constant encouragement. You inspire me every day.

CONTENTS

Unit Outline

Chapter 1: Safety Concerns and OSHA Standards,
Chapter 2: General Laboratory Equipment,
Chapter 3: The Microscope,
Chapter 4: The Metric System and Lab Calculations,
Chapter 5: Quality Control and Record Keeping,

Unit Objectives

Describe the role of the veterinary technician in the clinical laboratory.
List and describe the regulations related to safety concerns in the veterinary practice laboratory.
Describe the components of a quality control program for the veterinary practice laboratory.
Identify, use, and maintain common laboratory equipment.
Use the metric system to perform calculations and measurements.

Veterinarians depend on laboratory results to help establish diagnoses, to track the course of diseases, and to offer prognoses to clients. The veterinary practice laboratory can also be a significant source of income for the practice. The rapid availability of test results improves patient care and client service. Although some veterinary clinics use outside reference laboratories for test results, this may delay the implementation of appropriate treat ments for patients. Most diagnostic tests can be performed in house by a well-educated veterinary technician. Veterinary practice laboratories have become increasingly sophisticated. Analytic instruments are affordable and readily available for inclusion in even the smallest veterinary clinic.

The veterinary technician/veterinarian team approach works efficiently in a laboratory situation. A veterinar ian is educated in the interpretation of test results, whereas a veterinary technician is educated on generating accurate test results. The consistent generation of reliable laboratory results requires an educated veterinary technician. A veterinary technician must understand the value of quality control in the laboratory.

For additional sources for this unit see the Resources Appendix at the end of this textbook.

After studying this chapter, you will be able to:

- Discuss equirements hemical ygiene
- Identify chanisms or xposure eterinary ractice oratory.
- Describe eneral oncerns elated oratory esign.
- Identify, or ersonal rotective quipment.
- Discuss iteria or valuating nternet esources.

Biohazard
Bloodborne pathogen
Chemical Hygiene Plan (CHP)
Engineering controls
Material Safety Data Sheet (MSDS)
Occupational Safety and Health Administration (OSHA)
Personal Protective Equipment (PPE)
Zoonoses

comprehensive laboratory safety program essential to ensure the afety f mployees he linical aboratory rea. he afety policy include procedures precautions for maintenance of equipment. Safety equipment supplies—such eyewash stations Fig. fire extinguishers, spill cleanup Fig. dous **biohazard** e osal ontainers Fig. protective gloves—must be available. ll employees working clinical laboratory must be aware of location of ems roughly rained oratory safety olicies riting ced ccessible location within clinical laboratory area. Signs be posted to ify mployees ing, inking, plying osmetics, djusting ontact oratory rohibited.

In nited **Occupational Safety and Health Administration (OSHA)** mandates specific laboratory practices must be incorporated into laboratory safety policy. Many other ountries ve egulations. egulations focused on protecting safety of employees. responsible for determining enforcing protective standards. Some es ve egulations upersede ederal regulations. egulations stringent ederal ome ederal egulations lso ontain xemptions or acilities hat ave ewer employees. The regulations specifically include requirements for employers o ollowing:

- Comply elevant
- Correct ety orkplace.
- Educate mployees out otential orkplace
- Provide raining mployees egarding ety hazards.
- Provide equired **personal protective equipment (PPE)** employees.
- Maintain ccurate ecords ork-related uries
- Post ecific osters, ions, ury .

Fig. 1.1 Sink-Mounted Eyewash Station. This type of station is preferable

may not be of adequate volume to properly flush the eyes.

Biohazard waste disposal containers are available in a variety of

scalpel blades, hypodermic needles).

OSHA requires that this Job Safety and Health poster or an equivalent state version be posted in all workplaces. (From United States Department of Labor, Occupational Safety and Health Administration.)

Depending on the specific types of equipment present and the tests performed in the veterinary practice laboratory, veterinary technicians be exposed to variety of potential hazards. These include biologic physical hazards well hazards to musculoskeletal system related to improper ergonomics.

Methods for potential workplace hazards be categorized one of four types: **engineering controls** administrative controls, procedural controls, PPE. Engineering controls are focused on changing work envi ronment to eliminate or minimize exposure to hazard. An example would be of hood when handling ardous chemicals. Administrative controls involve creation of specific protocols to minimize worker exposure to hazards; protocols include found **Chemical Hygiene Plan** which discussed more detail later chapter. Procedural controls involve development of policies modify worker behavior. Examples would include restriction

from th etting ubstitution dous materials when easible. hen engineering, administrative, and procedural ontrols ly ffective or emoval hazard, E equired.

There are large number of specific standards related to veterinary practice ontained cupational afety ct. These ode ederal egula tions FR) in ction esignated Title 29. Each andard designated with part number. For example, standard regarding ormaldehyde or cations laboratories esignated art veral pendices. The ast rity ply ecifically workplace ety art hich vided subparts designated with letters through Summary informa tion egarding the andards with application to eterinary ractice oratory ontained hapter.

The standard titled Occupational Exposure to Hazardous Chemicals ommonly eferred Laboratory Standard. This standard requires each employer designate mployee hemical ygiene er; individual responsible for implementation of required CHP. The must contain specific details about chemical hazards present workplace, scope extent of worker training documentation of training, criteria for of PPE, precautions for handling hazardous chemicals, monitor f xposure, ecific ctions equired hen xposure occurs, luding equired.

The azard ommunication contains equirements or mployers valuate otential hemical hazards ommunicate ormation out propriate rotective ures mployees be otentially xposed nformation communicated to employees writing must include of all dous hemicals hich xposed. orker training rograms egarding hen hazardous chemicals are included standard. The standard mandates placement of specific types of labels on containers of hazardous chemicals; requires employer **Material Safety Data Sheets (MSDSs)** for all chemicals ccessible mployees are provided by manufacturers of potentially hazardous chemi they must contain specific information. The minimum information equired ollows:

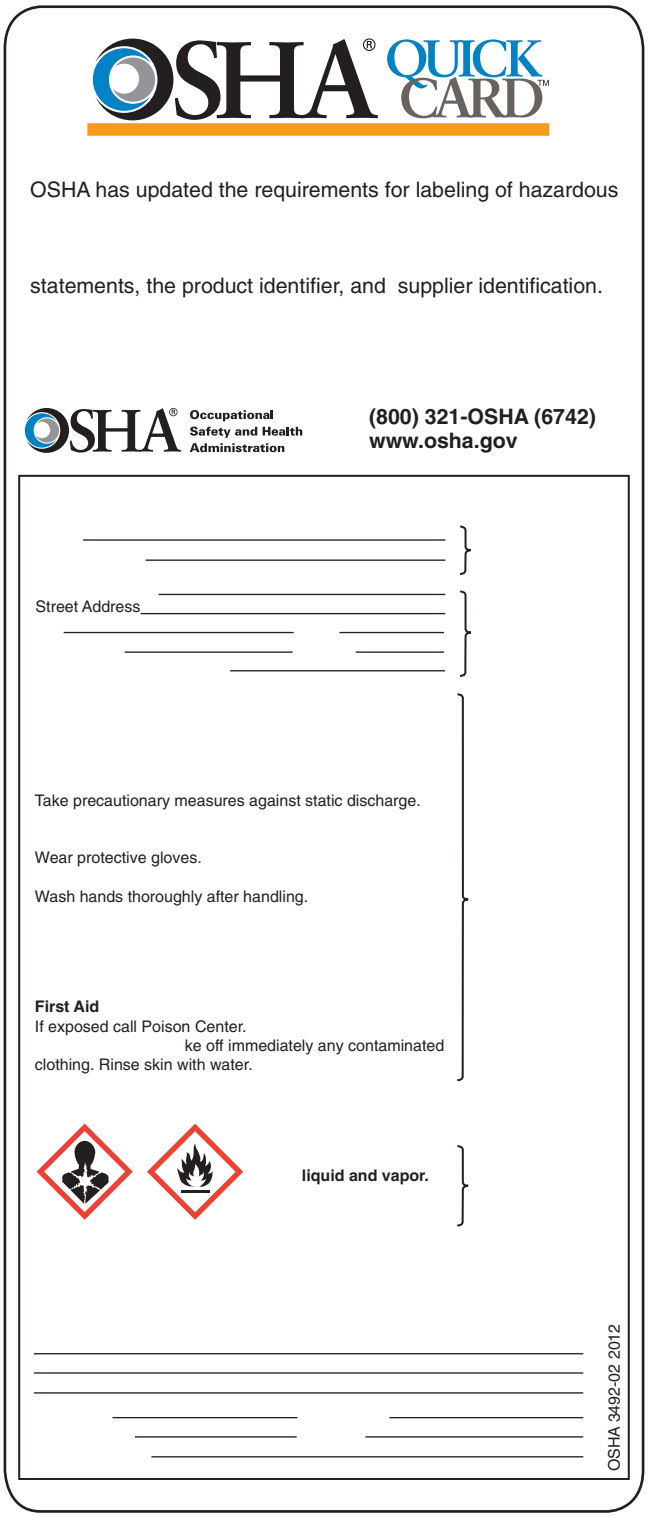
Material Safety Data Sheets must be available to employees.

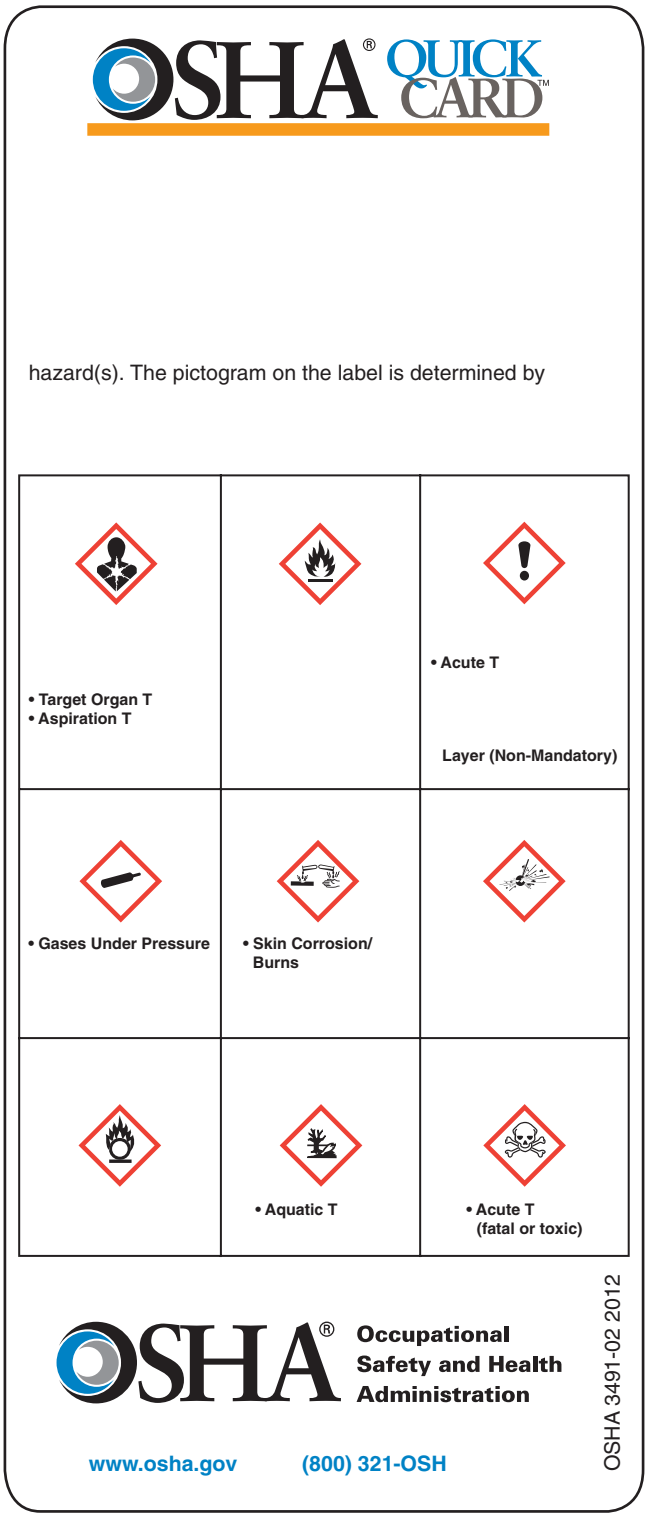
Safety Data Sheet

- Manufacturer's ontact ormation
- Hazardous redients/identity ormation
- Physical/chemical haracteristics
- Fire xplosion
- Reactivity
- Health
- Precautions or
- Control ures

Additional ormation resent. ecom mends of specific 16-section format for which ummarized ox

The Hazard Communication Standard contains detailed informa tion out roper eling ontainers hemicals hen chemicals are removed from their primary container ced condary ontainers or condary el must ontain ecific ormation. condary el required hen erial ork of erson led ontainer, hen erson filled ontainer ves ork hen ontainer moved to different work area from where filled





Pictograms allow for the rapid communication of specific hazard

Labor, Occupational Safety and Health Administration.)

possession of person who filled Pictograms are used o ommunicate ormation condary containers

The Bloodborne Pathogens Standard includes mandates to protect workers from infection with infectious agents resent loodstream; orporates equirements w, eedlestick afety Prevention ct eterinary ractice oratory,

exposures o **bloodborne pathogens** ould otentially occur uring ertain iological ontrol eri d uality ontrol rograms. owever, rity such ontrol erial nger ufactured based roducts. hemical raining ho ill otentially xposed equired. echanisms to control minimize exposure must be established com municated riting.

Although exposure to human bloodborne pathogens ommon roblem eterinary ractice boratory, iety of r ectious ents ncountered. **Zoonoses** e ransmitted etween The ents oonotic resent body eces, apings, ypes presented for analysis. Regulations related to other poten tially ectious erials ecific or bloodborne gens, xcept hen ve otential rious ety cterial organisms otulism, eterinary technicians may encounter such materials either by contact with infected nimals uring he ourse ollecting nd andling for analysis. Protocols must be place to prevent expo sure ust lude roper otentially infectious erials. rotocols enerally ocus of E, roper ection erials ork urfaces, mechanisms such autoclaving infectious materials before disposal r ration.

The ersonal rotective quipment tandard 29 FR 910.132) requires mployers rovide, or, nsure appropriate hen eded void ciated chemicals ury ough absorption, ion, hysical ontact. epending types f resent, lude ye rotection, ro tective lothing, riers yers required to provide training for workers documents

workers w equired, how o roperly or dditional elated to PPE are included Eye Face Protection Standard espiratory rotection Hand Protection Standard

are required to provide necessary PPE

Special onsiderations iven iomedical ustry. iohazards iological ubstances (e.g., d ypodermic edles, ient ontain infectious ents) ell ubstances ontainers iohazardous erial ked ecific mbol Fig. he enters or ontrol revention U.S. overnment ency lished recise elines for e ement ectious ents iomedical ustry. iosafety vels raded r her umber, reater risk. Brief summaries of precautions for each biosafety level are luded ollowing ctions. equirements or ch level increase requirements for lower levels are automatically included her vels.

The ents iosafety vel rdinarily ed, wever, otherwise harmless substances may affect individuals with immune eficiency.

Examples of products organisms found biosafety level include soaps cleaning agents, vaccines are admin istered o ecies-specific ectious infectious patitis).

There are specific requirements for handling or disposal of iosafety vel erials rmal ion

The universal biohazard symbol.

ould chen. ways ludes complete ounters, quipment,

The agents biosafety level are have potential to human handled incorrectly. The hazards included level may result from mucous membrane exposure, possible oral ingestion, puncture of Examples of organisms vel cterial ents oxoplasmosis salmonellosis. Substances group generally have low potential or erosol ontamination.

Although precautions will vary with specific substances, are eneral equirements or iosafety vel

- Limited ccess luding biohazards
- The wearing of gloves, laboratory coats, gowns, face shields of Class or Class biosafety cabinets to protect against otential erosol ontamination
- Appropriate ontainers
- Specific ructions or econtamination of equipment potentially dangerous materials, including nitoring eporting ontamination roblems
- Physical ontainment evices toclaving, eded

Agents biosafety level are substances serious potentially lethal The potential for aerosol respiratory ransmission rganism category *Mycobacterium tuberculosis* t level, primary secondary riers equired rotect ersonnel. neral requirements vel ollows:

- Controlled ccess
- Decontamination
- Decontamination lothing, quipment
- Testing f ersonnel valuate ossible xposure
- Use f lass iosafety inets hysical containment evices uring rocedures
- Use f ersonnel

It ely ersons ed xperience biohazards ill ver ncounter ubstances luded biosafety level IV. gents found category pose high risk of life-threatening Included level are Ebola Marburg viruses other dangerous exotic agents. Facilities handle substances exercise maximum containment. ersonnel ollow wer-in wer-out rocedures ess ody quipped ositive upply. ndividuals ork cilities ill undergo xtensive raining nsure ety.

Some eterinary ractices nostic ecimens tside laboratories or ysis. egulations elated ment f otentially dous ectious erials United es ed epartment rans portation enforced by Federal viation dministration. The .S. epartment ransportation onsiders rials e easonably xpected ontain oorganisms otentially hazardous ectious. nfectious erials lassified either ategory ategory epending egree risk associated with exposure to materials. Category poses her egree ategory ategory ludes erials wn ely ontain ec tious ent orm ermanent ility, life-threatening xposed erial. lude ures known o ontain ents uch *Bacillus anthracis, Coccidioides immitis, Mycobacterium tuberculosis,* est irus. Category ludes erials ontain ectious ent orm ermanent ility, threatening or healthy humans or exposed o erial. nostic om eterinary ients tside oratories or ysis Category . nfectious ents et iteria or inclusion Category will generally fall into Category B, they e xempt om egulations. xemptions lude following:

- Specimens hich gens ve een ctivated
- Specimens or known to contain infectious agents
- Specimens ontain nly npathogenic microorganisms
- Dried lood ecal ccult lood

The shipping of materials either category requires specific packaging eling efore resented rans portation rier edEx, ervice). eneral, specimens must be sealed, leakproof containers. If primary container roof, urrounded yer of ertight erial. rbent erial ced st yer, cond ertight yer dded. of ontents ched cond yer, erial then ced propriate ton. carton ust ry ectious ubstance el identifying kers ecified egulations.

The eterinary oratory cated parate om perations area ust e ell ell nough ccommodate laboratory quipment rovide omfortable ork Countertop ce ufficient nsitive quipment such chemistry analyzers cell counters are physically separated from centrifuges water Room temperature controls rovide onsistent nvironment urn provides for optimal quality control. draft-free area preferable to ne ith pen indows onditioning ducts lowing ry hich contaminate specimens interfere with test results. lthough each veterinary practice unique, every practice laboratory certain components, including storage space, electrical supply, nternet ccess.

The clinical laboratory should be separate from the main traffic flow in the clinic.

The laboratory area needs source of running water to provide place to rinse, drain, or specimens reagents to discard In every veterinary practice, caution be paramount; handling disposing of hazardous laboratory materials bear legal ethical responsibilities have increased substantially recent decades. Certain laboratory practices are essential for the protection of workers and the environment. Some of practices are simply good laboratory hygiene, whereas federal, state, local regulations have mandated others. thorough understanding of laws foundation of proper laboratory practices involve hazardous chemicals specimens. When doubt, veterinary tech never dispose of unknown reagents or chemicals down any drain.

Adequate storage space must be available for reagents sup to avoid clutter on laboratory counter space. Drawers cabinets be available needed supplies equipment are conveniently located site where they will be used. Some reagents specimens must be kept refrigerated or frozen. refrigerator freezer be readily available. compact countertop refrigerator sufficient for practice laboratories. Frost-free freezers remove from frozen thus them more concentrated they are left freezer too long. For long-term storage of (e.g., serum, chest freezer or freezer self-defrosting be used.

The placement of electrical equipment requires careful consideration. Sufficient electrical outlets circuit breakers must be available. Circuits must be overloaded with ungrounded three-prong adapters or extension cords. Veterinary technicians avoid working with around electrical wires or instruments. An uninterruptible power supply may be necessary sensitive equipment will be used or practice located an area that is subject to frequent power outages or fluctuations.

The diagnostic laboratory of progressive veterinary clinic have Internet access laboratory or another location within veterinary clinic. Many reference laboratories or to report critical results of submitted diagnostic tests. In veterinary clinic access to digital camera attachment for compound microscope, veterinarian veterinary technician Internet diagnostic Photographic images such scanned microscopic images of blood smears urine sediments may be sent attachments to outside reference laboratory for diagnostic assistance.

A computer with Internet access is a vital component

The Internet may be valuable resource for veterinary medical information. However, information on Internet may be oversimplified, incomplete, or inaccurate. The veterinary tech Internet sources for supplemental information in addition to consultation with the veterinarian. The veterinarian technician carefully examine all Internet resources together to determine quality of each website.

Two determinants are used to website quality. First, high-quality Internet sites are unbiased: group providing information have vested interest (e.g., selling product) slanting information certain way. Second, sources be staffed by recognized experts field, such

from government agency, college or university nostic laboratory, or the merican eterinary Medical ssociation. Other ns uality ebsite lude ollowing:

- Funding onsorship learly wn.
- Timeliness osting, evising, clear cate.
- Information bout he ource e.g., he rganization's ission statement)
- Authors contributors to references on site are clearly identified.
- References ces ormation ed.
- Experts ve eviewed e's ontent or ccuracy completeness.

Box ummarizes ortant iteria or valua tion f nternet esources.

Who is the author? Does the author list his or her occupation and

Chapter eview uestions ppendix

- A omprehensive oratory ety rogram mented ractice oratory nsure ety employees.
- MSDSs vailable or hemicals ccessible all otentially xposed mbers.
- Regulations related to laboratory safety involve multiple gov ernment encies.
- Personnel rovided propriate hen required.
- Chemical ontainer els ommunicate ecific dous information.
- Secondary hemical ontainers roperly eled.

General Laboratory Equipment

After studying this chapter, you will be able to:

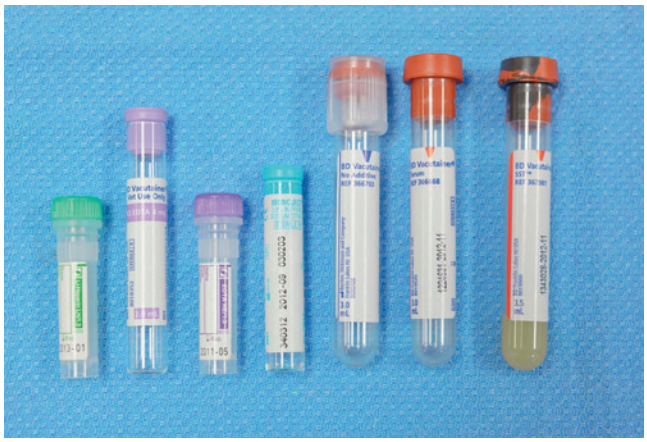
- List ypes quipment ommonly veterinary practice laboratory.
- Differentiate etween rizontal- led-head centrifuges.
- Describe roper entrifuge.
- Discuss lection roper ettes.
- Define erm efractive ex and describe the proper use of a refractometer.

Centrifuge
Incubator
Pipette
Refractive index
Refractometer
Supernatant

A variety of general laboratory equipment is needed for the in-house clinical laboratory. The size of the veterinary practice and the tests that are routinely performed in the laboratory determine the equipment and instrumentation needed. Minimal equip ment includes a microscope, a refractometer, a microhematocrit centrifuge, and a clinical centrifuge. Additional instrumentation that may be needed—including blood chemistry analyzers, cell counters, water baths, and incubators—depends on the type and size of the practice, the geographic locale of the practice, and the special interests of practice personnel. Test tubes, pipettes, heat blocks, and aliquot mixers are also commonly found in veterinary practices. The proper use and maintenance of this equipment are essential to ensure accurate test results and safety of personnel.

Test tubes that are used in the veterinary practice laboratory may be made of glass or plastic, and they are available in many sizes. Microhematocrit tubes, which are primarily used for evaluation of packed cell volume, may be plain or contain anticoagulant. Blood collection tubes are generally made of glass, and have color-coded caps to indicate whether any additives are present Fig. 2.1). Conical tubes have a narrow base and are most often used to centrifuge substances such as urine, which contain solid material within the solution (Fig. 2.2). Blood collection and conical tubes are available in a large number of sizes.

Centrifuges are vital instruments with many uses in the veterinary practice laboratory. The centrifuge is used to separate substances of different densities that are in a solution. The centrifuge spins samples at high speeds, which pushes the most dense components in the sample to the bottom of the tube. Liquid components are layered above the solid components, also according to their densities. When solid and liquid components are present in the sample, the liquid portion is referred to as the **supernatant** and the solid component is referred to as the sediment. The supernatant (e.g., plasma or serum from a blood sample) can be removed from the sediment and stored, shipped, or analyzed. Centrifuges vary in size, capacity (i.e., the number of tubes that can be spun at one time), and speed capabilities. Veterinary



Blood collection tubes are available in a variety of sizes and

Swinging-arm or horizontal-head centrifuge.

Conical Centrifuge Tube. This type of tube is used to centrifuge substances that contain solid material in solution.

cally designed for small sample volumes.

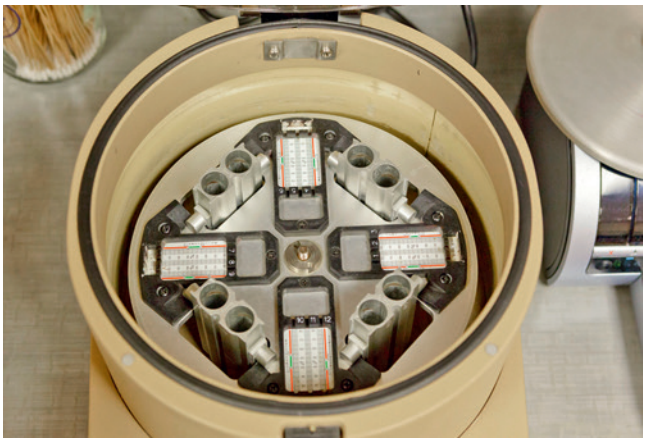
practice laboratories often have more than one type of centrifuge. A microhematocrit centrifuge is designed to hold capillary tubes, whereas a clinical centrifuge accommodates test tubes of varying sizes. Larger referral practices and reference laboratories may have additional types of centrifuges. A refrigerated centrifuge is used when materials must be kept cool during centrifugation (e.g., processing of blood components for transfusion therapy).

Centrifuges separate substances according to their

Clinical centrifuges that are used in veterinary laboratories are one of two types, depending on the style of the centrifuge head. A horizontal centrifuge head, which is also known as the "swinging-arm" type, has specimen cups that hang vertically when entrifuge uring entrifugation, the cups swing out to the horizontal position. As the specimen is centrifuged, centrifugal force drives the particles through the liquid to the bottom of the tube. When the centrifuge stops, the specimen cups fall back to the vertical position.

The horizontal head centrifuge has two disadvantages. At excessive speeds (i.e., greater than 300 revolutions/min), air friction causes heat buildup, which can damage delicate specimens. In addition, some remixing of the sediment with the supernatant may occur when the specimen cups fall back to the vertical position when the centrifuge head stops spinning.

The second type of centrifuge head that is available is the angled centrifuge head. The specimen tubes are inserted through drilled hold tubes fixed angle, usually of approximately 52 degrees. This type of centrifuge rotates at higher speeds than the horizontal-head centrifuge, without excessive heat buildup. The led entrifuge ually onfigured ccommodate just one tube size. Smaller-sized tubes require the use of an adapter unless a small-capacity centrifuge is available (Fig. 2.4). Microhematocrit centrifuges are a type of angled centrifuge. The microhematocrit centrifuge configured to accommodate lary tubes. In veterinary practice, the microhematocrit centrifuge is used for evaluation of the packed cell volume in a whole blood sample. Centrifuges that combine the features of more than one type of centrifuge are also available (Fig. 2.5



centrifuge is used to spin microhematocrit tubes.

In addition to a standard on/off switch, most centrifuges have a timer that automatically turns the centrifuge off after a preset time. A tachometer or dial to set the speed of the centrifuge is also usually present. Some centrifuges do not have a tachometer and always run at maximal speed. Most centrifuges have speed dials that have been calibrated in revolutions per minute (rpm) times 1000. Thus, a dial setting of 5 represents 5000 pm. Some laboratory procedures require specific relative centrifugal force (RCF) or G-force be used. The calculation of RCF requires measurement of the radius of the centrifuge head (r), measured from the center to the axis of rotation. The RCF is then calcu lated as follows:

118 10

A centrifuge may also have a braking device to rapidly stop it. The brake should only be used in cases of equipment malfunc tion, when the centrifuge must be stopped quickly. The centrifuge must never be operated with the lid unlatched. Always load the centrifuge with the open ends of the tubes toward the center

Verify that the load is properly balanced, with tubes of equal size and weight

of the centrifuge head. Tubes must be counterbalanced with tubes of equal size and weight placed directly opposite from each other. Water-filled ubes entrifuge. This ensures that the centrifuge will operate correctly without wobbling and that no liquid is forced from the tubes during operation. Incorrect loading of the centrifuge can cause damage to the instrument and injury to the operator. The centrifuge should be cleaned immediately if anything is spilled inside it. Tubes sometimes crack or break during centrifugation. Pieces of broken tubes must be removed when the centrifuge stops. If these are not removed, they could permanently damage the centrifuge. Box 2.1 contains general rules for centrifuge operation.

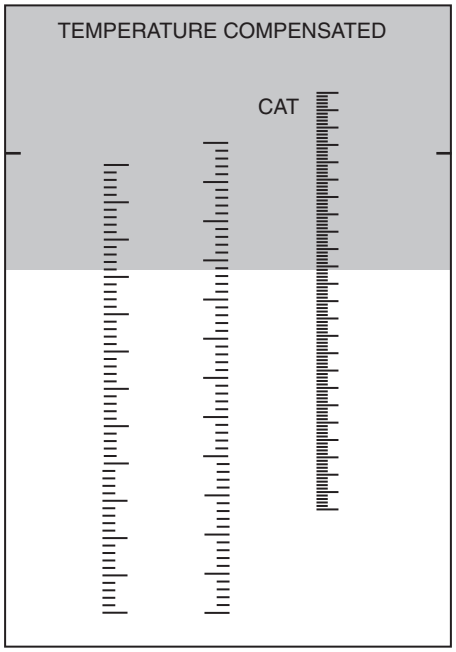
The operator's manual should list maintenance schedules for the different components of the centrifuge. Some centri fuges require periodic lubrication of the bearings, and most need the brushes to be checked or replaced regularly. Periodic verification entrifuge imer perating orrectly be performed with a stopwatch. Run the centrifuge at several speeds, and repeat each test run at least twice to ensure reproduc ibility. A tachometer can be used to verify that the centrifuge is reaching the appropriate speeds. A regular maintenance schedule prevents costly breakdowns and keeps the centrifuge running fficiency.

Centrifuges must always be balanced with tubes

Specimens must be centrifuged for specific time specific speed for maximal accuracy. A centrifuge that is run too fast or for too long may rupture cells or destroy the morphologic fea tures of cells in the sediment. A centrifuge may not completely separate the specimen or concentrate the sediment if it is run too slowly or for less than the proper time. Information about the speed and time of centrifugation should be developed for all laboratory procedures and strictly followed for maximal accuracy.

refractometer, or total solids meter, is used to measure the **refractive index** of a solution (Fig. 2.6). Refraction is the bending of light rays as they pass from one medium (e.g., air) into another medium (e.g., urine) that has a different optical density. The degree of refraction is a function of the concentration of solid material in the medium. Refractometers are calibrated to a zero reading (zero refractive index) with distilled water at a tempera ture of between 60° . The most common uses of the refractometer are for determination of specific gravity of urine or other fluids and the protein concentration of plasma and other fluids.

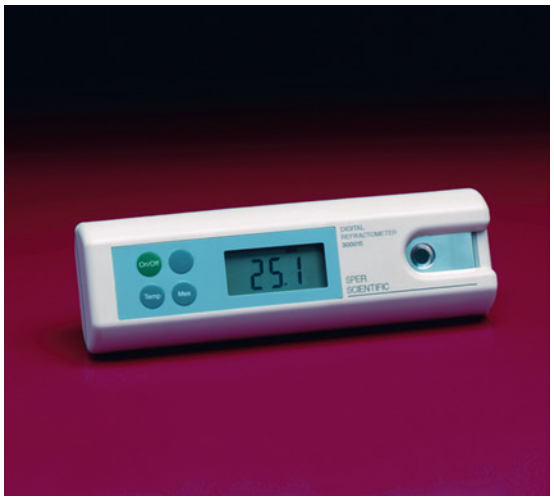
of urine specific gravity and total solids in plasma.



interface. (Courtesy B. Mitzner, DVM.)

The refractometer has a built-in prism and calibration scale Fig. 2.7). Although refractometers can measure the refractive index of any solution, the scale readings in the instrument have been calibrated terms of specific gravity ratio protein concentrations The specific gravity or protein concentration of a solution is directly proportional to its concentration of dissolved substances. Because no solution can be more dilute or have a lower concentration of dissolved substances than distilled water, calibration readings (either specific gravity or protein concentration) are always greater than zero. The refractometer is read on the scale at the distinct light–dark interface.

Various refractometer models are available. Most are temperature compensated between 60° . As long as the temperature remains between these two extremes, even as the refractometer is held in the hands, the temperature fluctuation will not affect the accuracy of the reading. Refractometers are available that are calibrated for canine, feline, and equine samples. The refractive index of some species correlates to a unique urine specific ravity eterinary-specific efractometers calibrated to account for those differences. Newer refractometers



Digital refractometer. (Courtesy B. Mitzner, DVM.)

are digital and contain a microprocessor that provides automatic calibration and temperature monitoring (Fig. 2.8

The procedure for the use of the refractometer is given in Procedure 2.1. The refractometer should be cleaned after each use and the prism cover glass and the cover plate wiped dry. Lens tissue should be used to protect the optical surfaces from scratches. Some manufacturers suggest cleaning the cover glass and plate with alcohol. The manufacturer's cleaning instructions should be consulted.

The refractometer should be calibrated regularly (i.e., weekly or y, epending istilled er oom empera ture placed on the refractometer should have a zero refractive index efore ead ecific ravity the light–dark boundary deviates from the zero mark by more than one-half of a division, the refractometer is adjusted by turning the adjusting screw as directed by the manufacturer. The refractometer should not be used if it is not calibrated to zero with distilled water.

Although most test kits and analyzers contain their own spe

pipettes and pipetting devices, some additional pipettes and pipetting devices may be needed in the veterinary practice laboratory. The primary types of pipettes used in the practice laboratory are transfer pipettes and graduated pipettes. Transfer pipettes are used when critical volume measurements are not needed. These pipettes may be plastic or glass, and some can deliver volumes by drops. Graduated pipettes may contain a single volume designation or have multiple gradations. Pipettes with single gradations are referred to as volumetric pipettes and are the most accurate of the measuring pipettes. It is important that the pipette be used correctly to ensure that the desired volume is measured. Always hold the pipette vertically, not tipped to the

side. er olumetric ettes ually esignated pipettes, which means that the pipette is designed "to deliver" the specific volume. small of liquid remain tip of the pipette after the volume has been delivered. Volumetric pipettes that have been designed to deliver microliter volumes are designated as TC pipettes, which means that the pipette is designed to contain" specified volume. These pipettes must only e d ecified olumes ubstances liquids. The pipette then must be rinsed with the other liquid to eliver ecified olume ccurately. olume fluid left in the tip of the pipette is then blown out of the pipette. Pipettes that contain multiple gradations are marked as either "TD" r TD low t," epending hether remaining in the tip of the pipette should remain or be blown out. with blow out pipettes usually contain double-etched or frosted band at the top.

Small incubator for use in the veterinary practice laboratory.

The ette hosen or ecific plication ways be the one that is the most accurate and that measures volumes closest to the volume needed. For example, if 0.8 eded, a 1-mL pipette rather than a 5-mL pipette should be chosen. Pipettes are designed for measuring liquids specified tem peratures, most commonly room-temperature liquids. Liquids that are nificantly older mer ill ure ccurately. Pipetting devices must also be used correctly, and fluid must not be allowed to enter the pipetting device. The pipette must be held vertically without tilting to the side when transferring liquids. Never pipette any fluid by placing your mouth directly on the pipette.

A variety of microbiology tests require the use of an **incubator** Incubators for the in-house veterinary practice laboratory are available ariety onfigurations. tor must be capable of sustaining a constant 37° which is the temperature at which the majority of pathogenic organisms grow. The incubator be fitted with thermometer, or one should be placed inside the chamber to monitor the tempera ture (Fig. 2.9). Heat should be provided by a thermostatically controlled element. A small dish of water should also be placed inside to maintain proper humidity. Some incubators have built-in humidity controls, but this type of equipment tends to be expensive. Larger laboratories may have incubators that auto matically monitor temperature and humidity as well as carbon dioxide and oxygen levels.

Many reagents and test kits that are used in the in-house vet erinary clinical laboratory require refrigeration, and some may

Aliquot mixer.

require storage in the freezer. Samples such as blood and urine may also require refrigeration. A basic tabletop refrigerator can be used for most items. This refrigerator may not contain any food for human consumption. Facilities that perform blood-banking services or transfusions must also have a special blood-bank refrigerator.

Some clinical chemistry assays, coagulation tests, and blood-banking procedures may require the use of a water bath or heat block that is capable of maintaining a constant temperature of

A variety of types of water baths are available, includ ing simple standard water baths, circulating water baths, and waterless bead baths. A rack must be placed inside the standard and circulating types to hold materials in place. Bead baths do not require a rack and have little need for maintenance. Heat blocks are generally designed to accommodate just one tube size, although some have multiple adapters that can be used for a variety of tube sizes (Fig. 2.10

A large number of automated analyzers are available for use in the in-house veterinary practice laboratory. These include hematology, clinical chemistry, electrolyte, immunology, coagula tion, and urine analyzers. The units may run single tests, or they may be capable of running multiple tests on the same sample. Analyzers vary considerably in test principle, and each one has specific dvantages dvantages. etailed ormation about yzers vailable or ecific ypes esting provided in their respective chapters.

Slide dryers can be a useful addition to the busy veterinary practice laboratory. The dryer minimizes the time required to prepare uch lood ell liquot ers helpful by keeping items well mixed and ready for use (Fig. 2.11

Chapter 2 review questions are in Appendix A

- Clinical entrifuges repare or ysis.
- Periodic calibration of centrifuge needed to ensure it is reaching the required speeds.
- The efractometer or veral ypes be calibrated on a regular basis to ensure diagnostic-quality results.
- A variety of additional supplies equipment may be needed veterinary practice laboratory, depending on specific tests performed.
- Pipettes veral ypes, ch led what differently.
- Proper ette nsures ccurate urement ubstances.

After studying this chapter, you will be able to:

- List oscope.
- Describe tions oscope.
- Describe roper oarse djustment knobs.
- List eps oscope
- Discuss enance oscope.

Care and Maintenance,
Calibration of the Microscope,
Digital Microscopy,
Capturing igital mages,
Review Questions,
Key Points,

Binocular
Compound light microscope
Condenser
Dark field microscope
Fluorescent microscope
Numerical aperture
Objective lenses
Phase-contrast microscope
Planachromatic
Resolution

Different ypes oscopes vailable or ut eterinary oratory enerally ype. Electron oscopes, hich lectron eate magnified bjects, rimarily esearch settings large human medical facilities. Light microscopes are tilize isible, raviolet, ce include **compound light microscopes** **fluorescent microscopes** **phase-contrast microscopes** **dark field microscopes** contrast, fluorescent, dark field microscopes are used primar ily eference oratories, ecially or iewing specimens. n eterinary ractice oratory, h-quality **binocular** ompound oscope ntial This oscope valuate lood, men, exudates, ransudates; ody eces; miscellaneous ecimens. etect ernal xternal asites ially haracterize cteria. practice eally oscopes. be d or erforming outine asite rocedures volve orrosive erials. second oscope eserved or tology matology valuations.

ompound oscope ecause en erates ombination ompound light microscopes have many components light The optical ube etween bjective eyepiece. In microscopes, distance The chanical valuated. microscope have smoothly operating mechanical stage to low ion eft- right-handed stages are generally available. Coarse Fig. ocus ocus object eing iewed.

The ompound oscope onsists parate lens stems: **ocular** stem bjective stem. ocular nses cated yepieces ften ve magnification of This ocular lens magnifies bject nocular oscope yepiece, whereas inocular oscope, hich ommonly used type, two eyepieces. The two eyepieces be adjusted to ch erpupillary r.

Most ompound ight icroscopes ave hree our **objective lenses** each with different magnification power. The

A binocular compound light microscope for use in the veterinary clinical laboratory. (Courtesy VetLab Supply, Palmetto Bay, FL.)

The coarse focus adjustment knob.

common objective lenses are (low power), (high dry), (oil immersion). The lens found on all microscopes. An optional lens, (low oil immersion), found on some microscopes. Some microscopes may have phase-contrast lenses. It important only immersion oil designed for microscopy be used on micro scope. Other oils may be damaging to optics.

Total magnification of object being viewed calculated by multiplying ocular magnification power objective magnification power. For example, an object viewed through the objective lens ocular lens times larger diameter unmagnified object:

The microscope head supports ocular lenses may be straight or inclined. microscope with inclined head ocular lenses that point back toward the user. This minimizes the need to bend over microscope to look through lenses. binocular head needed for nearly all routine laboratory evalu ations. Trinocular heads are available be used for training purposes or client education. The nosepiece objective lenses. It always rotate easily provide ready access to objective lenses for cleaning. The ocular lenses must be compatible with objective lenses be cautious about buying objectives oculars from different sources. Wide-field objective lenses provide larger visual field area standard type are recommended when user spends long periods looking through microscope, because they tend to reduce fatigue. High-eyepoint ocular lenses are for individuals who need or prefer to keep their eyeglasses on while microscope; however, who do wear eyeglasses may lenses to be advantageous well.

The important components of microscope are objective lenses. Objective lenses are characterized one of three types: achromatic, semi-apochromatic, apochromatic. The latter two are primarily used research settings for

The substage condenser control is used to raise and lower the

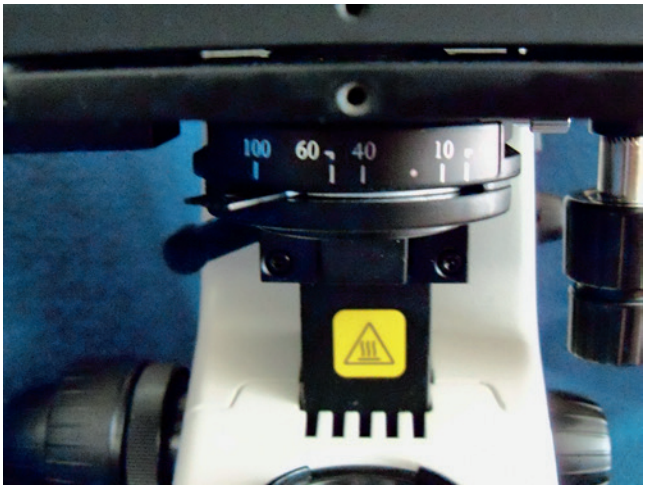


Fig. 3.6 The aperture diaphragm controls the amount of light illuminat

photomicrography. ype chromatic wn **chromatic** ns vailable. ype, hich referred o rovides re orm focus from center to periphery of microscopic image. However, h-quality chromatic cceptable or outine eterinary

The esolving ower oscope or image uality escribed erm **numerical aperture** A). he ommon ype **condenser** o-lens bbe ype. ondenser qual greater ower bjective. resolving ower stem ill reater NA f ower bjective. ecially ortant for bjectives reater btain resolution om bjectives, ondenser reater must be used, condenser must be raised makes contact with bottom of slide. Otherwise, air—which A f ill stem, reby elegating system o esolution

When viewed through compound light microscope, object appears own eversed. ctual ight image en ctual en right side. Movement of slide by mechanical stage reversed. ravel ve object (or portion of object) to be moved. hen stage ved o bject pears ve ight.

The substage condenser consists of two lenses focus light from light source on object being viewed. Light focused by aising r wering ondenser ithout stage ondenser, ings pear bjects. The erture hragm ually ype, hich onsists of umber ves pened losed ontrol f luminating bject

In odern icroscopes, he ight ource ontained ithin he microscope. he ost ommon ight ources ound ompound light microscopes are low-voltage tungsten higher-quality quartz-halogen light-emitting diode light. The light ce parate, ve heostat o djust ensity. any er oscopes that re currently contain ament light urces generally

that the eyepiece is at the correct interpupillary distance and that

the diaphragm until the circle of light just touches the edge of the

halogen or tungsten) are configured for Köhler illumination. To obtain high-quality images, microscope must be adjusted for roper lumination ox

Microscope rices ary epending uality accessories luded. oscope or ypical ractice often neither expensive nor expensive one. ccessories such dual-viewing options, phase-contrast or darkfield ilities, ras, hted ointers to rice ut ersatility oscope nostic oratory). econditioned oscopes are metimes vailable ough ptical quipment suppliers conomical ernative chase w oscope.

Regardless of features of individual microscope, care must be taken to follow manufacturer's recommendations for routine maintenance Procedure Only high-quality lens tissue vent

Adjust the condenser and diaphragm in accordance with the manufacturer's

Look for a suitable examination area using the 10 (low-power) objective

Do not use the coarse adjustment knob to focus on the specimen while

Rotate the nosepiece so that it is halfway between the high-power and

Do not use the coarse adjustment knob to focus on the specimen while

While looking through the eyepieces, adjust the distance between them

needed, methanol be used, or specially formulated lens-cleaning solution be purchased. Excess oil may require of xylene for cleaning. However, xylene may dissolve some of the adhesives that are used to secure the objective lenses must therefore be used sparingly. Note methanol xylene are flammable toxic. The microscope be wiped clean after each kept covered when dirty field of study may be caused by debris on eyepiece. The eyepieces be rotated one time while technician looks through them. If debris rotates, located on eyepiece. The eyepiece cleaned with lens paper. Cleaning adjustment by microscope professional be performed annually.

Extra light bulbs be available. Changing light bulb requires turning off power unplugging microscope. When defective bulb cooled, be removed replaced with new bulb according to the manufacturer's instruc tions. Replacement bulbs be identical to they are replacing. Avoid touching replacement bulb directly, because oils from shorten life of some types of bulbs.

Locate microscope area where protected from excessive humidity. With proper care, high-quality

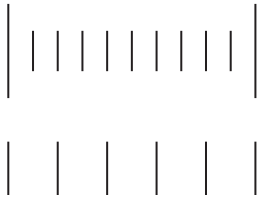
microscope lifetime. The microscope be placed area where be moved frequently, jarred by vibrations from entrifuges oors,
It ust e ept way om unlight oscope ried ith curely er upporting

The size of various stages of parasites often important for their correct entification. ome ggs *Trichuris* versus eggs of *Capillaria* species microfilariae *Dirofilaria immitis* ersus ofilariae *Dipetalonema reconditum* Calibration of microscope lenses be per formed on every microscope used laboratory. Each objective ns vidually rated rocedure
The e ometer oscope tched ked visions ometer quals ometer d nly nce rate bjectives oscope.

at low power (10), and focus on the 2-mm line if using the stage

on the stage micrometer that is aligned with the 10

), the distance between each hatch mark



2-mm line marked in 0.01-mm (10-

After cular ometer ompound oscope een rated 0 rated or service life of microscope; stage micrometer never used again. The stage micrometer therefore be borrowed from university or other diagnostic laboratory rather purchased.
The ocular micrometer glass into one of microscope yepieces. metimes eferred eticle. The impose image of net, or crosshairs over viewing area. The reticle be mounted in separate ocular lens be removed replaced with nonreticle assembly for imes hen eded. tched hatch marks are spaced equal intervals. The number of hatch arks he isk ay ry, ut he alibration rocedure does hange. ometer etermine e ometers etween ch ocular ometer or ch bjective oscope being rated. ormation ecorded eled f oscope or ture eference.

Digital oscopes ptics ra ture y omputer een nitor. here e umber dels vailable ary idely uality rice. xpensive dels ve nitors ce f yepieces oscope. end have oorest uality. icroscopes onnect computer via cable incorporate digital imaging technology o ra end rovide high-quality

Digital oscopy reatly nhance ractice ecord eeping become valuable tool for client education training. Obtaining hotomicrographs normalities en lood or tissue cytology preparations, parasite evaluations, urine sediment valuations, nostic to ocument ient ecord.
Photomicrographs dded lectronic ient records ermanently ocument Digital es ient ormation uring consultations eterinary rofessionals eate rary f or eaching poses.

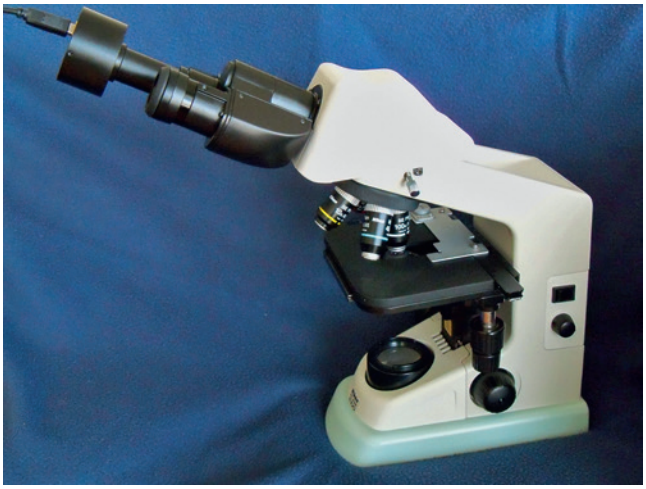
Digital oscopy ecome re ordable or ven small ractice. ommon ypes stems lude incorporated oscope, ch third yepiece rinocular oscope replace one of eyepieces on standard binocular micro scope. ome stems orporate iewing een ddition o ility erface omputer een monitor. lthough ossible btain dapter chments for icroscope yepiece hat llow tandard andheld igital camera o btain hotomicrographs, wer

attached digital camera. (Courtesy VetLab Supply, Palmetto Bay, FL.)

cameras be used way, cost of adapters may e thod rohibitive. omputer ftware included with digital microscopy systems allow images to be categorized chived. stems turing es ormats g, mp, iff). Some f rograms ectly xporting images o hoto-editing rogram.

Regardless of type used, systems are nearly all capable of capturing video addition to still images. Most of systems ve xibility low or rojection real time onto computer screen or monitor. This serve raining ool or mbers lowing ultiple individuals iew oscopic eterinary technician erforming oscopic valuation. eal-time streaming f nternet ossible, greatly enhance consultations with other veterinary professionals.

Digital microscopy systems vary with regard to their image reso lution ilities. erm **resolution** efers o egree detail isible larity esolu tion ured els, esolution umber providing e reatest etail larity. The greater the number of pixels, greater degree of detail clarity more e nlarged ithout larity. here primary types of digital imaging methods, different types f nsors. harge-coupled evice complementary metal–oxide–semiconductor image sensors ary egree pness produce. ras ecommended ecause end to rovide her-quality omparable camera resolution. In addition, camera may

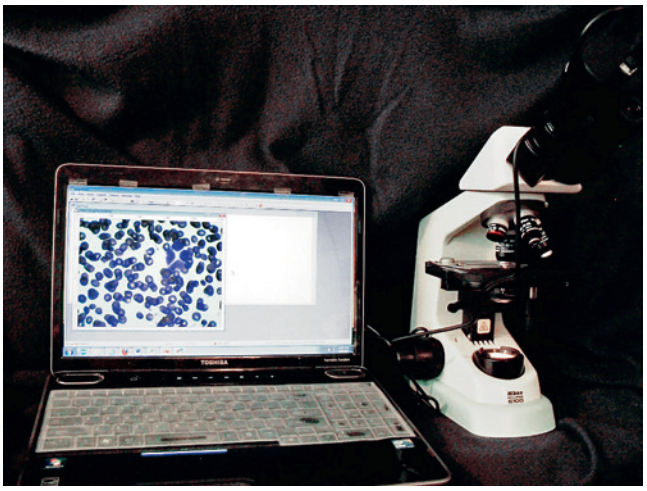


A digital eyepiece camera in place on a clinical microscope.

low or rojection resolution f ticular ed esolution output device used, such computer screen or monitor being d o esolution gapixels enerally ufficient or rinting ithout any of clarity. higher resolution will be needed for images ill e lished.

Digital icroscopes hat ncorporate gital amera nclude software to download save images to computer are generally compatible indows perating stems. egrated systems end reat re ostly chasing separate ra ch inocular rinocular clinical oscope. owever, ve dvantage ways being ready, they generally capture images quickly. The very busy ractice oratory her orthwhile. ariety f xpensive ypes ras vailable for hotomicroscopy dded microscope. Digital cameras attached to trinocular microscopes are efficient. The camera attachment mounted to third yepiece, stem ched omputer, often via attachment. Some systems will instead contain integrated evice emoved for ransfer omputer. hen eterinary technician encounters abnormality to be photographed, systems erform eded tions ery uickly.

Eyepiece cameras attach to binocular microscope usually involve emoval oscope yepieces replacing with eyepiece camera for capturing of images directly onto omputer Fig. 3.9). These systems highly ost-effective, ut end htly nger hen veterinary echnician ncounters ecorded, of oscope yepieces emoved, ra place, and the age captured computer Fig. 3.10 The technician then removes camera replaces eyepiece to continue emainder valuation. ttachments available low tphone let ched to yepiece or ture hotomicrographs



use of the software provided by the camera manufacturer.

The miPlatform system for obtaining photomicrographs using a smartphone or tablet. (Courtesy VetLab Supply, Palmetto Bay, FL.)

It ortant oscope btain hotomi crographs h-quality ptics. verall uality photomicrographs reatly nced uality microscope optics. The microscope have planachromatic ld) bjective oscope equires illumination, be sure to adjust before attempting to capture images. ithout proper illumination adjustments, image may pear venly luminated, esult bright dows ewer microscopes make of light sources tend to produce hest-quality esult nhanced olor balance reater ility tput chievable ht ces. egardless ype oscope ntial oscope rofessionally rviced ually.

Chapter eview uestions ppendix

- The linical oratory ve h-quality binocular ompound oscope.
- The linical oscope ve ocusable ubstage condenser chanical
- The roper oscope ntial ensure ccurate esults.
- The ration oscope eded low or accurate easurement ells rganisms hat ay resent
- Digital microscopy enhance veterinary practice's record keeping, lient ducation, raining.

The Metric System and Lab Calculations

After studying this chapter, you will be able to:

- Explain matical rinciples, uch ecimals, multiplication, vision, atios.
- Perform ions elated utions.
- Describe tilize tric nternational System f nits.
- Perform ions onvert etween ahrenheit Celsius urements.

Dilutions
Gram
International System of Units
Liter
Metric system
Ratio
Serial dilution

Veterinary echnicians equire wledge erform ariety ions oratory. eagent solutions ed repared uted, measured sometimes diluted, results must be calculated. All f hese athematical perations equire hat he eterinary technician have thorough understanding of metric system ell rong ckground ebra.

Abstract numbers are with designations. Concrete numbers ve ecific alue, uch ollars umber ithout esignation ract number. umber hat esignates pecific lue e.g., **grams** s concrete or denominate number. Numbers of different denominations ogether matical perations. When numbers of different denominations must be manipulated mathematically, onvert umbers esignation. umbers umbers, actions, or ed umbers.

Although several systems of measurement are used veterinary medicine, of calculations performed veterinary prac tice volve **metric system** he tric stem owers or erent stem.

The tric stem ecimal stem ion nly three or eight, olume, arious alues e xpressed tric stem refixes designate multiples or fractions of

To work metric system, some of more commonly used prefixes breviations morized.

The ee ure ummarized re:

The metric system multiples or powers of to describe magnitudes re ter,

Submultiples of Basic Units

gram, **liter** The prefixes for multiples submultiples of rovided able or kilogram rams, ligram / f ram. addition, entimeters ter, ters meter. ith regard to volume, there are deciliters liter, liters decaliter, decaliters hectoliter. Consistency ortant umbers, ut ecially tric system. lthough ram breviated orrect

To e rrors erpretations umbers, ew eneral or tric stem learned. he ften ncountered equivalence entimeter liliter. metric system, two are both used for volume, they designate olume. ecause tric ure of er efined olume entimeters or olume lthough erms *milliliter* *cubic centimeter* are often used interchangeably, milliliter orrect esignation or

Any ecimal umber umber of ecimal oint ve ero rted ceholder. Zeroes dded er ecimal umbers, void confusion medication orders. Fractions are written metric ystem. lways se ecimal umbers xpress umbers e

The ational nstitute echnology government ency romotes **International System of Units** his stem erived om rench Système International d'Unités abbreviated are designated or ven fferent ypes easurements: ngth, ass, time, electric rrent, temperature, luminosity, and uantity. In veterinary clinical laboratory, of importance are or emperature, uantity. kilogram; temperature reported kelvins quantity he oratory nstitute international ency elines or

It important to know which particular test result eported. or ve raditionally eported serum glucose results normal value for dogs could be oratory nstitute guidelines designate reporting of glucose results rmal alue

The eterinary echnician ed repare **dilutions** of eagents ient oratory. on centrations utions ually xpressed atios original olume olume. **ratio** one elative umber elative to hole. atios ritten umber ys; or example, / , nd .5 re ll quivalent. hese erms xpress ratio one two," one to two," or one half." ll three atios qual. erms atio ither ract numbers nly atio ually xpressed ecimal eterinary echnology specific gravity. Specific gravity ratio expressed decimal form epresents eight ubstance elative weight f olume er.

To repare ution ient ombine microliters of with of distilled water. This represents ution hich educes mati cally to Results from any tests involving dilution must n e ultiplied ield orrect esult or undiluted

Serial dilutions e metimes eded hen erforming certain unologic hen reparing ra tion ves or quipment. utions repared described reviously, oncentrations ubstances each ution ed. or tion f ilirubin ontains uted oncentration ch ution espectively.

Scientific ion thod ery ery small umbers. ion umbers scientific ion metimes hen umbers ve many decimal places. Certain laboratory tests are reported with results given scientific notation. Scientific notation involves f xponents epresent owers or iven umber.

Very large or very small numbers are usually written

Powers f ultiplying viding

10 10

10 10 100

The eps onvert umber xpo nential orm or ntific ion ollows:
1. Move ecimal oint erm re
2. The second term power of equal to number of imes ecimal oint ved.
3. The ign or etermines he irection hich ove ecimal. ecimal oint moved o ecimal oint ved ight.

For xample, onvert ntific ion, following steps occur:
1. Move ecimal oint ehind
2. Record erm
3. Count number of places decimal point moved.
4. Record umber ces xponent.
5. The orrect er iven

To onvert umber om ntific ion, ve ecimal oint umber ces ed xpo nent. For example, to convert move decimal place two ces o ight et or gative xponents, decimal ce ved or ould e

Logarithmic notation related to scientific notation, some plications eterinary ertain oratory tests (e.g., pH) clinical chemistry analyzers involve of logarithmic notation. Like scientific notation, logarithmic notation ion ery very small numbers. Logarithmic notation expresses numbers powers of For example, number expressed ritten ecause

The example of practical application of loga rithmic notation. defined negative logarithm of hydrogen n oncentration ution. ution an concentration of 10^{-6} as H f olution ith concentration of 10 f onsidered utral solution. ution; of more indicates or solution. Note difference between any two consecutive numbers on epresents ower erence oncentration. Another ommon pplication ogarithms ichter cale. This haracterize ensity thquakes, ch onsecutive umber epresents ower of en erence ensity rength thquake.

The emperature urement stem eteri nary clinical laboratory Celsius However, many items (e.g., est rovide itical emperature urements Fahrenheit or Kelvin systems, including such informa tion roper orage emperatures or eagents orrect temperature or erforming ticular

There e veral erent ions or conversions arious emperature ll calculations are based on fact there are points of equiva lence ng ee stems.

1. Absolute ero
2.
3. 0°

In ahrenheit er eezes oils elsius erence etween freezing (melting) boiling points Therefore, each degree elsius quivalent / degrees in ahrenheit oints quivalence, possible to derive umber of different equations that will allow for onversion rmometer eading om another. ou hichever thod

———— –

This equation be used to convert value to either Celsius or ahrenheit, rovided wn.

These quations oint quivalence oth stems:

/ 40 40

/ 40 40

These quations oint quivalencies

/

/

The elvin egins ute ero ga tive umbers. onverting elvins ccomplished o emperature elsius. emperatures eported kelvins o volve erm egrees; ather, nly tter or

- Metric system are used clinical laboratory measurements.
- Laboratory results are reported either metric system or
- Temperature measurements are made degrees Fahrenheit, degrees Celsius, or kelvins.
- Very small or very large numbers are written with scientific notation.
- Concentrations of dilutions are usually expressed ratios of original volume to new volume.
- The terms of ratio are either abstract numbers or of

Quality Control and Record Keeping

After studying this chapter, you will be able to:

- Describe omponents uality urance rogram.
- Differentiate etween ccuracy recision.
- Describe thods or erifying ccuracy results.

Accuracy
Controls
Hemolyzed
Icteric
Lipemic
Preanalytic variables
Precision
Quality assurance
Reliability
Standard operating procedures
Standards

The term **quality assurance** refers to procedures established to nsure esting erformed ompliance accepted standards processes results are properly documented. Unlike human medical laboratories, veterinary facili ties e ubject egulations equire uality urance programs. However, without comprehensive quality assurance program, ccuracy recision oratory esults be verified. comprehensive quality assurance program addresses l ects peration oratory. These aspects include qualifications of laboratory personnel; **standard operating procedures** for care of all supplies equipment; collection handling procedures; methods equency erformance uality ontrol assays; ecord-keeping rocedures.

Accuracy, recision, eliability erms equently used to describe quality control, they are standards for any quality ontrol rogram. **Accuracy** efers o w losely esults agree ith rue uantitative alue onstituent. **Preci sion** magnitude of random errors reproducibility of urements. **Reliability** ility thod accurate recise. actors ect ccuracy recision are test selection, test conditions, quality, technician skill, electrical urges, quipment enance.

The erm est lection efers rinciple method. any eterinary oratories ere adapted om oratory ddition, clinical nificance esults ary erent species. Regardless of test method used, care must be taken to follow analytic procedure exactly; any deviation seriously affect ccuracy esults. uality reatly ects uality esults. **lipemic icteric** r **hemolyzed** y equire ecial efore clinical analyzers. The collection of blood from properly fasted nimals sing ppropriate echniques nd quipment ill minimize ce rror.

Careful attention to proper sample collection methods

Although bvious ce rror, lectrical power urges opouts nificantly er quipment

function. epeated urges rten ces diagnostic quipment. ll lectrical quipment on nected to device designed to protect from surges electri opouts. rror erhaps esting parameter to control. Personnel responsible for performance of clinical testing must be appropriately trained test principles rocedures. echanisms ce rovide or the ontinual ducation ll linical aboratory ersonnel. he maintenance quipment luded uality control programs. regular written schedule of equipment tenance allows for changes equipment function to be detected before bvious rrors ccur. lways ollow ufacturer's recommendations or outine enance ruments equipment. The manufacturer will provide information regarding calibration procedures may be needed. **Standards** are nbiological erials or rating quipment.

Control serum used for technician instrument assessment. The production of valid results with control materials ensures procedure performed correctly all components (e.g., eagents, quipment) tioning orrectly. **Controls** are led xactly ient be egularly yed ch ch, y, eekly) ient rum yed

The frequency of control testing depends on laboratory's goals. To ensure reliability, control must be tested when ew ssay et p, hen ew echnician uns est, hen w umber eagents hen rument wn o erform rratically. deally, ontrol ill be ested ith ch ch ient roblem ticular equire equency control esting.

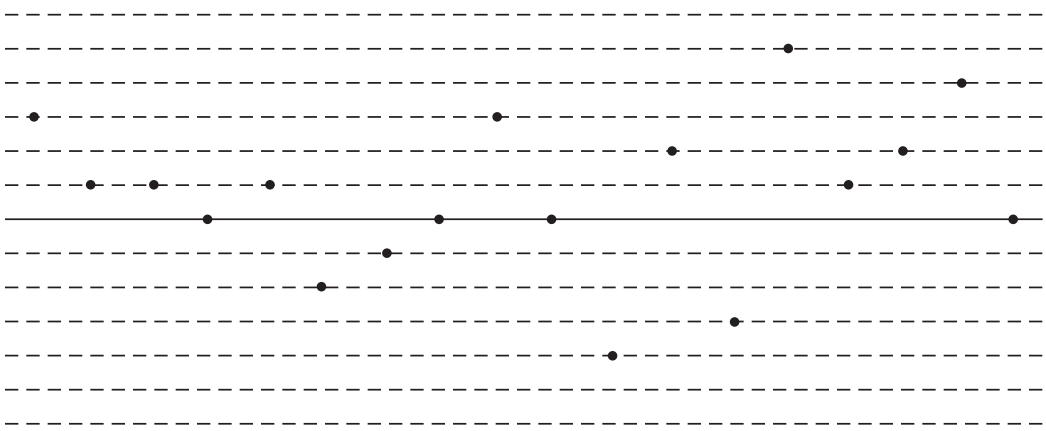
Control materials provided by the instrument manufacturer are assayed in the same manner as a patient sample.

After ompleted, ontrol alue within ufacturer's eported ange. oes assays of patient control must be repeated. The results f ysis ontrol rum ecorded chart r g or ch alues or er formed on control serum vary significantly each time ests e erformed. yzed ys: etection rends etermination whether esults or ontrol ange lished y ufacturer. ontrol rum esult oes fall ithin ange, etested. ill ange, eagents, ruments, echniques checked. hen ontrol alues uccessively ributed one r ed, systematic rror volved.

Depending on chemistry analyzer or electronic cell counter oratory, vidual uality ontrol rogram ill involve of number of solutions. Control serum consists of ooled, eeze-dried rum om ients ually ust e ccurately ehydrated efore ssayed ontrol serum een yzed epeatedly or ch on stituents present serum (e.g., glucose, urea nitrogen, calcium). e istically yzed, ange cceptable alues for each constituent established. The accepted ranges are spe or ch thod quipment ufacturer. manufacturer of control serum provides chart that range west cceptable alue cceptable value btained uring ys) average alue) or ch onstituent.

Controls ith rmal normal oncentrations constituents be evaluated, because assay method may perform all concentrations tested by particular method. ormal ontrol rum onstituent oncentrations proximate vels rmal or onstitu ent. bnormal ontrol rum onstituent oncentrations e ither her wer rmal. normal concentrations epresent oncentrations en linically with arious onditions. normal oncentration of onstituent ient esults be rusted normal ontrol rum oncentration assayed ange."

Individual laboratories may produce their own control serum. Serum obtained from clinically healthy of ne ecies ooled yzed umerous laboratory. ollected om istically analyzed o propriate anges alues.



The results of the analysis of a control serum are recorded on a chart or log for each assay.

procedure time-consuming, especially for smaller laboratories. For reason, purchasing of commercial control sera much more convenient.

Some manufacturers provide quality control service which test are sent to many laboratories for assay each month. The results from all of the laboratories are collected and compared. From results, manufacturer identify laboratories with accuracy problems.

Many factors other influence results of laboratory tests. These factors may be preanalytic, analytic, or postanalytic nature. Postanalytic factors are primarily related to entry record keeping.

Preanalytic variables may be biologic or nonbiologic. Biologic variables are factors are inherent to patient, such breed, age, gender. Because be controlled, they must be considered by veterinarian when test results are being evaluated. Other biologic variables involve factors be controlled when drawing blood such ensuring properly fasted. Nonbiologic variables are related to clerical errors well collection handling errors. Clerical errors are avoidable and include incor rect labeling, delays transporting incorrect tions, transcription errors, wrong patient. well-trained conscientious produces few clerical errors. Some of common problems related to handling are mislabeling incomplete or incorrect requisition forms. All tubes, slides, containers be labeled with owner's addition to patient's species, identification number available), date.

Analytic variables affect procedure by which analyte measured by instrument. The specific impact on test result will differ among laboratories, depending on type of instrumentation. Improperly maintained instruments errors are evident or trends results obtained with specific assay method. These errors often result gradual changes value of results to one direction to be elevated or decreased). Some factors systematic errors include inaccurate standard sera, reagent instability, method nonspecificity test method unsuitable for constituent being assayed).

Random errors are caused by variations found glassware pipettes, electronic optic variations of instruments, variations temperature controls timing. These errors occur all parts of system increase variability of results.

Instrument maintenance required to prolong life of instrument to prevent expensive downtime. All instruments are accompanied by an owner's manual. If the manual has been misplaced, manufacturer be contacted for replace ment. The manual instrument components must be inspected attended to regularly. notebook schedule with the types of maintenance required for each instru ment facilitates instrument maintenance. page dedicated to each instrument includes following information:

- Instrument
- Serial number

- Model umber
- Purchase
- Points o hecked
- Frequency hecks
- Record f eadings
- Changes made to restore accuracy precision of readings
- Cost time associated with necessary repairs restoration
- Name r erson erforming enance

Results btained ontrol rum ecorded ept ermanent ecord. eterinary echnician raph results hanges rends isually etected.

If attention to detail many sources possible of three types of errors be eliminated, then laboratory rovide eliable esults. loppy entive ork d o nostic rapeutic ers esult eath areful ention etail nsures eterinarian orrect ormation eded to e roper rescribe propriate reatment, ffer ducated rognosis.

Laboratory records are divided into internal external record systems. omplete o-date ecords cessary or both stems. umerous omputer stems vailable for lmost ll he ecords enerated he eterinary linic atient ormation, ventory, rdering ormation, ecords, oratory ored omputer. Clinics computer systems be sure to keep backup records omputer ure om omputer viruses.

By internal records, laboratory tracks assay results obtains methods. The records consist of standard operating pro cedures uality ontrol raphs. contain ructions or yses oratory. Each rocedure escribed eparate age. he asiest ay to book to insert instruction sheets accompany each commercial test three-ring binder along with pages for any other procedures performed laboratory. Each procedure performed with commercial described on separate page includes of test, synonyms y) or ationale or eagent ep-by-step ructions or ysis. ndividual pages e rted verlays or rotection.

book reviewed periodically updated needed. Those who eep ook omputer ure o-date d-copy ckup vailable.

Laboratory personnel communicate with people throughout veterinary linic oratories ough f xternal ecords. onsist equest orms accompany oratory, eport orms or results, oratory ooks vidual esults, book ontains ertinent ormation out to eference oratories. er omputer twork, ersonnel ccess uch information eded.

Information provided on request form includes patient's full entification ormation luding entification number, vailable) ell resenting thod btain ertinent ory, ests esired, ecial egarding ling, o hom thod elephone, written eport) esults eported.

The eport orm lude omplete ient entifica tion resenting esults luding propriate ion xtraordinary bservations explanatory comments, applicable. For additional backup, laboratory keep logbook to record test results. This way, riginal oratory eport orm ransit, results e etrievable.

Chapter eview ions ppendix

- Proper quality control procedures are essential to produc tion f nostic uality oratory esults.
- Factors that affect ccuracy and recision are est lection, test conditions, uality, echnician lectrical urges, quipment enance.
- The ollection lood amples rom roperly sted nimals with propriate echniques quipment ill minimize rrors esults.
- To nsure eliability, ontrol ested hen new assay set up, when new technician runs test, when new number of reagents used, or when instrument wn o erform rratically.
- SOPs uality ontrol raphs omponents of ernal ecords oratory.
- The ontains ructions or yses run oratory.
- Errors esults volve reanalytic, ytic, postanalytic ariables.

Unit Outline

Chapter 6: Hematopoiesis,
Chapter 7: Sample Collection and Handling,
Chapter 8: Automated Analyzers,
Chapter 9: Hemoglobin, PCV, and Erythrocyte Indices,
Chapter 10: Evaluating the Blood Smear,
Chapter 11: Morphologic Abnormalities of Blood Cells,
Chapter 12: Additional Hematologic Tests,
Chapter 13: Hematopoietic Disorders and Classification of Anemia,

Unit Objectives

List and describe the hematology evaluations that are commonly performed in veterinary practice.
Describe the components of blood.
Describe the development of the formed elements in blood.
Describe the appearance of normal blood cells and platelets.
Describe the appearance of commonly seen abnormal blood cells.
List the tests that comprise the complete blood count.
List and describe the equipment needed to perform a complete blood count.
Discuss aspects of quality control related to hematology testing.

Hematology is the science involved with the study of blood cells and their formation. Hematology testing represents an important role of the veterinary technician: providing accurate and reliable clinical laboratory test results to the veterinarian. An understanding of the principles of the various hematology tests and the methods used to ensure the accuracy of results is vital. The recent focus on the economic health of the veterinary clinic has also provided an opportunity for veterinary technicians to perform additional diagnostic testing, to improve overall animal care, and to provide an additional source of revenue for the clinic.

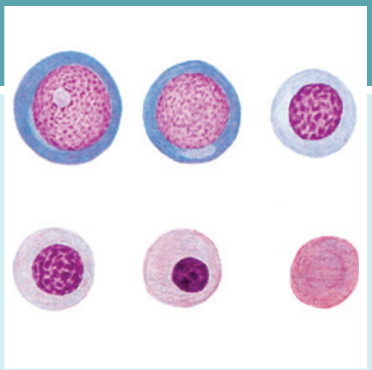
A complete hematology profile is indicated for the diagnostic evaluation of disease states, well-animal screening (e.g., geriatric), and as a screening tool before surgery. The complete blood count includes red and white blood cell counts, hemoglobin concentration, packed cell volume (PCV), a differential white blood film examination, and calculation of absolute values and erythrocyte indices. Additional tests that may be needed include reticulocyte counts, measurement of total solids, and thrombocyte (platelet) estimates. For some patients, additional information about the hematopoietic system is needed, and bone marrow evaluation must be per formed. Specific indications include unexplained nonregenerative anemia, leukopenia, thrombocytopenia, and pancytopenia (i.e., decreased numbers of all cell lines). Bone marrow evaluation is also used to confirm certain infections (e.g., ehrlichiosis) and to diagnose hematopoietic neoplasms (e.g., lymphoproliferative disorders).

Normal values, or reference ranges, for hematology results in common domestic animal species are located Appendix B. Please note that normal values are affected by a variety of factors, including the following:

- Testing thods
- Type f quipment
- Patient
- Patient ender
- Breed
- Reproductive

Laboratories should determine reference ranges for the tests performed in the clinic for the species that are commonly seen.

For dditional ces or esources ppendix extbook.



After studying this chapter, you will be able to:

- Define matopoiesis, ukopoiesis, rythropoiesis, thrombopoiesis.
- List rgans volved matopoiesis.
- Differentiate etween matopoiesis renatal dult
- Explain rythropoietin matopoiesis.
- List ells rythrocyte uration ries.
- List ells ukocyte uration ries.
- Describe ormation elets.

Hematopoiesis,
Erythropoiesis,
Thrombopoiesis,
Granulopoiesis,
Monopoiesis,
Lymphopoiesis,
Definitions,
Review Questions,
Key Points,

Agranulocytes
Erythropoiesis
Erythropoietin
Granulocytes
Hematopoiesis
Left shift
Leukemia
Leukemoid response
Leukocytosis
Leukopoiesis
Lymphopenia
Pancytopenia
Pluripotent stem cell
Thrombocytes
Thrombopoiesis
Thrombopoietin

The term **hematopoiesis** refers to the production of blood cells elets. lood omposed ells. component cellular component made of red blood cells (RBCs), which are also called erythrocytes; white blood cells (WBCs), which are called leukocytes; platelets, which are also called **thrombocytes** are further differentiated based on the presence or absence of granules that specific ways. The **agranulocytes** are lymphocytes monocytes. These cells may sometimes contain granules, but only ery l umbers. hey ommonly eferred mononuclear leukocytes. **Granulocytes** include the neutrophils, eosinophils, basophils. The granulocytes are commonly referred o olymorphonuclear ukocytes erm efers o gmentation bulation ucleus. However, erm ecifically uclear segmentation rominent irds eptiles.

Blood cells are constantly being produced have finite life Table They must be continually replaced. The life of blood cells varies among different types of cells different species f erstanding verall rocess or production of components will their evaluation. The rocess egins uring ly mbryonic volves umber f omplex hemical ways arious rgans. Some ariations rocess etween uvenile dult ematopoietic ctivity renatal ccurs ariety rgans, luding ver, en, ymus, red one row. ed one row ly very bone etus rimary for roduction lood ells onatal uvenile

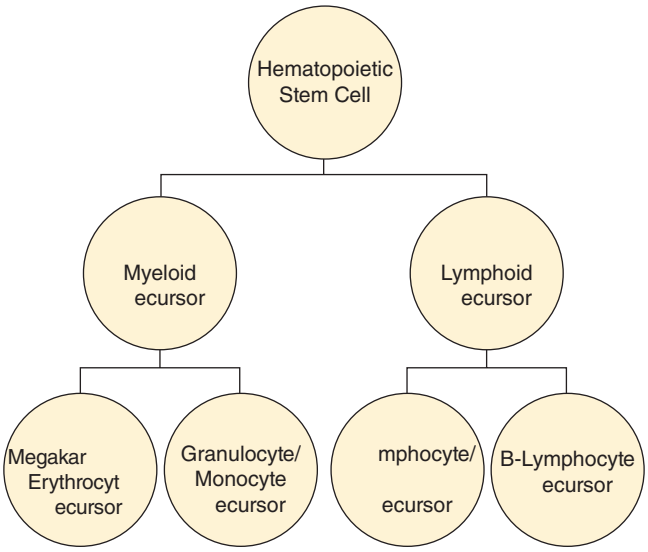
*Some species have values that are outside of the averages listed.

In dult ed one row rimary site or roduction uration lood ells elets. owever, ewer ones ontain ed one row dults. n ones, ed row onverted yellow bone marrow, which does actively produce cells. The marrow yellow appearance due to presence of yellow cells. ed row etained rimarily ones femur, ibia, umerus, ernum, During periods of hematopoietic stress, liver spleen may revert o ir etal roduce lood ells dult.

Erythropoiesis (the production of erythrocytes), **leukopoi** roduction ukocytes), **thrombopoiesis** production of platelets) involve different pathways chemical messengers. owever, lood ells om pluripotent hematopoietic stem cells **pluripotent stem cell** capable of developing into various types of cells. Pluripo tent evelop lood ells. capable f egeneration; umbers one row are relatively small but constant. initially differentiate into the ematopoietic rogenitor ells, hich onsist he ommon myeloid rogenitors ommon ymphoid rogenitors. The evelopment way etermined eractions various chemical messengers, which are referred to cytokines. Specific tokines volved roducing ch ypes of lood ells. urther erentiation esponse dditional cytokines results commitment to formation of specific cell types. Nearly two dozen different cytokines have been identified.

Pluripotent HSCs give rise to all of the blood cells.

The ommon ymphoid rogenitors ventually ive specific progenitors develop into various populations of lymphocytes. common myeloid progenitor will either develop into gakaryocyte/erythrocyte rogenitor ranulocyte/monocyte progenitor. The megakaryocyte/erythrocyte progenitor then erentiates ither ubriblasts, hich ive erythrocytes, gakaryoblasts, hich ive elets. The ranulocyte/monocyte rogenitor erentiates ither yeloblast, hich ives ranulocytic ukocytes, or noblast, hich ives nocytes Some eferences efer ly rogenitor olony-forming later stages blast-forming Myeloid

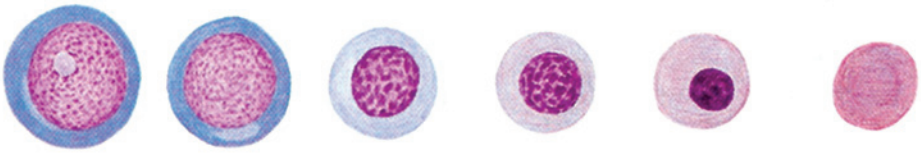


Blood cells arise from pluripotent hematopoietic stem cells.

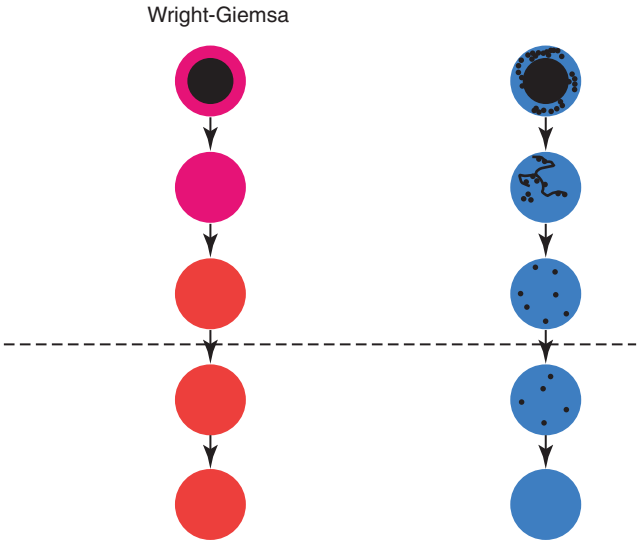
cells are relatively large cells, whereas erythroid cells e ler ve lumped philic uclei.

The primary cytokine responsible for production of **erythropoietin** EPO). PO redominantly roduced ertain cells kidney response to decreased oxygen tension blood. Those kidney cells then produce EPO, which circulates lood o one row. esser produced patocytes. eceptors surface of erythroid precursor cells bone marrow, which m vide ure. fter een imulated to evelop nto rythrocyte, he ell ndergoes dditional if ferentiation ubriblast. ubriblast ontains round ucleus, re ucleoli, basophilic cytoplasm. These cells continue to divide mature into rorubricytes, ubricytes, tarubricytes. Prorubricytes ler ubriblasts, htly re condensed ucleus ensely lue toplasm. ucleoli are nger isible. ells ubricyte ially ve basophilic toplasm derate lumping ucleus. As ell ures, rphologic haracteristics ubri cytes e inguished ked uclear lumping cytoplasm ecause orporation moglobin, hich begins uring

Metarubricytes re he mallest ells he rythroid eries nd have ondensed ucleus eep ed ytoplasm. etarubricytes are ell vision, moglobin ormation completed uring ell ill ventually xtrude nucleus ure eticulocyte. eticulocytes ure erythrocytes ontain ibosomal erial cell ures rganelles ccount or



Rubriblast Prorubricyte



blood as it occurs in most normal cats. Note that punctate reticulocytes do not appear polychromatophilic when stained with Wright-Giemsa

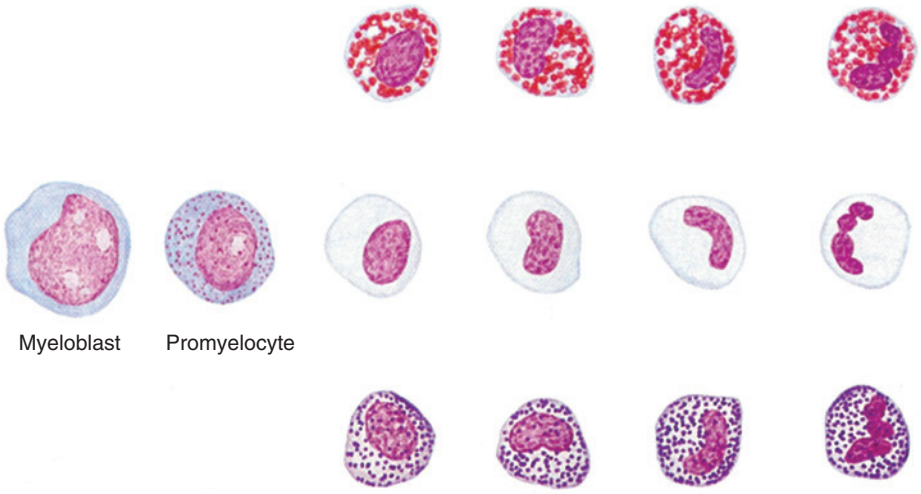
blue-gray or polychromatophilic of immature cells with Wright's hen upravital methylene lue), ly eticulocytes emonstrate twork reticulum pears gregated erial. epresents ibosomal erial. eticulocyte ergoes ther maturation, erial ecreases ubsequently pears l k lue ells eferred tate reticulocytes

The imulus or roduction ombocytes volves hormones thrombopoietin well numerous additional cyto **Thrombopoietin** primarily produced liver endothelial cells, ut eleased om ells sites. The progenitor cell develops into megakaryoblast, which contains single nucleus dark blue cytoplasm. The cell then develops o romegakaryocyte, hich ell contains o uclei. ucleus ontinues eplicate, ell ecomes rogressively er evelops gakaryocyte. ure gakaryocyte umerous nuclear lobes, cytoplasm reddish granules. The cells are ery ter), to xtends row here ed w f lood. ed agments eferred proplatelets; hey ventually ragment urther nto latelets hat are culation.

The imulus or roduction ranulocytes volves hormone leukopoietin well numerous additional cytokines. Cells ranulocyte ries vided roliferation pool, hich epresents ells maturation pool, which represents cells are longer capable of osis. roliferation ool ludes yeloblasts, romy elocytes, myelocytes. The maturation pool includes metamy elocytes ells. yeloblasts er ubriblasts ve ound val ucleus, rominent ucleolus, gray-blue cytoplasm. few reddish granules may be evident toplasm. romyelocyte ell with prominent reddish cytoplasmic granules prominent nucleoli. yelocytes ler ells uclei. ran are characteristic of mature neutrophil, eosinophil, phil egin pear uring yelocyte metamyelocyte appearance to myelocyte except nucleus indented. These cells are longer capable of mitosis. and ells ve rseshoe-shaped uclei allel sides. he uration roduces ure mented ranulocyte. uclei ells ontain re lobes .

Monocyte evelopmental lude noblast, promonocyte, nocyte. onoblasts pear to yeloblasts xcept ucleus regular Promonocytes ppear similar yelocytes and metamyelocytes. Monocytes may develop into macrophages when they are exposed to ecific tokine. owever, crophages erived from r ces.

The production of various populations of lymphocytes ymphocytes, ymphocytes, ural ler ells)



Granulopoiesis demonstrating the appearance of cells in Wright-stained bone marrow aspirate smears.

arises om ommon ymphoid rogenitor roceeds through ymphoblast rolymphocyte ell ially erentiated ither ymphocyte recursor or -lymphocyte/NK recursor. roduction volves ertain cytokines ell ecific ibodies. uvenile ymphocytes mature rimarily one row ecialized Peyer's ches ogs, ell bursa of Fabricius birds. lymphocytes mature thymus. cells mature bone marrow, but they may develop ymus ymphoid issues.

The ollowing efinitions matologic erms

-penia: ecreased umber ells lood. or neutropenia efers ecreased umbers utrophils lood. **Lymphopenia** escribes ecreased umbers lymphocytes lood, hereas **pancytopenia** refers to a decrease umber lood ell ypes.

-philia r tosis: ncreased umber ells lood. or example, eutrophilia efers ncreased umbers eutro phils blood. **Leukocytosis** refers to increased numbers of ukocytes lood.

Left shift ncreased umbers ure utrophils blood.

Leukemia Neoplastic cells blood or bone marrow. Leuke are often described with terms *leukemic, subleukemic,* or *aleukemic,* thereby indicating variation tendency for oplastic ells eleased lood.

Leukemoid response ondition en or kemia. he ukemoid esponse haracterized ked leukocytosis ually esult inflammatory

Chapter eview uestions ppendix

- Hematopoiesis efers roduction lood ells platelets.
- Erythropoiesis roduction rythrocytes), ukopoi esis the roduction eukocytes), nd hrombopoiesis the production elets) volve ecific tokines.
- Red one row rimary or roduction maturation lood ells elets dult

- All lood ells erived om uripotent
- The rythrocyte evelopmental ubriblasts, rorubricytes, ubricytes, tarubricytes, eticulocytes.

- Platelet roduction roceeds ough gakaryoblast, romegakaryocyte, gakaryocyte
- Mature gmented ranulocytes roduced ough myeloblast, romyelocyte, yelocyte, tamy elocyte, ells recursors.
- T lymphocytes, lymphocytes, cells develop through ymphoblast rolymphocyte

Sample Collection and Handling

After studying this chapter, you will be able to:

- Describe rocedures or ollection lood from l
- List ommonly lood ollection or arious species.
- List ommonly icoagulants pose de f ction or ch.
- List quipment eded or ollection lood
- Describe rocedures or reparing lood or evaluation.
- Calculate lood olumes ithdrawn om patients.

Anticoagulants
Citrate
Ethylenediaminetetraacetic acid
Heparin
Oxalate
Serum
Sodium fluoride
Vacutainer

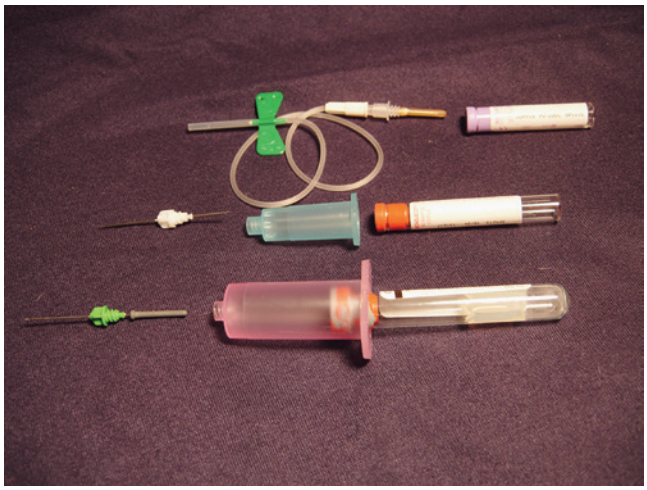
When reparing ollect lood, echnician determine ecific rocedures ill eded. will determine, part, equipment supplies needed choice of particular blood vessel from which to collect he awn efore iation medical reatment. reatments ave een iven, hese ust noted on blood collection record. Some test methods e ccurately erformed er ient eceived certain harmaceutical rapies.

The referred lood ce ways enous lood. Jugular lood ollection propriate ommon eterinary species. In some exotic species, there are readily accessible veins, y e cessary ollect eripheral lary lood ommonly lood ollection are ummarized able lood ollection

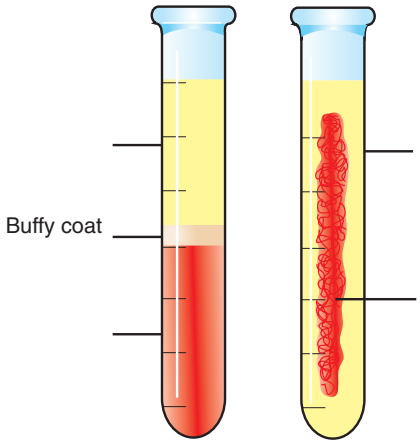
cleaned swabbed with alcohol before collection. The alcohol be allowed to dry before proceeding with col lection. The must be restrained, preferably with minimal manual restraint. Every effort be made to minimize stress ient, ecause ften ompromises

Venous blood is preferred for most blood cell testing.

Traditionally, amples ave een ollected sing eedle nd syringe. hen thod edle hosen always e he argest ne hat he nimal an omfortably ccom modate. he ringe hosen equired olume. er ringe collapse patient's vein. The preferred method of blood collec tion acuum stem **Vacutainer** . system omposed edle, edle er, ollection



The vacuum system is composed of a needle, a needle holder,



The difference between blood plasma and blood serum. Plasma

The sheathed end of the needle is inserted into the holder.

tubes. The sheathed end of needle inserted into holder Fig. he revents lood om ntering er when venipuncture made. The of blood collection tube enetrated edle er edle lumen f lood essel. ollection ubes sterile ubes, hey ay ontain **anticoagulants** he ubes re

available ange om ew oliters
The correct-sized tube must be used to minimize damage to or possibility of collapsing vein. The tubes be lowed o orrect olume rength of acuum ressure ube) nsure propriate ratio f nticoagulant nd lood. dvantage his ystem ultiple ollected ectly ollec tion ubes ithout ultiple enipuncture rocedures. quality est hen ollected acuum ubes roper echniques, ecause otential for elet ctivation.

The Vacutainer system is preferred for blood sample

The veterinary technician obtains whole blood by with drawing lood uitable ontainer roper anticoagulant revent lotting. on lood lected, lood icoagulant ed entle rocking motion. vigorously hemolysis, which turn affect results of assays when chemicals e rmally rythrocytes eleased into

ortion lood hich ells are uspended. omposed proximately er dissolved constituents, such proteins, carbohydrates, vita hormones, enzymes, waste materials, antibod other ions molecules. **Serum** from which fibrinogen, rotein, een emoved. uring clotting rocess, uble rinogen onverted to uble rin rix hen lood ueezed ellular rum. Specific rotocols or btaining rum be ound

Hematology esting rimarily volves lood emostatic esting lood lood nticoagulants are required when whole blood or e eded. icoagulant hemical hen added o lood revents elays lotting (coagulation) hoice ticular ico de eded. ome anticoagulants ect ys, rocedure be consulted for proper anticoagulant. Some anticoagulants erfere ertain oagulation egardless anticoagulant hosen, icoagulant be ell ed entle version efore ested ollection efrigerated. Refrigerated must be brought back to room temperature emixed entle version efore ysis. lood ozen, ecause lood ells yse uring eez wing rocesses. able ummarizes ommonly used icoagulants.

Heparin uitable icoagulant or equire plasma amples, articularly lood hemistry nalyses. eparin anticoagulant ver or erential lood analysis, ecause icoagulant erferes of hite lood ells eparin vailable dium, potassium, nium eparin cts reventing conversion of prothrombin to thrombin during clotting processes. ecause parin lumping platelets erferes rmal ern or yses rphology. Heparin be used ratio of of blood to be collected. or olumes, onvenient thod or parin oat ringe heparin efore awn om ient. acuum collection ubes ontain roper parin commercially vailable. ubes onvenient hen many parinized eded.

Ethylenediaminetetraacetic acid A) referred coagulant or matologic ecause oes er ell morphology hen roper atio or be analyzed soon after collection. It be used e or hemical ys. vailable sodium r otassium revents lotting orming uble omplex hich cessary or formation. ubes vailable ither owder forms. he orm eates ution prevent lotting, dded lood be ollected. acuum ollection ubes ontain roper of EDTA are commercially available. Excess EDTA cells o ink validates ell erformed automated yzers.

Oxalates e vailable dium, otassium, nium, lithium **Citrates** e vailable dium These ubstances revent lotting orming nsoluble omplexes with um, hich cessary or ormation. otassium oxalate commonly used oxalate To prevent clot ting, used to of blood to be collected. Sodium citrates are commonly used, especially transfusion medi acuum ollection ubes ontain roper of oxalate citrate anticoagulants are commercially available. Unfortunately, oxalates may also ind metallic ions cessary for enzyme activity. Potassium oxalate may inhibit lactate dehydro genase phosphatase activity. In addition, because otassium lood assayed or otassium. odium rates ly erfere sodium assays well with many of commonly performed blood chemistry tests. Note different laboratories may have tubes ith ering oncentrations rate, hich ect certain oagulation

Sodium fluoride which best known glucose preservative, anticoagulant properties. anticoagulant, used

o lood ollected. acuum ollection tubes ontain roper dium ride or anticoagulation are commercially available. Sodium fluoride may e dded lucose reservative, ven erent icoagulant resent. or lucose reservation, sodium ride lood. odium ride erferes nzymatic erformed blood rum.

The lood ollected om epends quantity of serum or required for assay hydration us ient. or ell-hydrated with cked ell olume ield lood ells lood yield ehydrated moconcentration results ler atio ells. ehydrated with cked ell olume ields lood ells nly obtained om lood

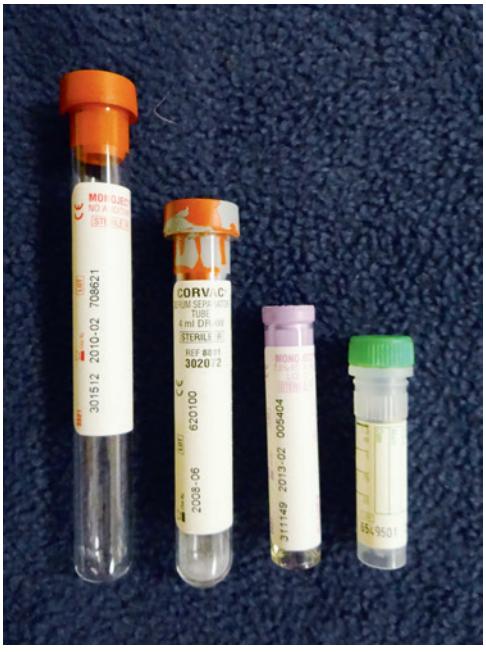
Ideally, nough lood ollected ield nough serum, lood ys three times. This allows for technician error, instrument failure, or need to dilute without having to collect another om

Blood dequately ed efore erformance of any tests; inadequate results erroneous For xample, ed lood ells om rses seconds, cked ell olume erformed ed y rroneous. ubes lood ed gentle version ube on ommercially vailable ilting ack otator.

After olume eded een etermined ecific types of testing required have been identified, prepare equipment obtain appropriate number types of blood collection tubes. Perform venipuncture with tissue injury possible to e ontamination issue hemolysis. If acutainer used, allow tube to fill to capacity to nsure roper lood-to-anticoagulant atio. estraint necessary o revent vement esult ceration lood essel rgan rious omplications. or small patient placed sternal recumbency. Hair ved om enipuncture contamination of introduction of bacteria from ient. estrainer ccludes essel r ourniquet enipuncture site. he ourniquet ced ight nough end ein without occluding blood flow. Hemoconcentration occur ourniquet ce or xcessive The echnician ollecting cate essel enipuncture Do ouch ers er een cleaned. ilize ein rt edle evel facing proximately egree nter ein ne oth ion. ringe, ate lood the yringe. hen he roper olume as een btained, emove edle diately ply entle ressure eni puncture site to ensure hemostasis. Remove needle from syringe efore ransferring he lood he ial, ecause orcing blood through needle may result hemolysis. If vacuum system used, needle inserted into vessel described previously, er ilized ube ently pushed nto edle er. After ube ompletely, emoved tube inserted. The prevents blood from dripping into holder hen hanging ubes.

All lood ollected ube ontains ico ently verted veral diately after ollection ribute icoagulant. eled diately er ollection. ube be labeled with date time of collection, owner's ient's ient's entification number. If to be submitted to laboratory, include request form with includes all necessary identification clear indication of which tests are requested.

When ultiple ypes equired, ways ollected acuum stem, must be collected specific order. The vacuum system ensures propriate olume ch ype btained. However, ubes ed ollected ecific rder avoid otential ontamination dditives from other tubes. Tubes contain citrate additives are drawn first. These tubes usually require small of first e en ube ded. rate tube eded, ed-top ube awn



tube, ethylenediaminetetraacetic acid tube, and heparin tube.

for Commonly Used Blood				
of Draw	Cap Color			Primary Use
		Light blue	Sodium citrate	Coagulation studies
			Glass: no additive Plastic: silicon-coated	Serum for blood chemistry
		Red/gray, gold, or red/black "tiger-top"	Gel separator and clot	
		Green or tan		Plasma for blood chemistry
		Lavender, royal blue, or tan		
			Potassium oxalate or sodium fluoride	Coagulation testing Glucose testing

Table ummarizes rder aw or ubes commonly used veterinary practice. Note some individuals prefer o ollect ed-top ube efore rate ube ather ube. cceptable rovided red-top ube ontains el dditives, hich otentially contaminate rate ube.

Chapter eview uestions ppendix

- Sites or lood ollection ary erent ecies, ut jugular ein essel hoice or lood ollection om
- The referred thod lood ollection acutainer system.
- The preferred anticoagulant for hematology testing EDTA; referred icoagulant or oagulation esting rate.
- Plasma whole blood minus cells; serum whole blood minus ells lotting lements.

After studying this chapter, you will be able to:

- List ypes matology yzers vailable or eterinary ractice.
- Describe rinciple lectrical edance yzer.
- Describe tometry rinciples.
- Describe rinciple uantitative oat ysis.
- Describe enance tomated hematology yzers.
- Describe rocedures or ounting ells.
- Define ogram ograms.

Anemia
Complete blood count
Histogram
Impedance analyzer
Laser flow cytometry
Neubauer rulings
Polycythemia
Quantitative buffy coat analysis
Red cell distribution width

Instrumentation designed for veterinary available to acilitate he eneration ematologic ata or he **complete blood count** ptions ost-effective onvenient for uations hich veral erformed er day. he enefits rumentation lude educed or investment, re omplete ormation, rovement of eliability.

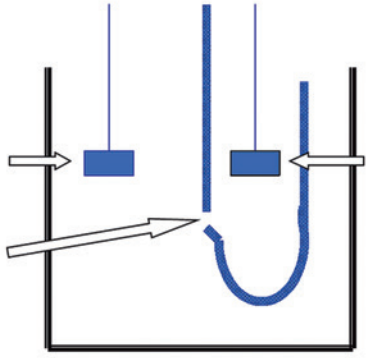
The ounting rythrocytes ed lood ells kocytes white lood ells WBCs]) outine art he BC. Cell ounts ually erformed tomated thods. total alues etermined Total platelet (thrombocyte) counts are performed tomated thods. anual ell thods are outinely erformed, xcept vian xotic animal ractices. ince any automated nalyzers not rovide accurate platelet counts, may be performed with manual methods cilities.

An ease umber culating ed lood ells termed **polycythemia** or erythrocytosis. It accompanied by increased packed cell volume hemoglobin concentration. Such increases esult rimary condary gy, or they may be relative polycythemia. Relative polycythemia seen with conditions such splenic contraction (which releases large umbers rythrocytes culation) ehydra tion. rimary olycythemia, hich led olycythemia vera, yeloproliferative rder haracterized roliferation rythroid recursor ells. econdary of olycythemia lude ariety enal rders conditions lead to increased erythropoietin levels (e.g., chronic ypoxia).

The term **anemia** refers to decrease oxygen-carrying capacity f lood, ually esult ecreased umbers of culating ther xplored hapter

Hematology rumentation or eterinary into ee eneral egories: **impedance analyzers** **laser flow cytometry** yzers, **quantitative buffy coat analysis** stem. ome ufacturers rovide yzers

The Genesis hematology analyzer (Oxford Science, Oxford, CT) combines impedance and laser-based methods.



The principle of impedance analysis for cell counts.

The Coulter AcT hematology analyzer (Beckman Coulter, Brea,

species, makes use of impedance technology.

ombine veral thods or erforming ommonly vailable stem volves edance methods or numeration ells, ell r-based methods or erforming erential hite lood ell Fig. ome matology yzers ontain hotomet ric ilities or valuation moglobin. ch thod ecific dvantages dvantages. egardless hich analyzer erstanding esting rinciples ecific yzer ntial. nowing ions analytic stem nhances alidity esults. egular quality ontrol ntial or nsuring ccuracy test esults.

umber lectronic ell ounters laboratories ve een dapted or eterinary

Adaptation cessary ecause ariation lood cell e erent ecies. ome ompanies ve developed dedicated veterinary multispecies hematology systems ount ells etermine matocrit, moglobin concentration, orpuscular moglobin oncen tration. ome rovide tial erential

Electronic ell ounters edance thod based n lectric rent cross lectrodes separated y ube pening erture ctrolyte ither erture onducts rent. ounting ccurs ving ecific olume cells lectrolyte ution ough erture f acuum ositive ressure. ecause ells elatively poor onductors lectricity ompared lectrolyte they impede flow of current while through aperture. These transient changes current may be counted to determine blood cell concentration. In addition, volume or size of cell proportional to change current, thereby allowing stem erentiate ell ypes sizes. ize ormation yed raphical ormat **(histogram)** f ell opulation. eukocytes, rythrocytes, elets numerated stems. owever, om ccurately valuated esult of ity eline rythrocytes eline platelets.

Impedance analyzers are calibrated to count cells specified size anges efined ttings hich revents erroneous erpretation ebris lectronic ells) roperly parate ell opulations dilution ., elets, rythrocytes). ecause ell opulations vary e ecies, ttings species ecific. ttings lished ufac turer ually tomatically stem ftware when user selects species or lysis software menu. Comprehensive matology stems esigned ecifically or veterinary plications ommonly eterinary facilities. These systems incorporate advantages of individual cell analysis provides sophisticated quantitative information about lood ell opulations.

The lood uted ells. or ution reated ytic ent estroys ell branes, ving nly uclei or ounting. ythrocytes analyzed ith stems provide ell ormation uch reater lood dilution o hich ytic ent dded. ythrocyte ysis on automated systems provides diagnostic information about cell volume ernative thod or etermining cked cell olume hich eferred matocrit (Hct). he orpuscular olume ectly measured om ysis rythrocyte olume ri bution. he matocrit ed ultiplying y rythrocyte oncentration. re phisticated systems, volume distribution curve of erythrocyte popula tion displayed. some analyzers, **red cell distribution width** W) y rovided. alue etermined by matic ysis ribution, tions index of erythrocyte volume heterogeneity. bnormally high values indicate increased volume heterogeneity underlying disturbance of erythron. hen used conjunction with alue, rt eterinarian of rythrocytes er re dvanced stems, ounting tions or valuating platelets.

Many tomated matology stems rovide omplete analysis f elet, rythrocyte, opulations, lud erential ormation. rovide raphic displays of cell population size analysis. Differential information ed om opulation ribution. any of stems rovide imate elative ercentage of ranulated ngranulated alue ed application for evaluation of patients with pathologic con dition. ariations size of cells introduce error into measurement. ddition, umerous rphologic normali ties e resent entified tial differential rough xamination lood must e luded hen valuating ients.

Impedance yzers omposed umerous tubes, alves iluting dusty glassware may be contaminated with particles are large enough to be erroneously counted cells. daily background count" enerally equired. volves lectro lyte ution ough yzer ticles may e dentified nd ubsequently ot ounted he nalyzer. The erture ecome tially otally bstructed require leaning. tting roperly on ounter with variable hreshold control. Cold gglutinins may ecreased esult lumping. Before rocessing, efrigerated lood med to oom emperature. ragile ymphocytes, hich en some orms ymphocytic eukemia, ay upture he ysing solution yse upturing esult decreased count. The presence of spherocytes abnor mally small, round may alter MCV, thereby reducing calculated hematocrit. Elevated serum viscosity may interfere with cell counts. Platelet counts are obtained with impedance counters e ected elet lumping resence nucleated ed lood ells, ften ccurate. mall clumps of platelets, large platelets, nucleated red blood cells may e ounted rythrocytes.

The uantitative oat stem ucker iagnostics, ort Matilda, A) erential entrifugation provide imation ellular lements. easurements made expanded buffy coat layer specialized micro hematocrit ube. rovides matocrit alue ell estimates of ukocyte oncentration and atelet oncentration. It extrapolates tube volumes to estimated concentration based on fixed cell volumes. Partial differential count information provided form of total granulocytes lymphocyte monocyte egories. ion ukocyte roup ings normalities ymphopenia) undetected lood xamined efined or um stems eening tools, because they provide estimation of cell numbers rather ctual ell

Quantitative buffy coat analyzers provide estimated

Laser w tometer yzers volve ocused beams to evaluate size density of components. Cells scatter light differently depending on shape volume of cell nd he resence bsence ranules nd uclei. he aser beam ected hannel ough hich ells flow single The degree direction of light scatter from vidual ells lows or numeration nocytes, lymphocytes, granulocytes, erythrocytes. hen certain dyes are added to variations laser light scatter allow for enumeration of mature immature erythrocytes Fig. hese stems ually rovide rythrocyte indices, RDW, platelet parameters (e.g., platelet volume, platelet distribution width, plateletcrit). More informa tion out elet ameters resented

Many automated analyzers offer histograms of cell plate let ounts. ogram raph rovides isual eport of es umbers various cellular components. nother version of histogram orm f er here ch epresents ecific cell. The histogram be used to verify results of differential blood ell rovide ion roblem with test results. For xample, when megathrombocytes or let gregates resent, erformed automated analyzers will be falsely elevated, because large platelets e ually ounted ukocytes. ogram

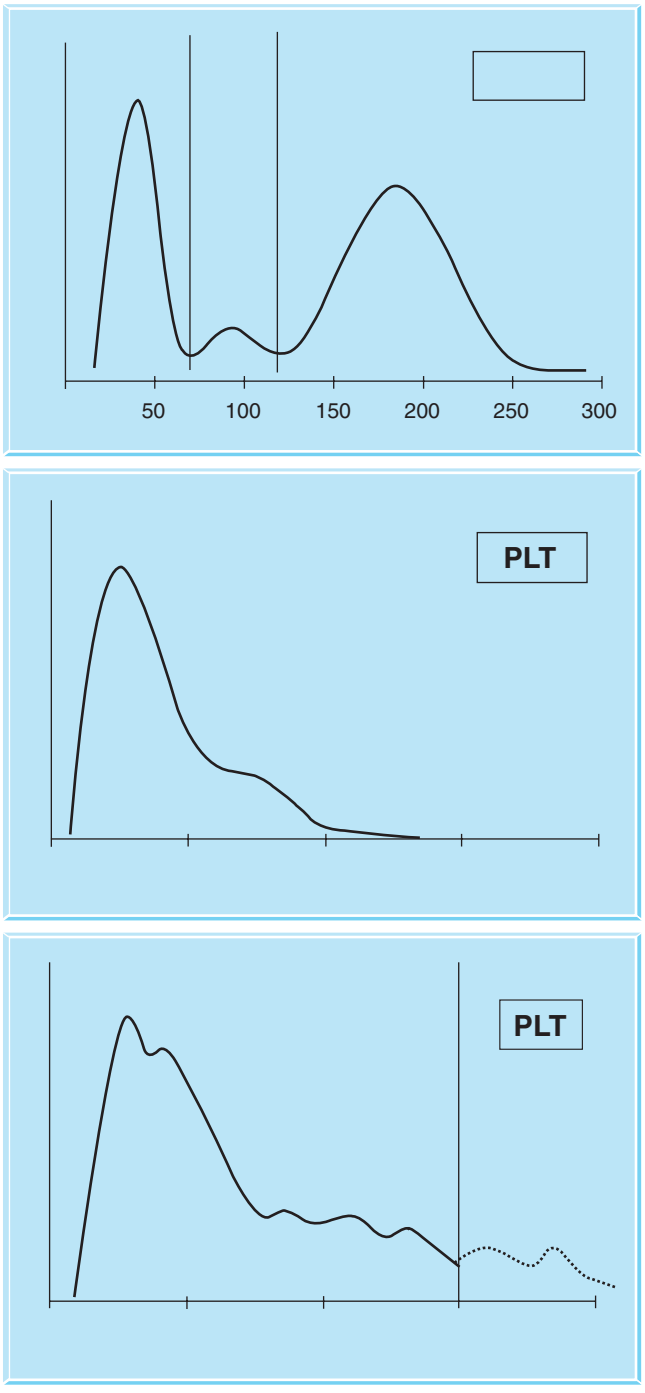
A laser-based analyzer for use in the veterinary practice laboratory.

provide vidence maly, ecause ve
ogram ill ered

The counting of erythrocytes leukocytes routine part of ell enerally erformed methods xcept vian xotic ractices. anual count methods for exotic be performed with eukopet stem. stem ludes pipette redetermined lood ervoir ontains uting ysing ent. rocedure describes thod or ounting vian ukocytes ells eukopet stem

The Leukopet system premeasured volume of phloxine diluent. hen dilution tube filled with appropriate volume of blood mixed, small of blood–diluent mixture placed on hemocytometer. The hemocytometer con ptical uality over ured rid contains ecific olume.

Hemocytometers ounting hambers determine umber ells er oliter of lood everal dels vailable, ut common type used two identical sets of grids of parallel erpendicular tched led **Neubauer rulings** . ch rid vided uares. orner squares are divided into smaller squares, center square vided iny uares roups ch). area of each grid each Neubauer ruling) designed to recise nowing umber cells t rid area for calculating number of cells per microliter of lood. echanical ounters vailable ually eep track f umber ells bserved



Platelet histogram with evidence of platelet aggregates.

Using the pipettor, aspirate 25 L of freshly drawn anticoagulated blood.

Dispense the blood sample into the tube of phloxine, and rinse the pipette

Make sure that the hemocytometer and its special coverslip are clean and

Using the rinsed pipette, aspirate a sample from the tube and charge (fill)

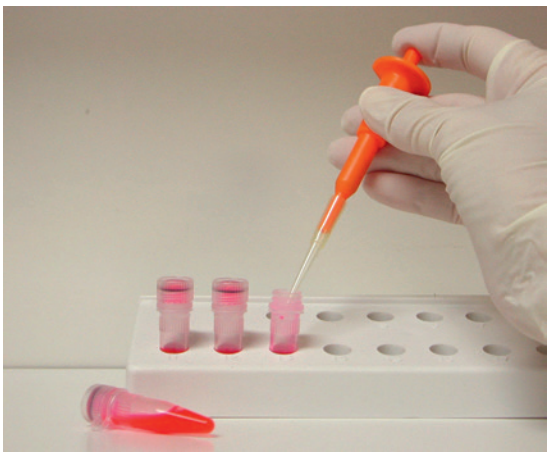
underfill the counting chamber, because this can cause uneven distribution

Allow the sample to stand for up to 10 minutes so that the cells can

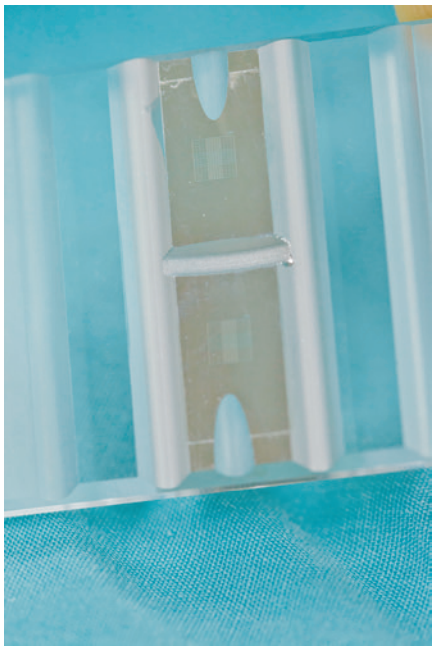
objective lens, count the heterophils and eosinophils in both chambers of the Neubauer hemocytometer. Cells that touch the lines between two squares are considered as within that square if they touch

/

From Sirois M: Principles and practice of veterinary technology, ed 3,



The Leukopet system used for the counting of avian white blood



The hemocytometer contains two grid areas.

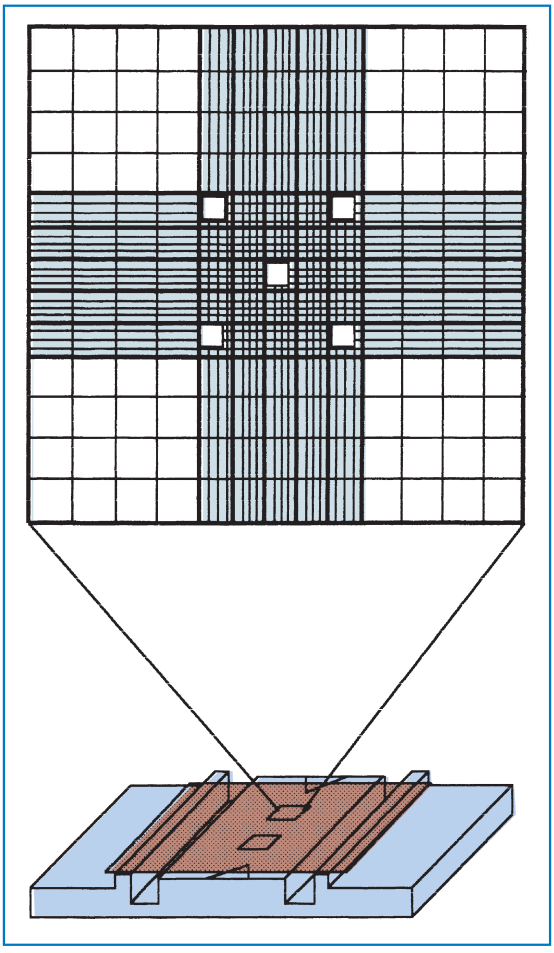
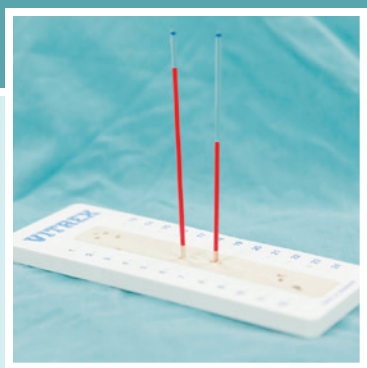


Fig. 8.8 Neubauer hemocytometer. (From Sirois M: *Principles and practice*

The hand tally counter is used to keep track of numbers of cells counted.

- Most cell counters for veterinary practice laboratory involve either impedance or laser-based technology.
- Buffy coat analyzers provide estimates of cell counts.
- Impedance analyzers work by measuring change current cells electrolyte solution through aperture.
- Impedance analyzers classify cells according to their sizes.
- Laser flow cytometers classify cells on of their size density they through focused laser beam.
- Histograms provide visual representation of numbers sizes of cells present
- Manual cell counts are performed with hemocytometer.



Hemoglobin, PCV, and Erythrocyte Indices

After studying this chapter, you will be able to:

- Describe rocedure or erforming cked ell volume est ohematocrit thod.
- Describe rocedure or rating entrifuge or optimum ohematocrit
- List yers entrifuged ohematocrit ube order om ottom op.
- Explain nificance eddish, ellow, loudy olors entrifuged ohematocrit ube.
- Differentiate xyhemoglobin, themoglobin, sulfhemoglobin.
- List ions btain orpuscular volume, orpuscular moglobin, corpuscular moglobin oncentration.

Buffy coat
Erythrocyte indices
Hemoglobin
Icteric
Lipemic
Mean corpuscular hemoglobin
Mean corpuscular hemoglobin concentration
Mean corpuscular volume
Methemoglobin
Microhematocrit
Oxyhemoglobin
Packed cell volume

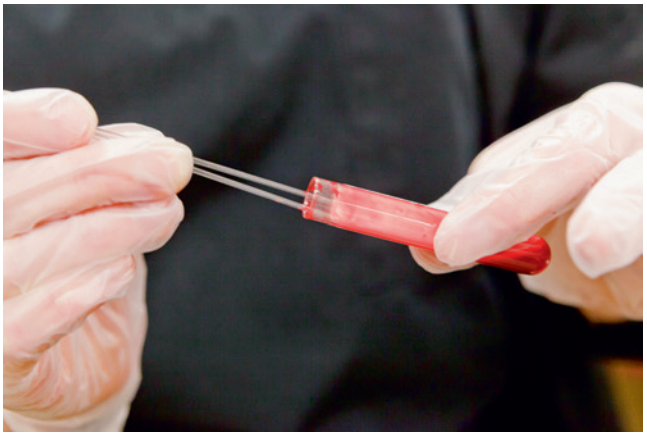
The omplete lood rovides values hat ay etermined eliably nd ost-effectively he setting. The tests included be performed manually or by automated analyzers. variety of procedures are available or thods.

The onsist ollowing ormation:

- Total ed lood ell **Packed cell volume**)
- Plasma rotein oncentration
- Total hite lood ell
- Blood examination: differential count, erythrocyte ukocyte rphology, elet imation
- Reticulocyte hen ient
- Hemoglobin oncentration
- Erythrocyte

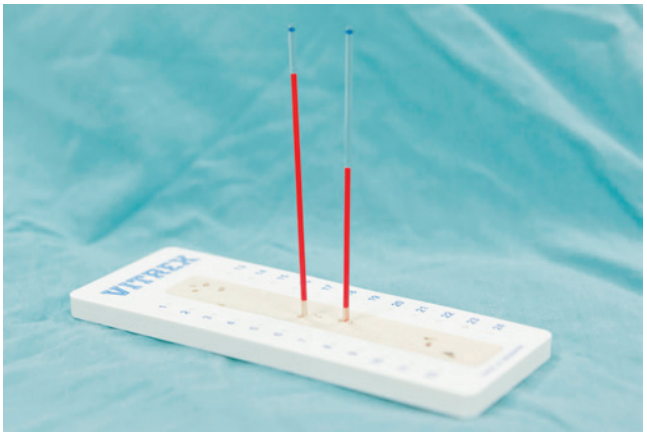
The percentage of whole blood composed of rythrocytes lthough luded CBC, t s lso requently rdered ingle est. he ommonly performed ersion eferred **microhematocrit** t) r matocrit erformed by cing icoagulated lood lary tube. eparinized ubes vailable entified ed ing op ube. ohematocrit tubes are identified with blue ring Fig. Microhematocrit tubes led proximately ee-fourths sually two ubes led om they e entrifuged ogether fter ube been led o propriate olume, ugged

Microhematocrit tubes and clay sealant.

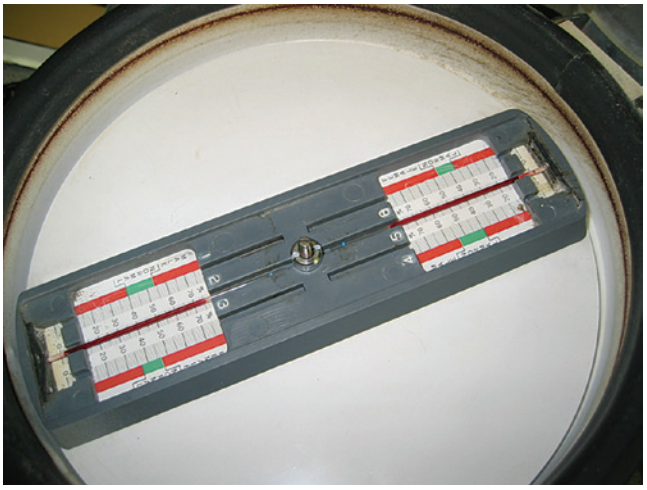


clay ube ced entrifuge with ugged tward entrifuged microhematocrit entrifuge or utes, epending type of centrifuge used Fig. Small-capacity centrifuges that se 0-mm ubes re lso vailable Fig. .5 ee rocedure or w rate ohematocrit entrifuge.

lood ells, ve ecific ravity. They ravitate ottom ube uring entrifugation pear ed yer. hitish-gray yer ove yer led **buffy coat** onsists elets. ight oat yer rovide rough stimate he otal BC ount. he resence ncreased numbers of nucleated imparts reddish tinge to buffy coat. he ellow op obtained by method be used to determine protein concentration by refractometry. color transparency may be helpful for determination of diagnosis be recorded. Normal clear (transparent) or



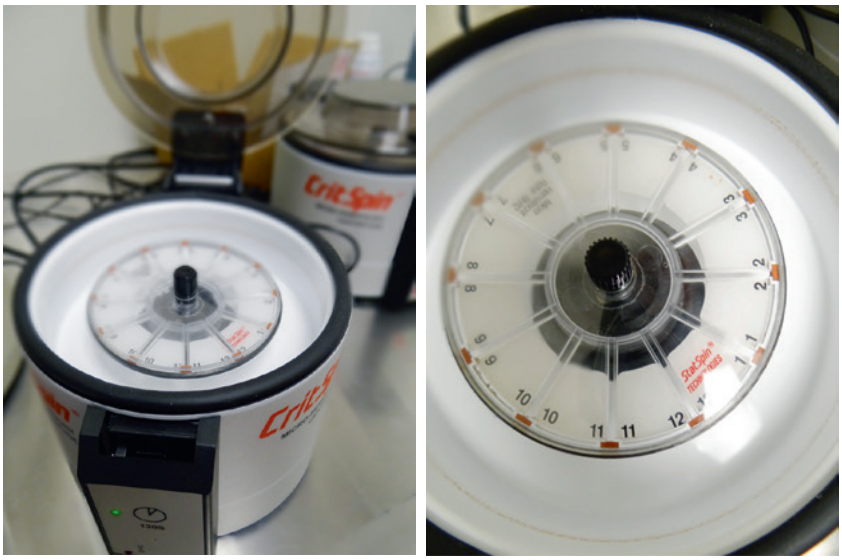
Microhematocrit tubes that have been sealed with clay. These without balance tubes of equal size and weight.



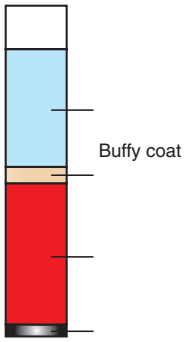
are required to balance the centrifuge.

raw-yellow olor erum pears loudy escribed **lipemic** his y esult gic condition, r en tifact ient properly fasted before blood collection. reddish-tinged layer escribed molyzed. tifact lood roperly ollected led, y e vidence gic ondition molytic anemia). eep ellow escribed **icteric** be seen with liver or hemolytic anemia. Abnormal olors ed, ecause olor interfere with chemical analyses photometric methods are used or valuation onstituents. ny uring device ., uler) etermine

Special matocrit ube eader vailable, which have of blood tube need be exact. The bottom of layer be zero



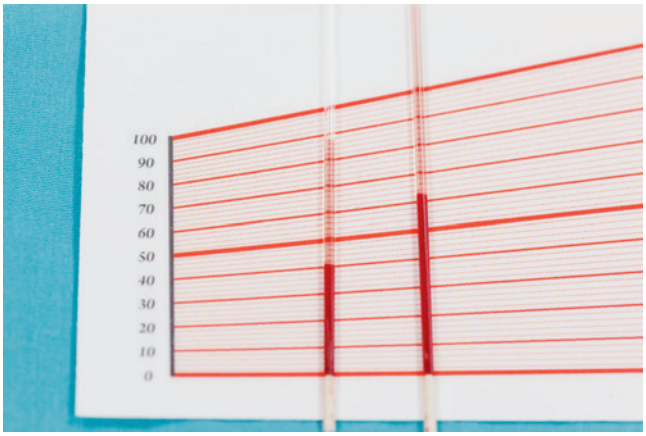
A microhematocrit centrifuge designed for small-capacity tubes.



op op ercentage
then be read level with top of
layer . btain uler, ure
height f olumn ight om op
the lay lug he op he lasma olumn. ivide he eight
of olumn ight ultiply
obtain ercentage. imate
obtained y viding or
imated vided hich qual
to hich epresents lion
The crohematocrit thod or etermining
commonly erformed, ecause equires
blood, enerally lood ced
introbe ube entrifuged or utes pm.
The cale n he introbe ube ead he evel he acked
number multiplied by to determine PCV.

The often used screening tool to determine whether
gic ondition uch resent. ormal
values for common domestic species are listed Table alues
e ed anges

whereas alues reater vels
polycythemia. More often, increased seen patients who
are ehydrated, esult wer olume resent
in he sculature. CV ill lso alsely ecreased nad
equate olume lood ollected icoagulant.



the clay plug on the zero line and locating the intersecting line where the packed red cells and the buffy coat meet.

Values for Common Species

Although specifically considered hematologic test, materials needed for protein concentration are already available result of performing PCV. protein con centration imation efractometry ortant omponent of ecies. etermine collected by breaking hematocrit tube just above uffy oat–plasma erface. lowed onto efractometer rism rocedure refractometer right eading made viding etween right The rotein alue ead ectly om efractometer. emic ontains hylomicrons light to diffract many different directions usually results rotein eading.

The rotein **hemoglobin** tional erythrocyte. The molecule consists of two components: heme portion, which contains iron, globin portion, which omposed ed hemoglobin ccurs uring uration bone row. fter ntering culation, erent orms hemoglobin emoglobin xygen referred o **oxyhemoglobin** hen xygen elivered

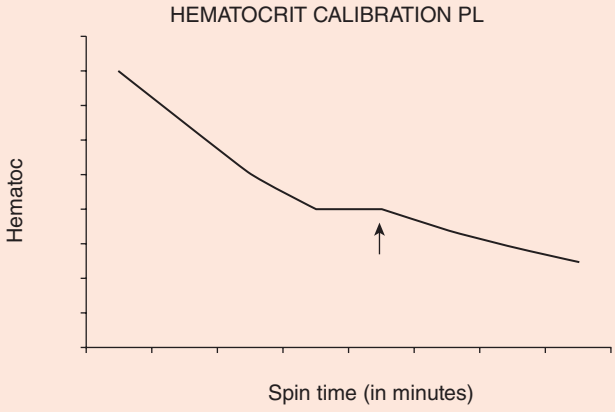
Microhematocrit Centrifuge

he minimum time needed to achieve optimal packing of the red cells should

ose two fresh ethylenediaminetetraacetic-acid–anticoagulated blood

duplicate microhematocrit determinations at increasing times, beginning at 2 minutes. Centrifuge times should be increased by 30-second

curve after the curve flattens out (see figure below). This is the optimum



issues bon xide ce oxygen. he bon xide eplaced xygen during respiration. Other forms of hemoglobin may be present lude **methemoglobin** ulfhemoglobin. orms fficient hen omes xygen ransport. Sulfhemoglobin results from normal aging processes. Methe moglobin occurs naturally both within but be converted to hemoglobin used for oxygen delivery. Carboxyhemoglobin results from exposure to carbon monoxide. Hemoglobin methemoglobin have much higher affinity for carbon monoxide for oxygen or carbon dioxide. Therefore, eaction eates boxyhemoglobin reversible.

ariety f thods vailable or etermination hemoglobin. The oldest methods involve color matching of lysed . ome tomated yzer ypes rovide nly estimate of hemoglobin concentration on of ount. tomated yzers etermine moglobin concentration lood tion o yse lood ells omparing olor ith xposure anide onverts orms

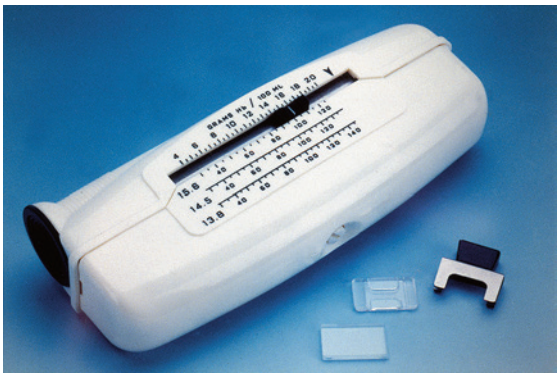


Fig. 9.9 The hemoglobinometer uses a color-matching method to deter mine the hemoglobin concentration in a sample of lysed red blood cells.

Fig. 9.10 The HemoCue is a type of photometer that is used to measure

of moglobin anmethemoglobin. ysing utions contain anide tion onvert orms of moglobin anmethemoglobin refore rovide measure f orms moglobin umber of tomated miautomated yzers edicated hemoglobin urement. dification anmethemoglobin hotometric rocedure, quite accurate properly maintained. Small dedicated analyzers are available Fig. some of provide results only for oxyhemoglobin color-matching technology. Other types cyanide-free hemoglobin–hydroxylamine method photometric rocedures rated proximate cyanmethemoglobin procedure. Normal hemoglobin values for common omestic ecies cated able

Common Species

The determination of **erythrocyte indices** helpful for classi fication of certain ypes of anemia. rythrocyte indices include **mean corpuscular volume**), **mean corpuscular hemoglobin** **mean corpuscular hemoglobin concentration** rovide bjective measure f verage moglobin concentration. ccuracy ion epends accuracy f the ndividual measurements total RBC count, PCV, moglobin oncentration. alues or rythrocyte ways ompared rphologic eatures ells n lood or alue or vel vident rythrocytes pear re rmal ypochromic) lood

is measure of average size of erythrocytes. calculated by dividing by concentration multiplying olume emtoliter For xample, og of lion/mL, Many f tomated matology yzers etermine lectronically urement .

eight moglobin ontained verage hich ured ograms calculated y ividing he emoglobin oncentration he BC concentration ultiplying

/
/

concentration of hemoglobin average eryth rocyte r atio eight moglobin olume hich ontained). ed dividing moglobin oncentration (percentage) ultiplying

/

exception f ep mbers
elidae ls), hich ve alues

For xample, og moglobin oncentration
of The normal
range for to for all with

Chapter eview uestions ppendix

- PCV ommonly erformed matology
- PCV eased esult ehydration olycythemia.
- Decreased
- The ohematocrit lary ubes led with lood entrifuged.
- The layers microhematocrit tube are packed uffy oat,
- Plasma color must be evaluated recorded when per forming
- Hemoglobin esting erformed tomated lyzers r ith ters.
- Erythrocyte ed alues rovide measure f verage moglo bin oncentration.

Evaluating the Blood Smear

After studying this chapter, you will be able to:

- Describe rocedure or reparing edge perform erential lood ell
- Describe rocedure or reparing overslip perform erential lood ell
- Describe rocedure or roper blood .
- Troubleshoot uality lood
- Describe pearance rmal lood cells.
- Describe rocedure or erforming ukocyte absolute alue ion.
- Describe rocedure or erforming elet imate.

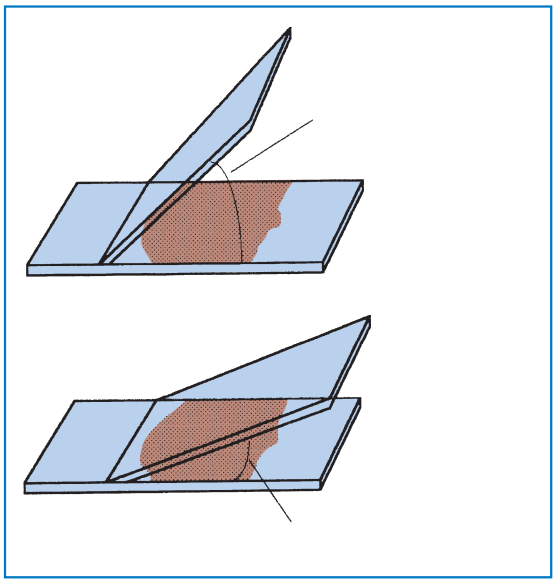
Absolute value
Basophil
Coverslip smear
Eosinophil
Heterophil
Lymphocyte
Megathrombocytes
Methanol
Methylene blue
Monocyte
Neutrophil
Neutrophilia
Platelet
Romanowsky stain
Wedge smear
Wright's stain
Wright-Giemsa stain

The blood used to perform differential white blood cell (WBC) count, to estimate platelet numbers, to evaluate morphologic features of red blood cells platelets. eripheral lood repared of ither wedge smear echnique r overslip echnique. he wedge ommonly ype reparation. The overslip echnique ften repare om blood btained om vian xotic ecies.

To prepare blood smear, drop of blood withdrawn from ethylenediaminetetraacetic-acid (EDTA)–anticoagulated blood collection ube. op btained ither transfer ipette lacing wo ooden pplicator ticks nto lood ube hen icks ogether ithdrawn, op lood propriate ill be etween lood op ced oward osted end f lean oscope ient information ritten ectly osted pencil. he cond ced urface of st egree awn ck drop f lood cond modified o ccount or hanges onsistency lood from mic ient rocedure hen lood read reader slide, then pushed forward with steady, even, rapid motion. The e ently ved low quickly. roperly repared lood ven distribution ells.

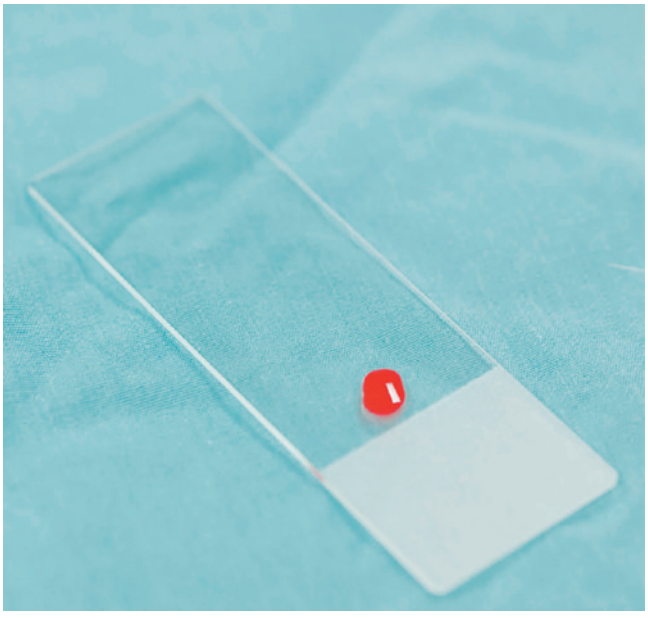
Coverslip smears e de y tting op lood enter f uare overslip. ce cond overslip diagonally on top of first, blood to spread evenly

cator sticks into the tube and holding them together when withdrawing them from the tube.

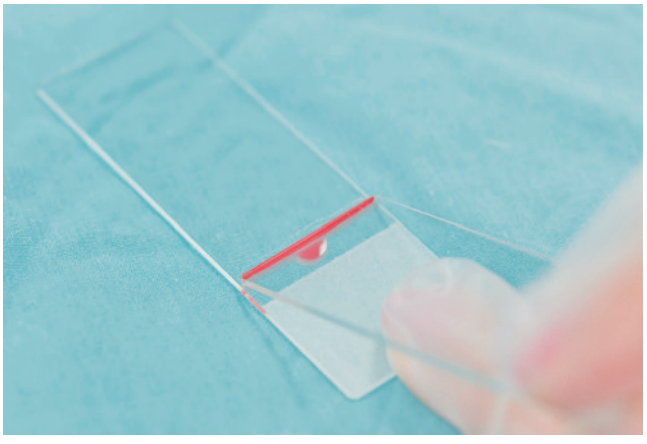


The difference in slide angle necessary for making blood smears

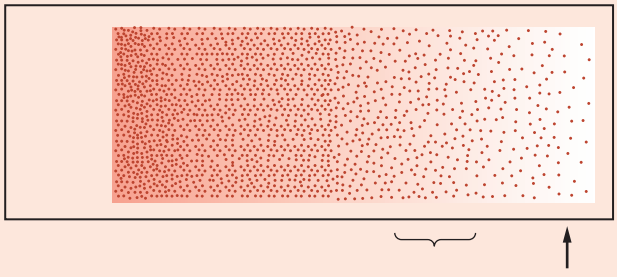
A small angle is used for hemoconcentrated blood.



Place one drop of blood toward the frosted end of the glass slide.



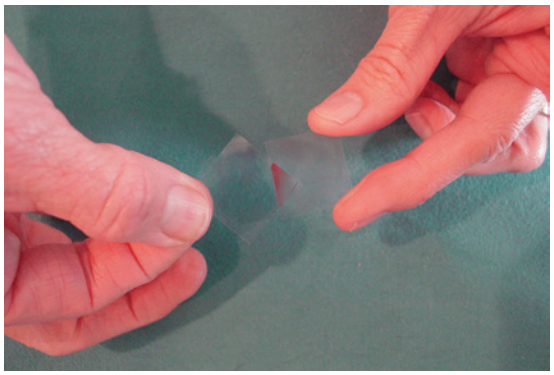
and draw it back into the blood drop.



A blood smear showing the label area, the monolayer counting area, and the feathered edge. (From Sirois M: *Principles and practice of*

Label the slide at the thick end of the smear. If the slide has a frosted edge,

After drying, stain the smear with Wright's stain or a Romanowsky-type stain, which is available in commercial kits (e.g., Wright's Dip Stat #3



Preparing a blood smear with the use of the coverslip method.

between urfaces. hen overslips single oth ion efore lood ompletely read Fig. rocedure ave ently o romote ying.

After y y, lood learly distinguish vidual ells entify normal cellular haracteristics. lood of omanowsky-type ommonly vailable **Romanowsky stains** include **Wright's stain** **Wright-Giemsa** omanowsky vailable ither ne-step three-step formulations. The components of may vary somewhat, ut hey sually nclude xative nd uffered olu tions f **eosin** **methylene blue** he ive ually **methanol** The eosin component buffered acidic and t tains he asic omponents he ells, uch emoglo bin eosinophilic granules. The methylene blue component buffered o omponents of cell, such leukocyte nuclei. three-step gives acceptable esults iemens lto, When ee-step insed distilled water between each of three components. Care must be taken to avoid dripping water into any of components, or ill ecome egraded. illed water one lowed to y efore oscopic xamination

The est uality chieved hen ed methanol or conds efore time o ariable ected umber factors, uch or ee-step average ime rse conds or ch om ponent. t cessary ars. Table problems related to staining. Cells appear dark they are overstained, whereas extensive rinsing may them to ok ded. hanging egularly cessary or on sistent esults or revention recipitation . ered emove xcess ebris. Refractile artifacts on are another common problem. These are ually isture ive ution, hich may be result of water dripping off of slides into jars or om ars eing overed hen ake onfuse tifacts ellular normalities.

Although ost eterinary ematology nalyzers rovide east tial erential lood ill prepared valuated. umber normalities ill e outinely eported tomated yzers, luding nucleated oxic ranulation, elet lumps, et ells, moparasites.

Examining slides way each time important to avoid mistakes when counting cells or important observations. lways begin examination by slide under low-power magnification general assessment of overall ell umbers btained.

The ntire or resence platelet clumps, large abnormal cells, microfilariae. Locating feathered edge monolayer performed next, high-power nification eathered dge blood contains cells are usually greatly distorted erratically ributed. nolayer lood here ells venly andomly ributed not istorted. fter hese wo reas re dentified, he echnician focuses on one microscopic field monolayer just adjacent to feathered edge. The differential count performed the monolayer by oil-immersion magnification. minimum of are counted, identified, recorded during ecause ounted, umber of ch ype bserved ecorded ercentage. led elative arious ounting evices available o elp erform he ifferential BC ount Fig. 0.7).

The differential blood leukocyte count provides the

After elative ercentages ch ell ype ve een eter mined, **absolute value** f ch ell ype ed. Calculation ute alues ccomplished ultiply otal ercentage ch ell ype. For xample, utrophils ere ounted lood n ute value or utrophils utrophils/ of lood.

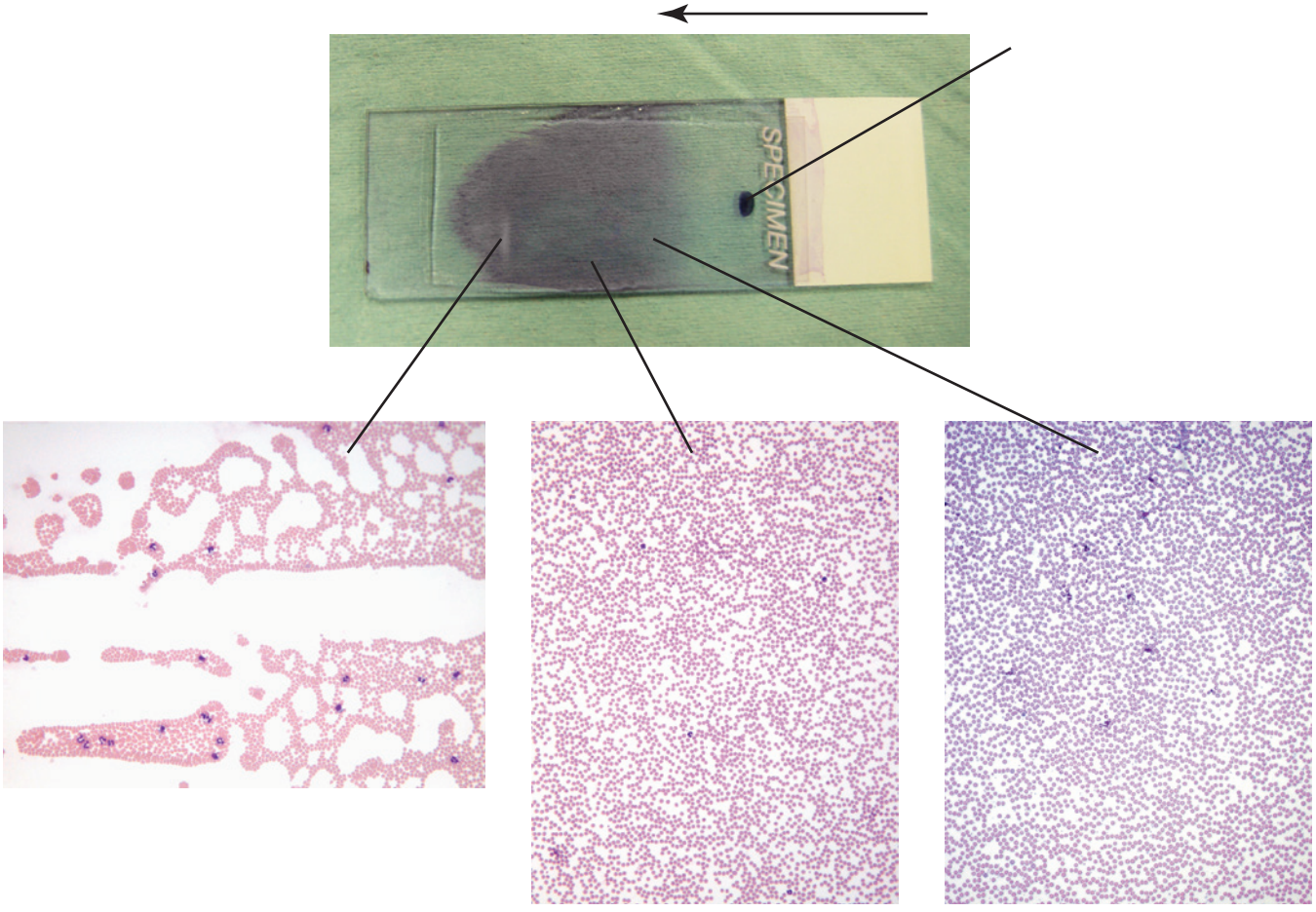
Troubleshooting Staining Problems

Excessive Blue Staining (Red Blood Cells May Stain a Blue-Green Color)

Precipitate on Preparation

Cowell and Tyler's diagnostic cytology and hematology of the dog and cat,

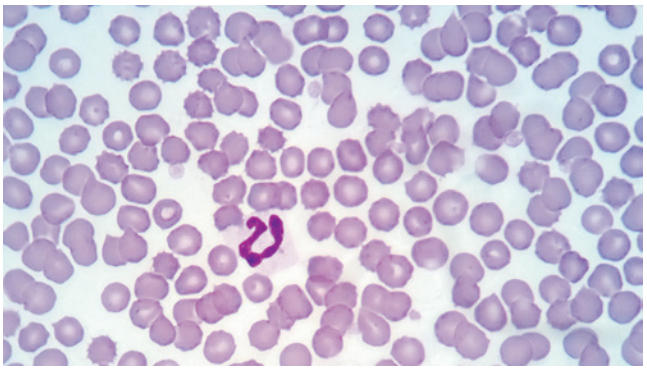
The relative percentage of each cell type from differ ential count may be misleading, especially when evaluating which total count or relative percent ages of differential count are normal range. For example, consider normal range of segmented neutro phils on blood to with lute value of to normal ranges for lymphocytes are to with absolute value of to If patient relative neutrophil count of lymphocytes, would appear to be **neutrophilia** with normal lymphocyte count. However, patient total count of (within normal range), absolute value for neutrophils normal level), absolute value for lymphocytes low level); patient actually lymphopenia. Simi larly, patient with relative lymphocyte count of would appear to have lymphopenia. However, patient's total count then absolute value for lymphocytes for patient which normal absolute value.



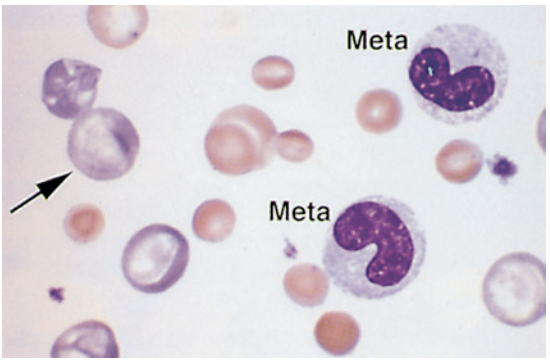
. The three major areas of the blood smear *(Feathered edge, Count area, and Thick area)* are indicated by the lines connected to the respective microscopic views. (From Valenciano A, et *Canine and feline blood smear analysis: a practical atlas,*

Mature ure utrophils, ymphocytes, nocytes, eosinophils, phils ukocytes found lood ch ype ell ys ortant ody's efense stem, concentration of each type extremely valuable for diagnosis of various Functions of include phagocytosis, release of substances modulate immune system, production of antibodies. More information about tions of the various populations of leukocytes located in nit 4.

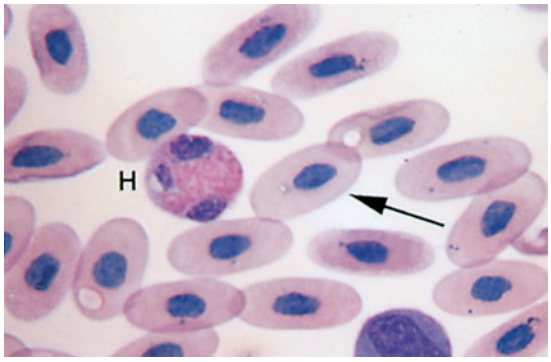
Neutrophils are abundant peripheral lood ucleus trophil regular longated, rue etween nuclear bes are resence ee nuclear bes haracteristic utrophils eripheral culation. ucleus quine utrophils vily lumped, oarse hromatin. toplasm ith ranules. ovine utrophils ve darker-pink toplasm. rimary tion utrophils phagocytosis. ncreased umbers eutrophils sually ndicate infection r ion.

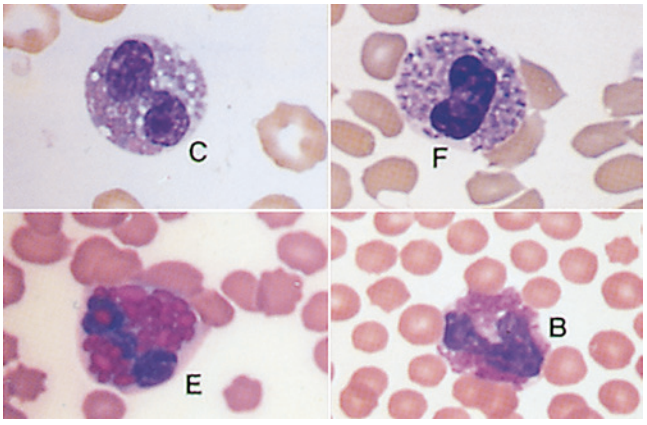


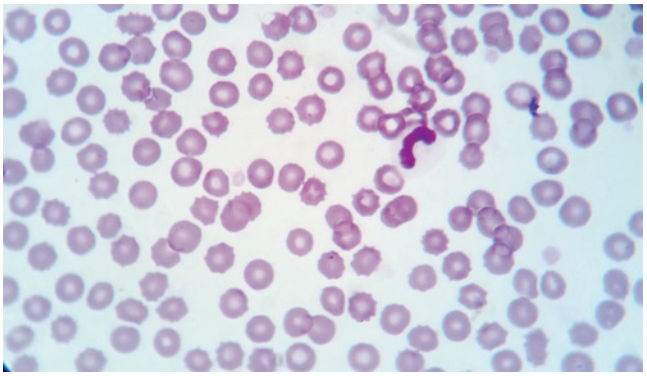
A neutrophil in a blood smear from a normal canine.



are also present,







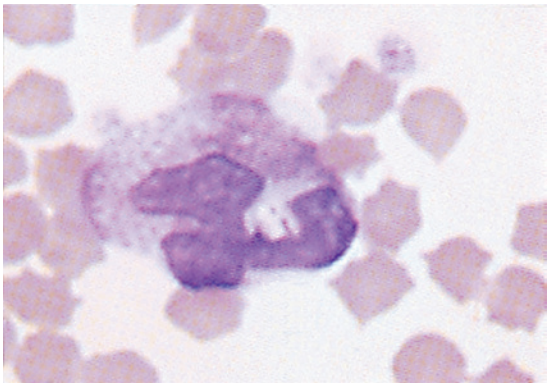
In vian, eptile, ecies (e.g., rabbits, pigs), cell functionally equivalent to neutrophil referred to **heterophil** Heterophils have distinct osinophilic ranules toplasm

Band neutrophil. The ucleus utrophils horseshoe-shaped, ith rge round nds ig. 10.10). lthough slight entations resent ucleus, on striction re ne-third nucleus, ell ually lassified gmented utrophil. The designation of neutrophil or mature segmented cell mewhat ubjective. ch cility learly iteria or hich utrophil ill esignated ply iteria onsistently oubt about whether particular cell or mature segmented cell, ell lassified ure ell. ore ure neutrophils (e.g., myelocytes, metamyelocytes) are common eripheral lood

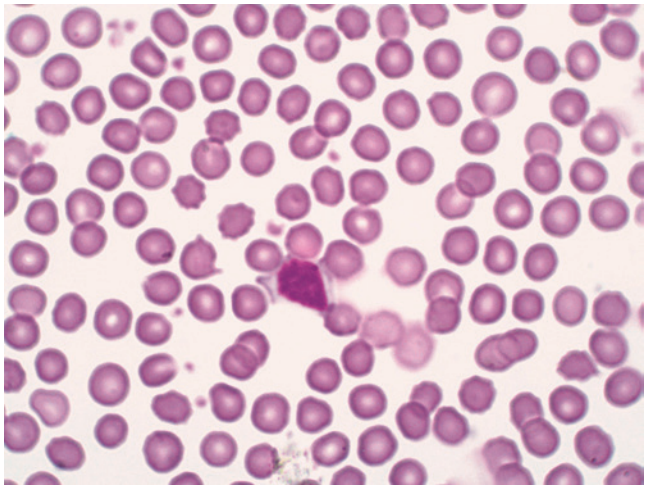
Eosinophils ontain ucleus f utrophils, ut hromatin ually oarsely clumped. he osinophilic ranules aries on siderably ecies ranules eosinophils often vary size with small large granules within ell, ensely species; y ually ed eline osinophils contain ranules od-shaped, umerous. Equine osinophil ranules val, they ense range-red olor. osinophil ranules ep, uch ler found rses, ense osinophils are le hagocytosis, ut rimary tion dulation stem. ncreased umbers eosinophils are commonly seen patients with allergic reactions asite ections estations.

The size, color, shape, and number of granules present

The uclei **basophils** e monocytes. Basophil granules dogs are few number purple to blue-black color. Equine bovine basophil granules



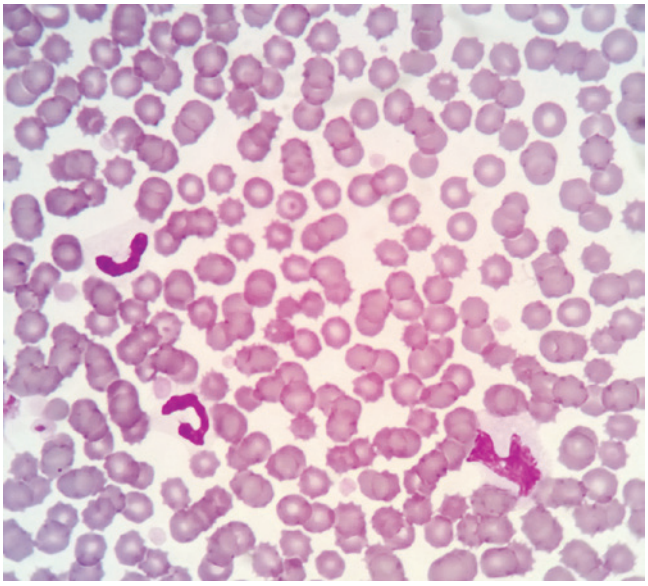
Normal feline basophil.



are sually ore umerous, hey end tain lue-black olor, y ompletely ck toplasm. eline phil granules e vender olor asophils volved diation system, increased numbers are seen with variety of matory ections onditions.

Basophils are not commonly seen on the blood smear.

Lymphocytes e resent ariety eripheral lood. hey from ruminant patients. Small lymphocytes are approxi mately o ter ogs ve slightly ented uclei hromatin oarsely clumped, cytoplasm light blue quite scanty. Chro mocenters, which are areas of condensed chromatin, be confused with nucleoli; chromocenters appear dark clumps within ucleus. edium-size ymphocytes diameter, with more abundant cytoplasm. The cytoplasm may contain pink-purple granules. Normal bovine lymphocytes may ontain ucleolar ings, to inguish om nocytes oplastic ymphoid ells. major tion ymphocytes roduction ibody. Increased numbers of lymphocytes often indicate viral infection.

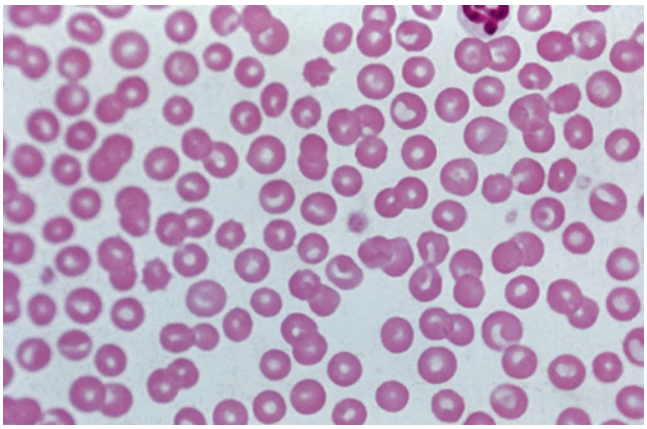


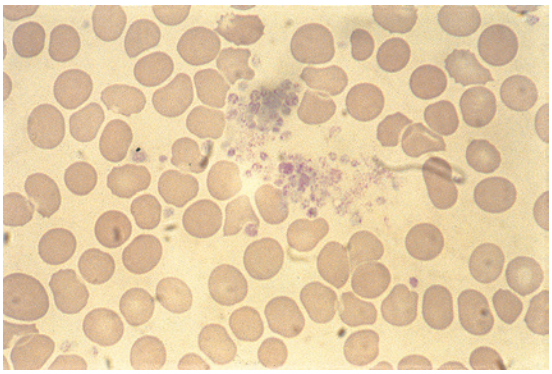
Normal canine monocyte

TECHNICIAN NOTE A variety of sizes of lymphocytes are usually present

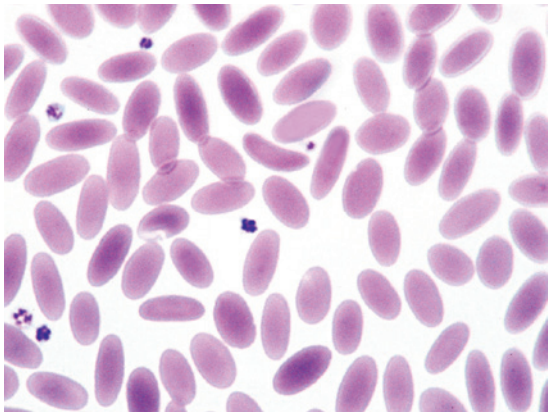
Monocytes e peripheral lood, ontain ariably ed uclei he ucleus ccasionally but ften longated, bulated, eboid. uclear chromatin re nocytes utrophils, hich oarsely lumped. toplasm nocytes lue-gray olor, ontain acuoles ranules. onocytes from utrophils, ymphocytes, tamyelocytes e oxic. resent, ells uestion are robably nocytes. tion nocytes phagocytosis. ncreased umbers nocytes en variety f hronic ections.

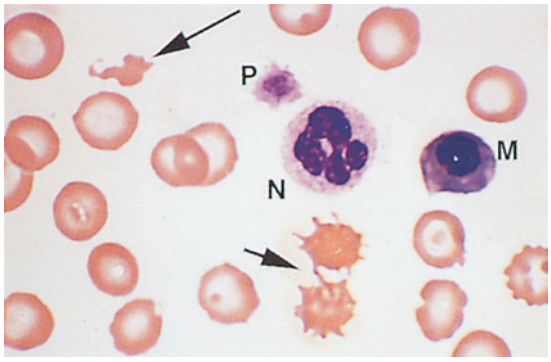
Normal erythrocyte morphologic features vary among different species of domestic Normal erythrocytes have biconcave inct entral lor eline rythrocytes entral pallor. Unlike of avian, reptile, amphib species are nucleated (see Fig. Oval, elliptical, longated rythrocytes en arious ypes anemia; metimes eferred encil ells. llama mbers y, predominant ell ype, gic condition ormal oat ep lood contain val-shaped rythrocytes. emoglobin ment





A platelet clump in a canine blood smear.





appear venly ursed oughout ell oncentrated each nd f val, entral lor.

ange en ultiplying imated elet number averaged from fields) by used indirect ure elet ernative thod for ect urement elet umber volves counting umber elets en er blood . umber elet estimate ith ollowing quation:

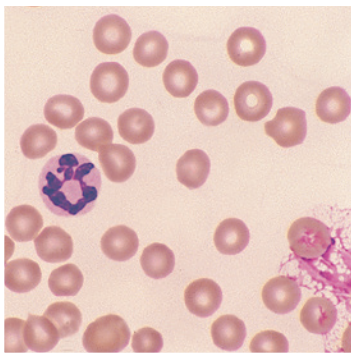
Platelets (thrombocytes) are important component of hemo stasis. ne rocedure or latelet valuation he xamination the lood ear. hen latelet umbers ppear ecreased, determining platelet concentration by more quantitative proce dure appropriate. Platelet numbers be evaluated counting area of blood smear. The numbers of platelets minimum of microscopic fields be counted. The size of oil-immersion field depends on type of microscope used. n verage elets er il-immersion more common normal patients. Platelet estimates be reported average number seen microscopic fields or

Platelet clumping common among If clumps are observed Fig. platelets are probably adequate number. The resence nusually arge latelets i.e., **megathrombocytes** Fig. uggest ly elease elets om bone row ed. elets, ecially may e er rythrocytes. resence ga platelets y ect elet epend n ype yzer ecreased elet umbers are uspected lood xamination, platelet ount ed.

Chapter eview uestions ppendix

- The wedge common technique used to prepare differential blood cell count.
- Differential blood cell smears are usually stained with Romanowsky
- Blood cell smears are used to determine estimated platelet numbers.
- A minimum of are counted classified when performing differential count.
- The differential count provides relative percentage of each type present
- Absolute values are recorded by multiplying relative per centage of each cell type by total count.
- Eosinophilic granules vary size, color, shape, number of granules present among species.
- Monocytes tend to have amoeboid nucleus, they are largest of circulation.
- Neutrophils are largest of granulocytes, they contain nucleus with three to five lobes.
- Basophils are commonly seen on blood cell smear.

Morphologic Abnormalities of Blood Cells



After studying this chapter, you will be able to:

- Describe methods for semiquantifying morphologic changes.
- Describe ypes rphologic hanges en hite blood ells.
- Describe ypes rphologic hanges en ed blood ells.
- Discuss erm oxic hange.
- List escribe erms escribe normal changes ed lood ell
- List escribe erms escribe normal changes ed lood ell
- List escribe erms escribe normal changes ed lood ell rangement.
- List escribe erms escribe normal changes ed lood ell olor.
- List escribe asites en lood smear.

Acanthocyte
Anisocytosis
Anulocyte
Apoptosis
Atypical lymphocyte
Autoagglutination
Basophilic stippling
Codocyte
Dacryocyte
Döhle body
Drepanocyte
Echinocyte
Heinz body
Howell-Jolly body
Hyperchromatophilic
Hypersegmented
Hypochromasia
Hyposegmentation
Karyolysis
Karyorrhexis
Keratocyte
Leptocyte
Macrocytosis
Microcytosis
Nucleated erythrocyte
Pelger-Huët anomaly
Poikilocytosis
Pyknosis
Reactive lymphocyte
Rouleaux
Schistocyte
Smudge cell
Spherocyte
Stomatocyte
Target cell
Torocyte
Toxic granulation

In ddition o numerating ach ype hite lood ell WBC) estimating platelet count, differential blood cell count requires morphologic features of cells be evaluated. The resence normal ells oxic hanges semiquantified.

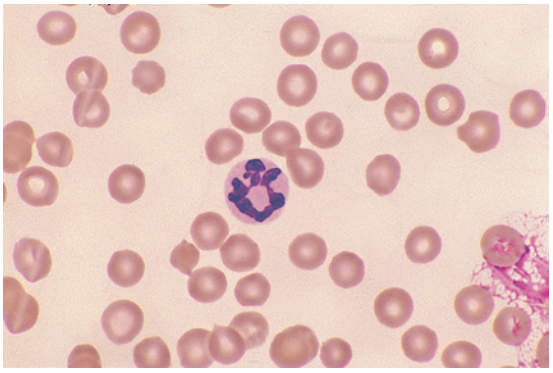
Two thods ommonly egree phologic changes. method of to indicate relative percentage of cells with morphologic change. he esignation enerally quates ells eing ected; es es approximately es re ells e ected. ubjective nother method esignations slight," moderate," marked" to e proximately re cells eing ected, espectively.

Pelger-Huët anomaly ongenital reditary efect haracterized **hyposegmentation** f l ranulocyte nuclei. Nuclear chromatin appears condensed but unsegmented, toplasm ected ells pears rmal Eosinophils phils ected. maly elieved o esult om tosomal-dominant rait, ommon ustralian pherd ogs. homozygous for trait generally suffer from skeletal abnormali ties rtly er irth. yposegmentation reflect early release of neutrophils. Pseudo–Pelger-Huët anomaly een eported ither ariant rmal inflammatory esponse atic eaction. general, ith udo–Pelger-Huët maly, ewer utrophils are yposegmented en ongenital maly.

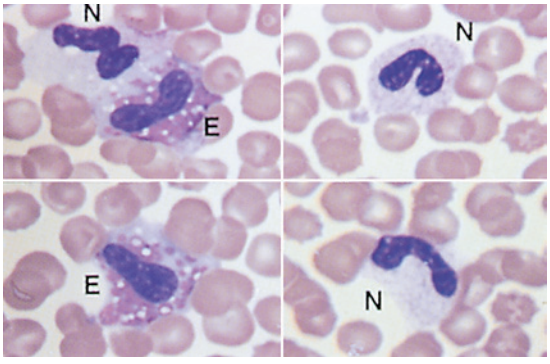
Canine eline utrophils re bes considered **hypersegmented** Fig. This usually attribut able to aging of neutrophils, either vivo would be seen with ndogenous xogenous lucocorticoids, hich rolong half-life of circulating neutrophils) or vitro result of rolonged orage lood efore lood de). Hypersegmented utrophils en lood of oodles oodle **macrocytosis**

Nuclear hypersegmentation of neutrophils is a com

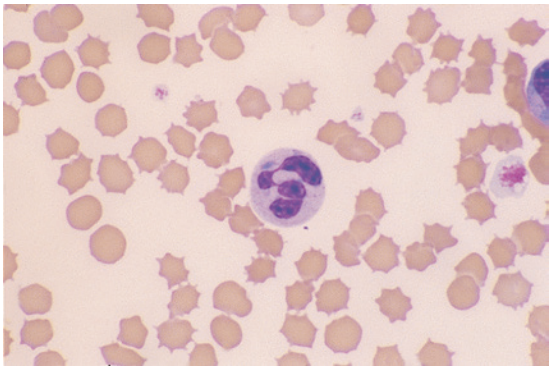
The common disease-induced cytoplasmic changes neu trophils e eferred oxic hanges ciated conditions uch ion, ection, oxicity. These hanges re hen ccur ogs. they e vere, ften uggest cterial ection. owever, toxic hanges uite ommon verely Types f oxic hange lude toplasmic philia, **bodies** vacuoles or foaminess" Fig. rarely, intensely stained rimary ranules eferred **toxic granulation** . ffected ells pear uch er rmal segmented neutrophils Fig. These toxic changes are thought to e d ecreased utrophil ration ithin row. riteria or valuating egree toxicity e resented ox



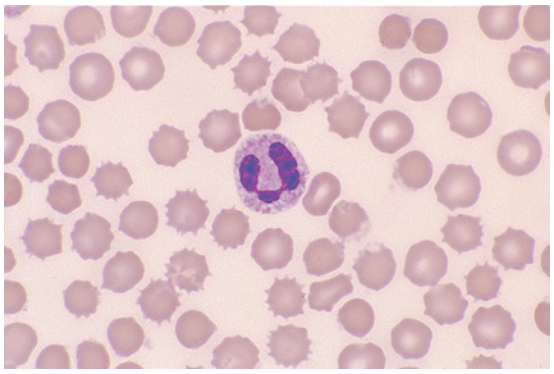
A canine neutrophil with a hypersegmented nucleus.



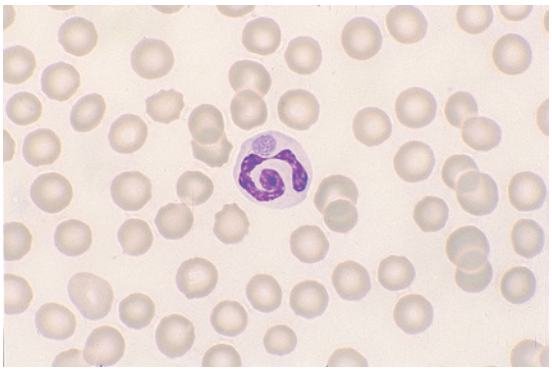
from a dog with Pelger-Huët anomaly. (Wright's stain.)



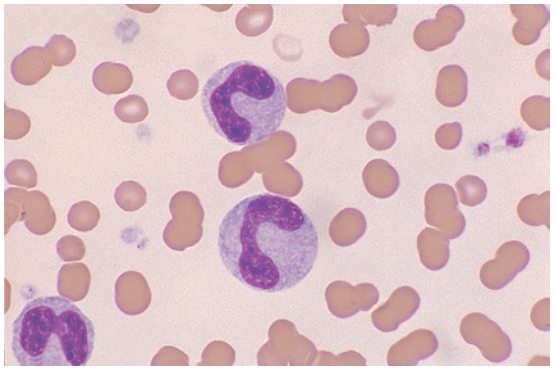
A toxic neutrophil showing cytoplasmic basophilia and a large Döhle body. The red blood cells are crenated.



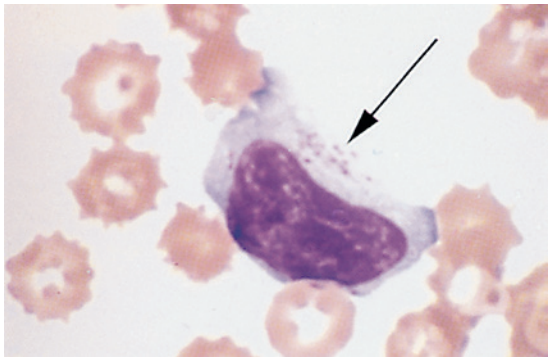
A neutrophil with toxic granulation.



A canine neutrophil that contains an



A giant neutrophil adjacent to a normally proportioned feline



of Toxic Changes in the Cytoplasm

*One or two Döhle bodies are sometimes seen in a few neutrophils from cats that do not exhibit signs of illness.
May also contain Döhle bodies.

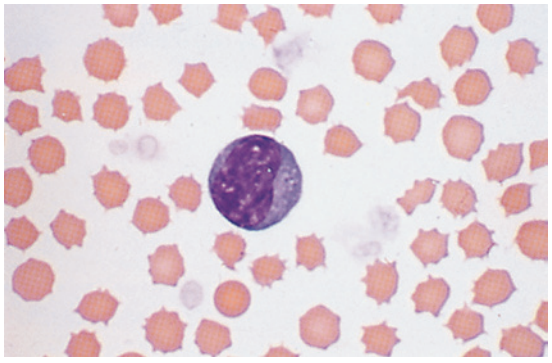
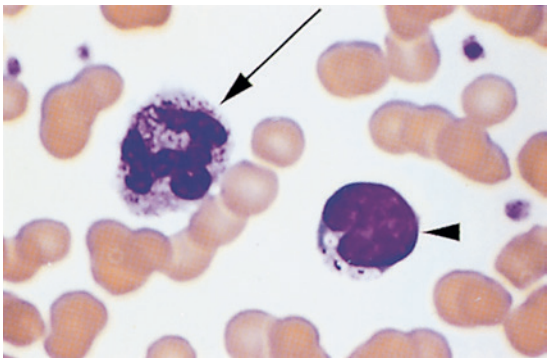


Fig. 11.8 A reactive lymphocyte in a canine blood smear. Numerous

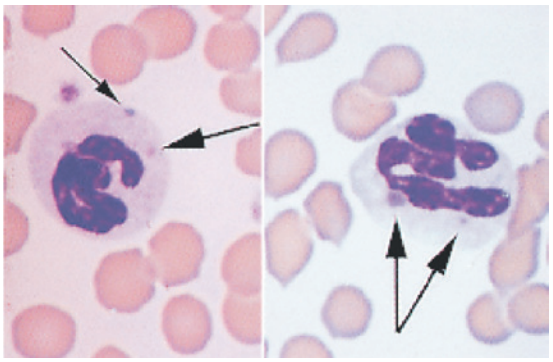
lusions utrophils nocytes lude *Histo plasma capsulatum, Francisella philomiragia, Mycobacterium,* gametocytes of *Hepatozoon canis,* amastigotes of *Leishmania infantum.*

Canine distemper inclusions may appear red blood cells or utrophils, lue enta olor. The rulae ickettsial rganisms *Ehrlichia Ana plasma* species) may be seen within cytoplasm of neutrophils Fig. ther ectious ents emonstrated

Azurophilic ranules toplasm ymphocytes e ften ciated hronic igenic imulation, especially ith hrlichiosis. zurophilic ranules be resent rmal ovine ymphocytes. **Atypical lympho cytes** y ve philic toplasm leaved uclei, and hey ay how vidence synchronous aturation he nucleus toplasm. **Reactive lymphocytes** . have increased basophilia cytoplasm; they may have more



as well as a neutrophil with toxic granulation



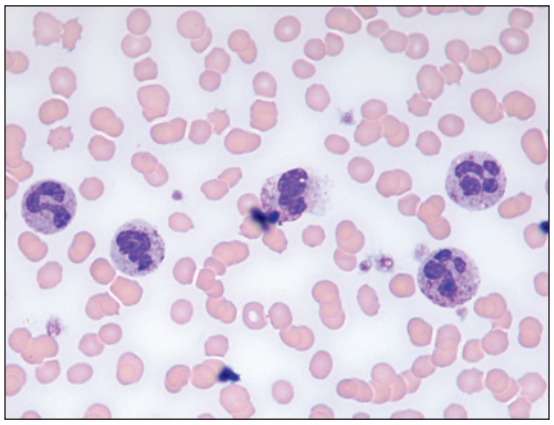


Fig. 11.10 Cytoplasmic granules associated with Birman cat anomaly. *Atlas of canine and feline peripheral blood smears*

abundant toplasm, metimes ontain er more onvoluted ucleus. hanges ually antigenic stimulation econdary ccination fection. eac tive ymphocytes eferred unocytes.

With group of rare inherited substance abnor mally ored ells, ually esult racellular enzyme eficiency. umerous ypes onditions ve been eported ary epending specific nzyme eficiency. ypes volve ither eletal abnormalities rogressive urologic ecause cells f ody ected, ored ubstance en leukocytes (usually monocytes, lymphocytes, or neutrophils). The pearance ukocytes aries epending ype of ysosomal orage ymphocytes acuolated, or y y ontain ranules; utrophils ontain granules

Neutrophils from affected by Birman Granulation nomaly contain eosinophilic to magenta granules. Fig. This anomaly inherited autosomal-recessive trait. Neutrophil function rmal, ected y. ranu lation ust inguished om oxic ranulation granulation en utrophils ucopolysac charidosis gangliosidosis, which are two of lysosomal storage rders.

Neutrophils rited rder Higashi ome ve ysosomes cytoplasm lightly or eosinophilic Fig. Approximately ee utrophils ontains lysosomes. ranules osinophils pear htly large. ffected ve endency leed, ecause their elet tion normal. lthough utrophil tion normal, ected enerally y. The ome ften een eported ersian but een emonstrated ccur oxes, other ecies.

Granules of hemosiderin may be present neutrophils monocytes f nimals ith emolytic nemia. hey ppear imilar to odies, ut erentiated Prussian lue odies russian blue. Siderotic inclusions occur erythrocytes; affected cells ould eferred erocytes.

Smudge cells hich e metimes eferred et ells, are degenerative leukocytes have ruptured Fig. Their presence onsidered umbers seen n lood mall umbers udge ells be roduced tifact hen lood oo efore de xcess ressure hen . umbers udge ells ciated leukemia.

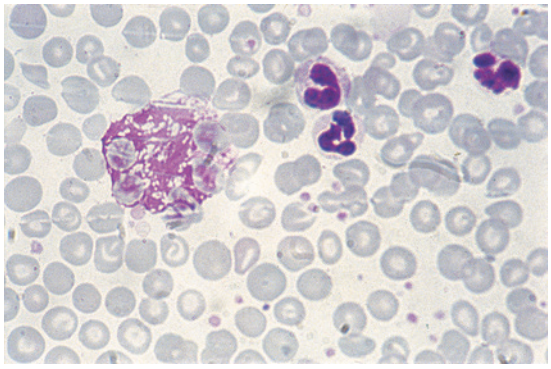
Karyolysis egenerative hange ucleus acterized by dissolution of nuclear membrane. It usually affects neutrophils, associated with presence of septic exudates. The term **karyorrhexis** refers to fragmentation of ucleus er ell **apoptosis** erm **pyknosis** refers to condensing of nucleus cell Fig.

The orphologic haracteristics rythrocytes ategorized according o ell rangement lood color, e, resence ructures rythrocytes.

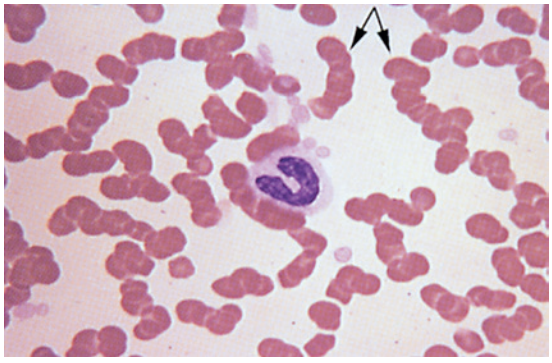
Rouleaux ormation volves rouping rythrocytes stacks . ncreased ormation en increased rinogen lobulin oncentrations. or mation ccompanied rythrocyte mentation ate. arked ormation en

horses, may be present on blood smears from healthy s. en tifact lood ld oo efore lood repared lood een efrigerated.

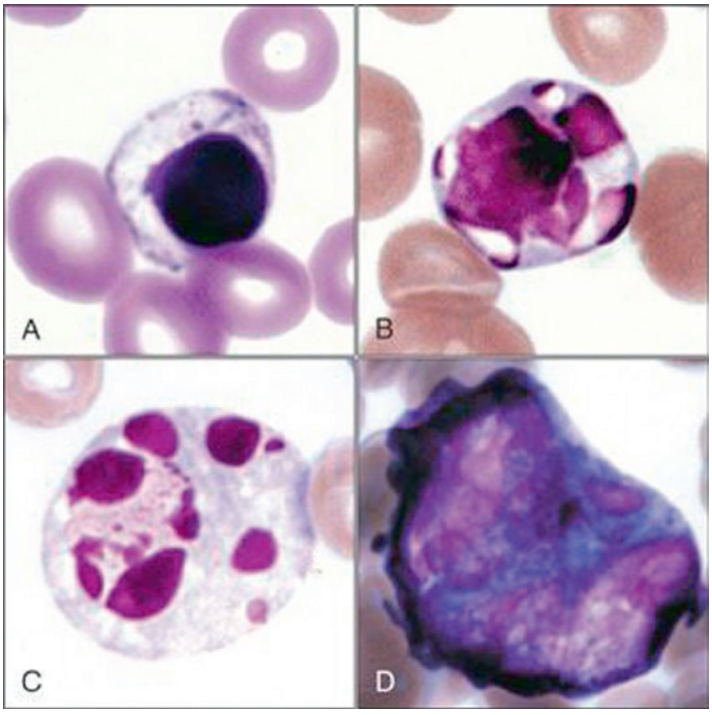
The glutination rythrocytes inguished om rouleaux ormation **Autoagglutination** ccurs immune-mediated disorders which antibody coats eryth rocyte, esulting ridging lumping sometimes bserved croscopically oscopically. differentiate om glutination, op to op f lood, oscopically for glutination. ormation ill erse



A smudge cell and several neutrophils in a canine blood smear.

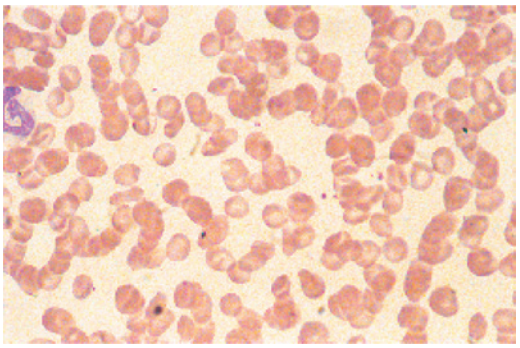


A neutrophilic band cell is also present.

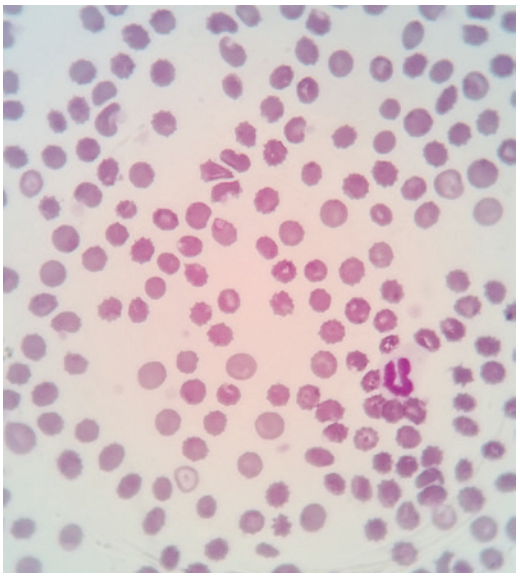


Pyknotic cell with condensed chromatin in blood from

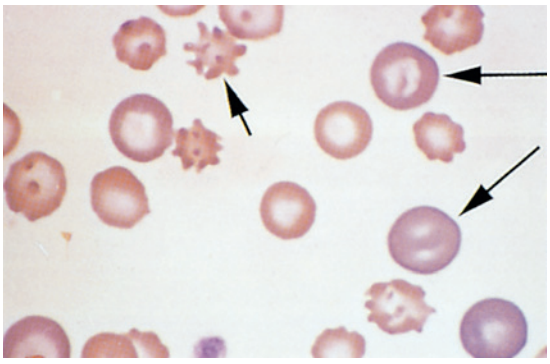
Pyknosis and karyorrhexis of a cell in blood from a cow with leukemic lymphoma. (Wright-Giemsa stain.)



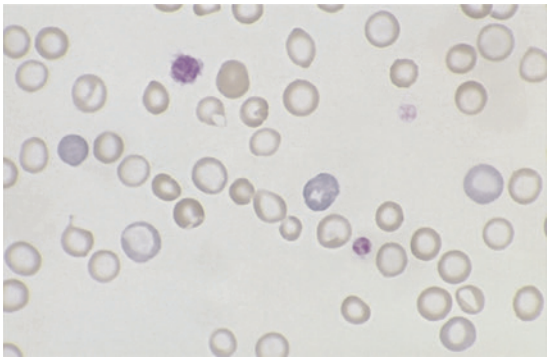
Autoagglutination in a canine blood smear.



Autoagglutination will not disperse when RBCs are



Macrocytic polychromatophilic red blood cells



. lue esult resence organelles remain within cytoplasm; therefore, are young ells. hen thylene lue rilliant cresyl lue, ells ually pear eticulocytes.

Anisocytosis variation size of Fig. may indicate presence of macrocytes large cells), micro cytes small cells), or both. nisocytosis common in ormal ovine lood. acrocytes re BCs hat re arger han normal, ith eased orpuscular olume Macrocytes are usually young, polychromatophilic erythrocytes reticulocytes). Microcytes are with diameter smaller of normal erythrocytes, with decreased MCV. Microcytic ells en on eficiency.

Anisocytosis may involve microcytes or macrocytes,

Hypochromasia ecreased ensity by ufficient moglobin ell The cell will normally appear more darkly stained along eriphery, radually ers uch entral region. ron eficiency ommon macrocytic rythrocytes ften pear ypochromic ecause their large diameter. Hypochromic cells be distinguished from bowl-shaped cells **anulocytes** or punched-out" cells **torocytes** hich e enerally onsidered tifacts result from improper technique Fig. with true ypochromasia ways ve **microcytosis** hich determined ecreased ormochromia rmal ensity.

The erm olychromasia efers erythrocytes hat exhibit bluish tint hen omanowsky-type right's

The word **hyperchromatophilic** refers to cells appear to be more arkly tained han ormal ells. his ives he ppearance

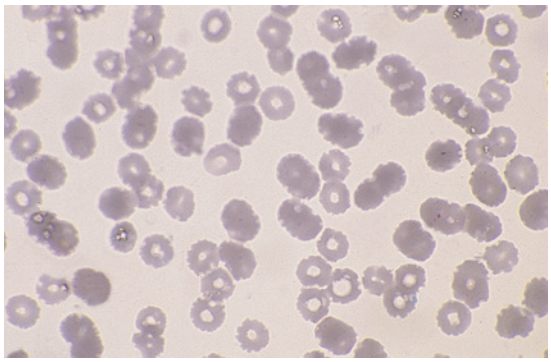
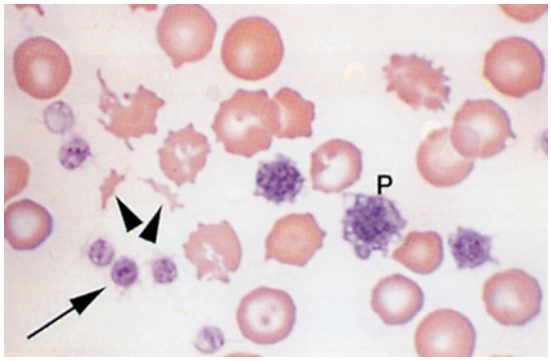


Fig. 11.19 The punched-out appearance of many of these red blood cells is an artifact caused by inadequate drying of the blood smear.



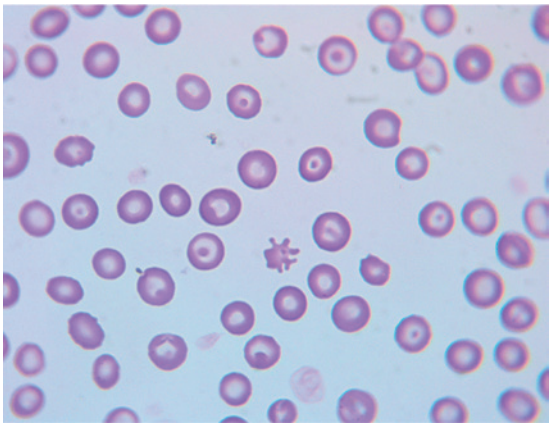
and giant platelets
are seen on this blood smear from a dog with iron-deficiency anemia.

cells are oversaturated with hemoglobin. Because
ed city or moglobin, versaturation
ccur. ells ually ocytes herocytes.

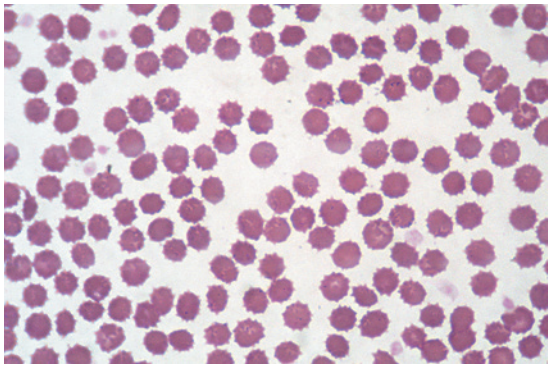
Abnormally haped rythrocytes re alled oikilocytes. owever, erminology lpful, ecause oes uggest specific nosis rovide ormation egarding of shape change. The origin of abnormal shape depends in art n he pecies eing xamined. hape nd olor hanges are considered important when they are associated with specific disorders. he erm **poikilocytosis** e nly hen rphologic normalities escribed f re ecific erms.

Schistocytes Fig. which are fragments, are usually formed esult ravascular trauma. histocytes bserved minated ra vascular oagulopathy hen rythrocytes roken fibrin strands, with vascular neoplasms (e.g., hemangiosarcoma), with iron deficiency. with usually have concur rent ombocytopenia.

Acanthocytes hich e led ells, regu lar, ed ew venly ributed urface



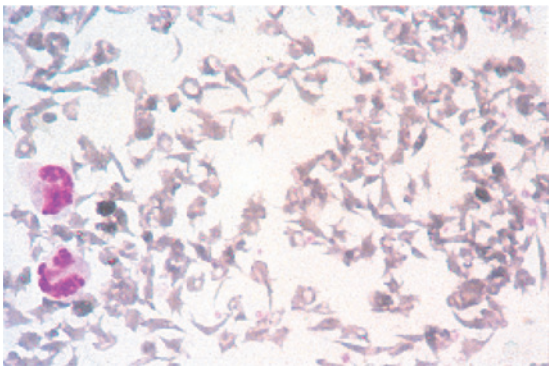
(Wright-Giemsa stain.) (From Harvey JW:



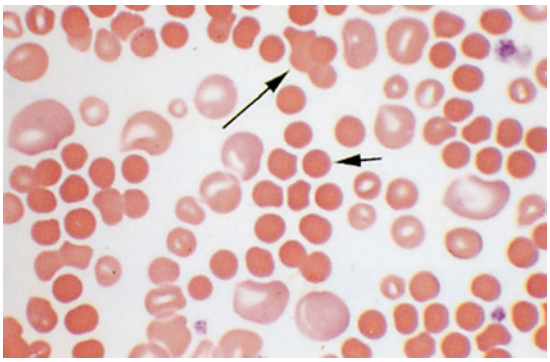
Echinocytes in a feline blood smear.

projections ariable ter hey are en ients ered tabolism with patic ogs mangiosarcoma liver). The presence of acanthocytes middle-aged to large-breed dogs with concurrent egenerative anemia suggestive hemangiosarcoma.

Echinocytes which are called burr cells, are spiculated cells with numerous short, evenly spaced, blunt to sharp surface projec tions of uniform size shape Fig. Echinocyte formation e tifactual, itro rocess ciated drying f lood rolonged orage common erm or normality enation. hinocytes e roduced tifact ube er filled. hinocytes ve een en enal lymphosarcoma dogs; after exercise horses; from rmal, hey ve ccurred er snake, coral snake, water moccasin, viper envenomation ogs.



Drepanocytes (sickle cells) in a blood smear from a normal deer.



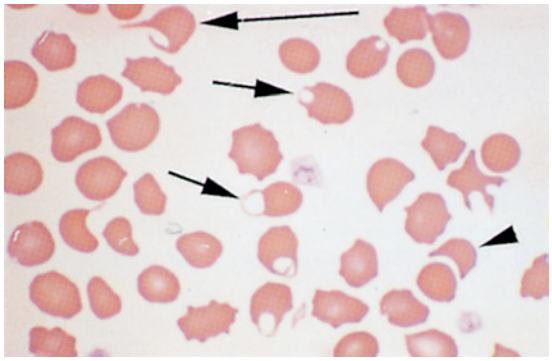
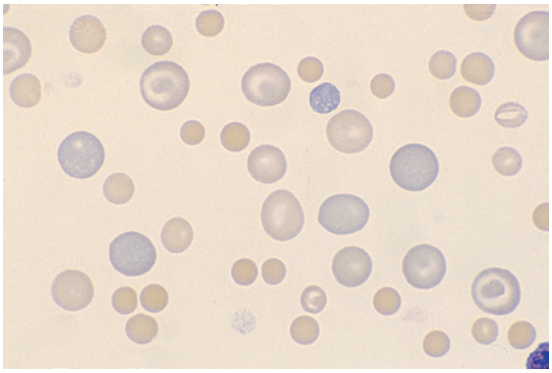


Fig. 11.24 Numerous keratocytes *(arrows)* and a schistocyte *(arrowhead)* are present in this blood smear from a cat with iron-deficiency anemia.



Mixed anisocytosis and polychromasia is also present.

Drepanocytes hich e led ells, bserved lood rmal eer ora oats. to e itro henomenon xygen tension .

Keratocytes e ommonly eferred lmet ells, lister cells, r ite ells. resence eratocytes een ciated ith mangiosarcoma, oplasia, lomerulonephritis, arious patic ell pear ontain acuole. eratocytes elieved orm om ravascular trauma volves isection ell rin rands. The pposing ell dhere ch orm udovacuole eratocytes ve been emonstrated lood om ients ver disorders, yelodysplastic ome.

Spherocytes are darkly with reduced or central pallor Fig. Spherocytes are easily detected species other ogs. hey ve educed mbrane surface ea esult tial hagocytosis crophages, which occurs response to presence of antibody or comple ment n urface herocytes y uggest une-mediated estruction resulting molytic hey en er transfusion ched roperly ored lood, er snake envenomation, association with parasites, with oxicity. mmune-mediated molytic ually regenerative ked olychromasia reticulocyte owever, nonregenerative result of antibodies against precursors within he one arrow. hese ases, pherocytes re ften if to detect, because presence of large polychromatophilic cells acilitates ecognition mall pherocytes. lthough spherocytes ve ecreased ter pear their olume ormal. ddition, ogs ith mmune-mediated hemolytic ve ecreased

These cells are characterized by increased membrane surface area relative to cell volume, affected cells may take variety of es. **Target cells** hich e eferred **codocytes** are **leptocytes** ith entral ment urrounded clear ea ense eripheral toplasm ew en rmal lood may e ciated ver inherited rders. eptocytes pear olded ells **stomatocytes** olded ells ve ransverse, aised that xtends cross he enter he ell ell lear, litlike region center of cell Fig. Folded cells stomatocytes are considered artifacts areas of pallor are all perpendicular eathered dge. arr ells eferred to ocytes. ype ptocyte pears ve hemoglobin cross enter ell.

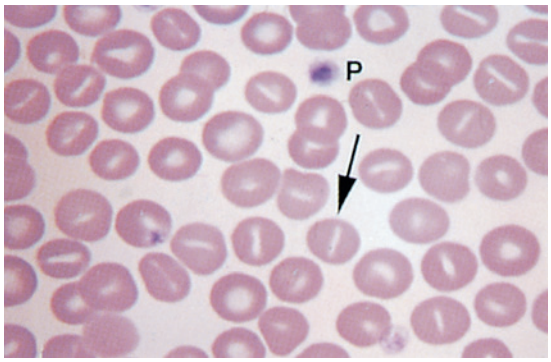
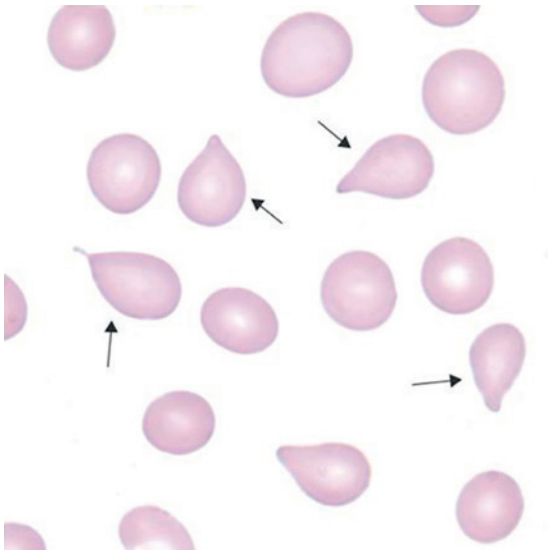
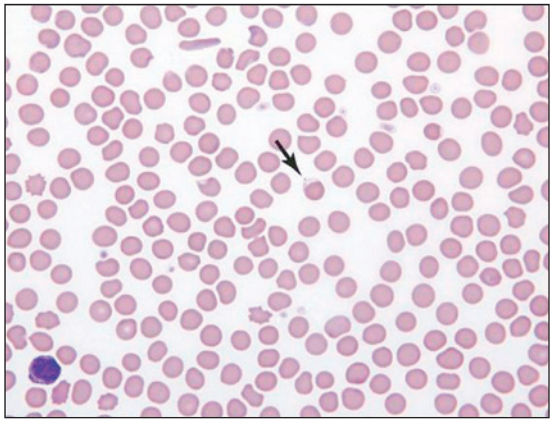
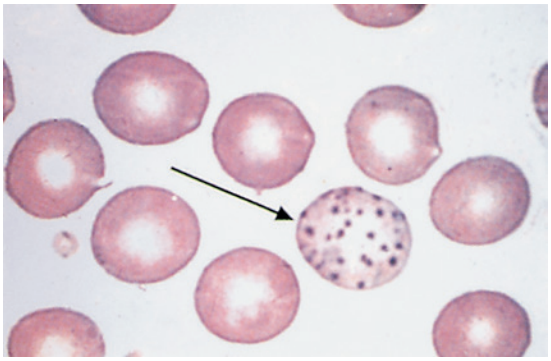


Fig. 11.27 Folded cells and stomatocytes and a platelet on



6th





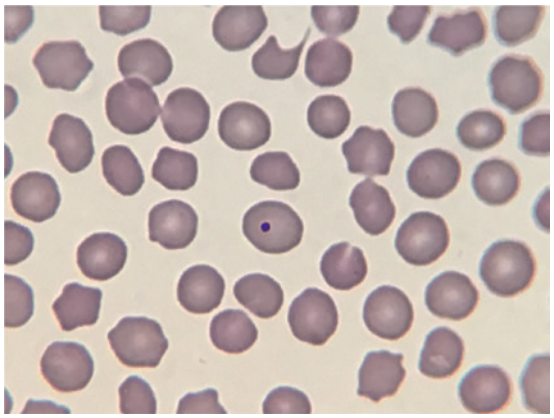
Erythrocytes om ecies mally oval or elliptical shape (see Fig. In other species, ells ciated ymphoblastic ukemia, patic lipidosis, ortosystemic lomerulonephritis.

Eccentrocytes have been described patients with diabetic keto acidosis or neoplasia, with *Babesia canis* infections, after ingestion of oxidants such garlic, onions, acetaminophen. The ells pear ve moglobin rimarily one e

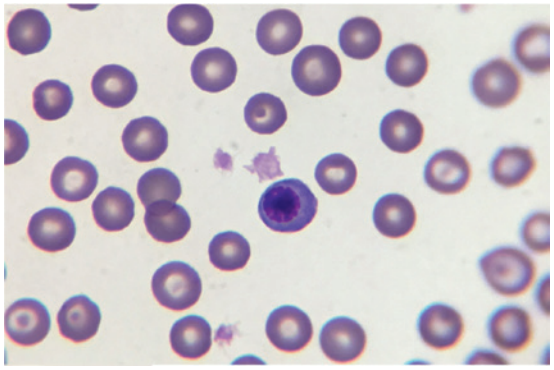
Dacryocytes re eardrop-shaped ells hat re een ith yelo fibrosis certain other myeloproliferative They have been identified blood from llamas alpacas are iron deficient. hese ells roduced tifacts, ut be dentified he irection heir longated ails; acryocytes ve een roduced tifact ve ointing ection

Basophilic stippling—or presence of small, dark-blue bodies within erythrocyte—is observed right-stained cells represents esidual ommon ure ruminants ccasionally uring esponse to anemia. It characteristic lead poisoning Fig. 11.30

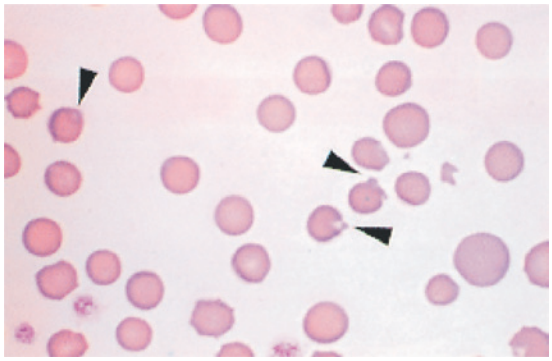
Howell-Jolly bodies e philic uclear emnants seen oung rythrocytes uring esponse As ells ontain uclear emnants ough spleen, hagocytic ells emove emnants. onsequently, increased umbers en er emoval en or ith nic rders.



Howell-Jolly bodies on a canine blood smear.



Nucleated red blood cells in a canine blood smear.



on a feline blood smear. (Wright's

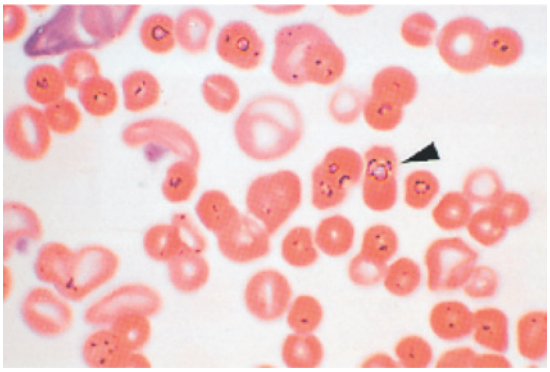


Fig. 11.35 Drying artifacts. These will appear refractile under the

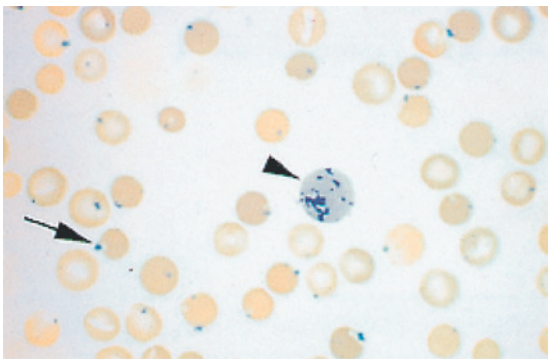


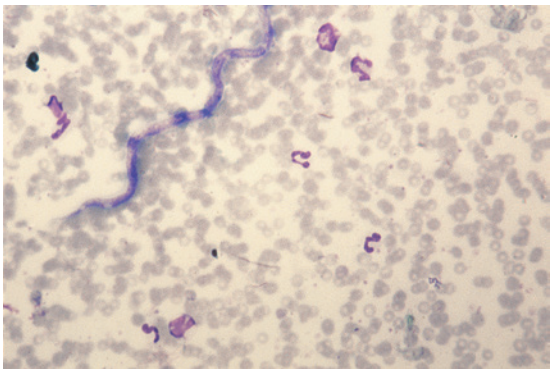
Fig. 11.33 Heinz bodies on a feline blood smear. A reticulocyte is also present. (New methylene blue stain.)

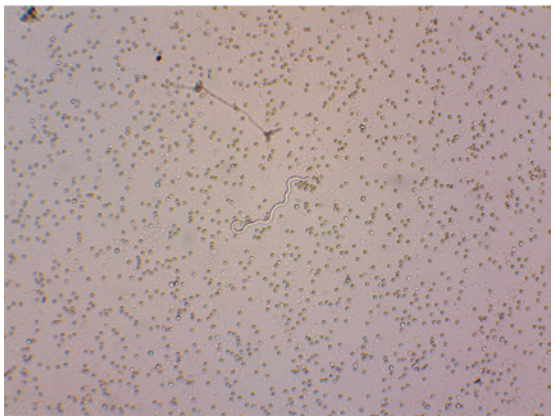
y einz odies ften increased concentration with eases such as lymphosarcoma, hyperthyroidism, etes llitus

In **nucleated erythrocytes** represent early release of ure ells uring ut cca sionally bserved nanemic ll of nmammalian ecies irds, eptiles) ontain uclei. Nucleated luded performed by hemocytometers electronic cell counters. hen performing erential ell ounted separately eported umber ncountered ounting orporated following equation to calculate corrected leukocyte count:

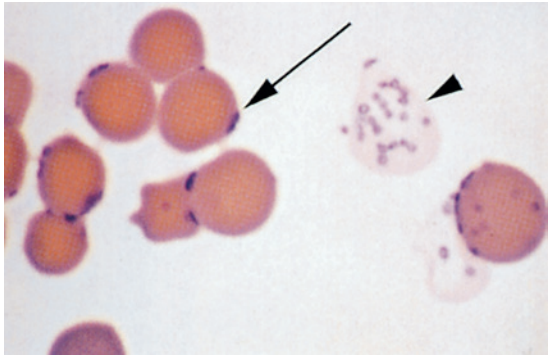
Heinz bodies e ound ructures epresent enatured hemoglobin ertain ugs chemicals. he enatured moglobin ecomes ched cell membrane appears area with right's Fig. hen thylene lue technique or eticulocytes, einz odies pear lue Fig. ogs, einz odies ter. Unlike r omestic rmal ve einz odies

Parasites may be present or on erythrocytes. precipitate drying artifacts are sometimes confused with parasites. Drying tifact ually pears efractile ommonly en lood asites *Ehrlichia*





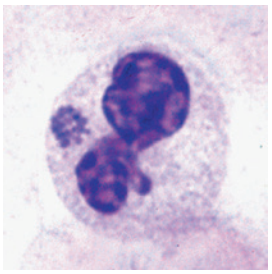
Microfilaria of *Dirofilaria immitis* in a canine blood smear.

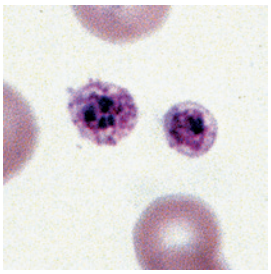


. Ring forms are visible in the lysed erythrocyte

Mycoplasma. Occasionally, microfilaria of *Dirofilaria immitis* may e en eripheral lood ther parasites ncountered lude *Eperythrozoa, Anaplasma, Cytauxzoon, Babesia* dditional ormation parasites ect lood ells hapter

Mycoplasma haemofelis ly ommon asite eline erythrocytes. eferred bartonellosis or feline infectious anemia. The organisms appear l occoid, od-shaped, inglike structures dark purple with right's Fig. They equently pear rt ods eriphery RBCs. he arasitemia yclic; emobartonellosis uspected, blood be examined several times different times of y efore ection uled lood





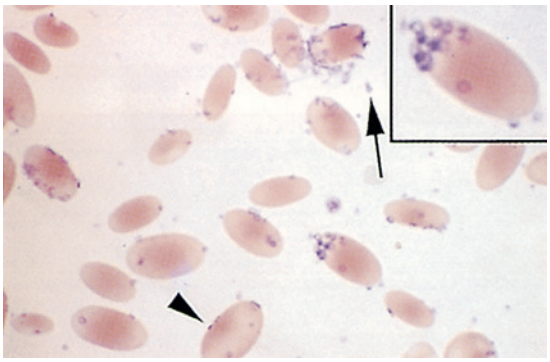
been contact with anticoagulant preferred when evaluating ample for uspected emobartonellosis. organisms often detach from surface of when anticoagulant. *Haemobartonella canis* infection rare dogs, usually observed nly nectomized unosuppressed ogs. The organism commonly appears chain of small cocci or ods retch cross urface rythrocyte. chains y pear ranch.

ariety f ecies *Ehrlichia* *Anaplasma* e le of infecting dogs. *Anaplasma platys* (previously *Ehrlichia platys* affects nly elets ectious clic ombocytopenia. ther *Ehrlichia* ecies y ect ukocytes. *Ehrlichia canis* ommonly ects nocytes utrophils Fig. The organisms are transmitted by tick vectors; they appear lusters led rulae cytoplasm. *Anaplasma marginale* intracellular blood parasite wild ruminants. It appears l, occi must e erentiated om owell-Jolly odies, ecause sizes are similar. Early during course of many f ontain asites.

vere, ually ected.

Anaplasma *Ehrlichia* parasites belong to group of rick ettsial rganisms. menclature arious ecies changed ignificantly uring ecent ears dditional nformation about iochemistry rganisms een overed. The esult utropenia, ombocytopenia, anemia. hronically ected verely with arked eukopenia nd hrombocytopenia, ut ometimes nly matologic normality ymphocytosis.

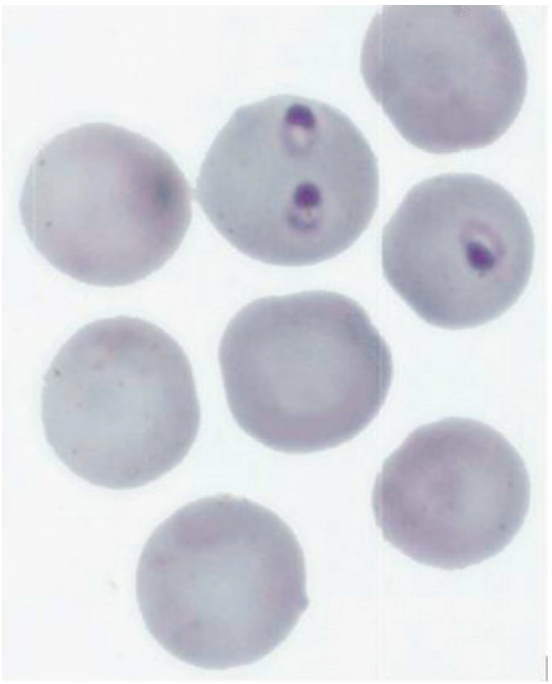
protein level usually increased. The organisms are best demonstrated during acute phase, but they are usually present l umbers. mears de om oat with diagnosis. However, organisms are seen, de unologic esting.



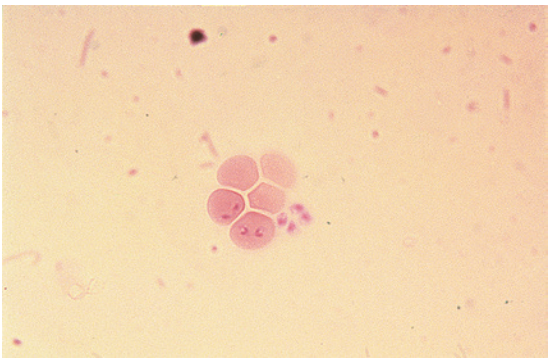
Eperythrozoonosis wine, attle, lamas uite imilar to hemobartonellosis; organisms are closely related. *Eperyth rozoa* pear occi, ods, ings on urface ee orm ommon

Cytauxzoon felis are molytic The rganism pears regular forms ithin rythrocytes, ymphocytes, crophages Fig.

Babesiosis *Babesia bigemina* *B. bovis.* he led exas ever, ed er ever, ick ever. *Babesia* p. pear pleomorphic, eardrop-shaped racellular rganisms frequently en abesiosis rses piroplasmosis) *B. equi* *B. caballi,* hich e o *B. bigemina.* abesiosis are rses, ew have been reported United States have been seen South (especially Florida). Babesiosis dogs caused by *B. canis* *B. gibsoni.* hese rganisms pear ance o *B. bigemina,* *B. gibsoni* htly ler, appears ings. ercentage rythrocytes be ected. rganisms re ommonly bserved eathered dge.



Feline erythrocytes infected with the characteristic signet-ring organisms. (Wright-Giemsa, 330.) (From Little S: *The cat*, St Louis,

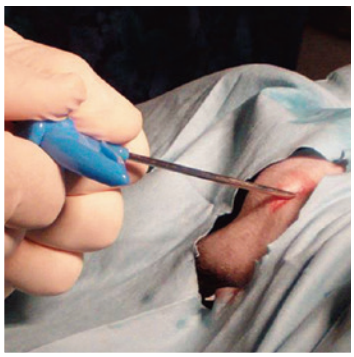


organisms in bovine red blood cells.

Chapter eview uestions ppendix

- Changes ukocyte rphologic eatures ect nucleus r toplasm volve lusions cell.
- Changes erythrocyte morphologic features affect cells' size, shape, olor, rangement.
- Morphologic hanges en lood be miquantified.
- Nuclear hanges ukocytes lude yposegmentation, hypersegmentation, yknosis, yolysis, yorrhexis.
- Inclusions en ukocytes lude ysosomes, variety f normal ranules, lood asites.
- Changes rythrocyte volve ocytes, cro cytes, r oth.
- Alterations erythrocyte behavior include rouleaux forma tion toagglutination.
- Inclusions en rythrocytes lude owell-Jolly odies, Heinz odies, philic ippling, ariety lood parasites.

Additional Hematologic Tests



After studying this chapter, you will be able to:

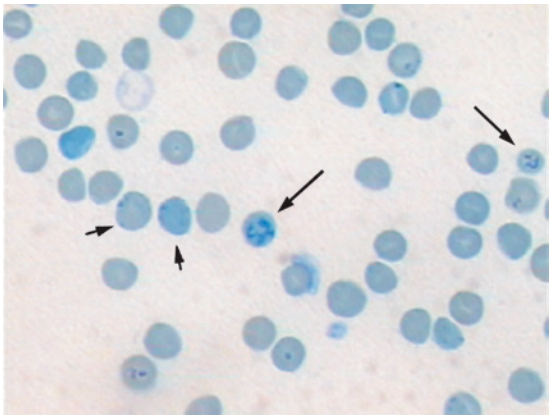
- Describe rocedure or erforming eticulocyte count.
- Differentiate etween gregate tate eticulocytes.
- Discuss ions or one row valuation.
- Describe ollection one row aspiration ore
- Describe thods or reparing one row or evaluation.
- Describe iteria haracterize one row cellular, ypercellular, ypocellular.
- List escribe ells ommonly en one marrow

Aspiration biopsy
Core biopsy
Erythrocyte sedimentation rate
Illinois sternal needle
Jamshidi needle
New methylene blue
Reticulocyte production index
Reticulocytes
Rosenthal needle

Reticulocytes are immature erythrocytes contain organelles (ribosomes) ells ure organelles account for blue-gray or polychromatophilic of immature cells with right's Romanowsky does cellular components. supravital such **new methylene blue** r rilliant esyl lue, Supravital ontain ives. hen eticulocytes stained ith upravital rganelles lump isible granules are referred to reticulum. This reticulum present aggregates or chains of blue granules. The must be fresh, ered efore ill artifacts uch recipitate cteria resent upravital

Some automated analyzers laser-based methods provide eticulocyte everal thods used to prepare reticulocyte smears. Special reticulocyte are available, or blood be stained by placing few drops of blood equal number of drops of new methylene blue or brilliant cresyl lue ube. ure lowed or proximately utes. op ure then used to prepare conventional air-dried blood smear, which n e xamined ounterstained right's Because enerally erformed ients, blood drop used be larger made bit more thickly standard blood smear. lternatively, e dded op lood ectly lood ell ed lowed or several utes efore lood eated. intensity f eticulocytes nhanced re pared eticulocyte or ew conds following with placement methylene blue component of Diff-Quik (Siemens Palo lto, for seconds.

Cats, e ecies, ve rphologic orms reticulocytes. he ggregate orm ontains arge lumps eticu lum, eticulocytes ecies, epre sents cell polychromatophilic with right's



he tate orm, hich ontains to ight philic ranules. ells olychromatophilic right's rmal, anemic proximately ed lood ells are gregate eticulocytes, hereas are punctate reticulocytes. For meaningful reticulocyte count only aggregate form of reticulocytes be counted. eticulocyte xpression ercentage eticulocytes. ercentage eticulocytes er erythrocytes determined with of oil-immersion lens. eticulocyte erformed lood of l mic omestic xcept rses, hich release eticulocytes om one row, ven ce of egenerative eticulocyte oncentration or one row's esponse

Reticulocyte erpreted ccording degree of anemia, because fewer mature erythrocytes are present anemic reticulocytes are released earlier persist longer they do normal Higher percentages may be seen hemolytic anemia compared with hemorrhagic types of mia. lthough eticulocyte esults ften eported ercentage, re thod orrected reticulocyte **reticulocyte production index** orrected eticulocyte ed ultiplying bserved eticulocyte ercentage bserved cked cell olume vided rmal or ogs, d rmal or or og bserved eticulocyte then corrected reticulocyte count obtained follows:

——

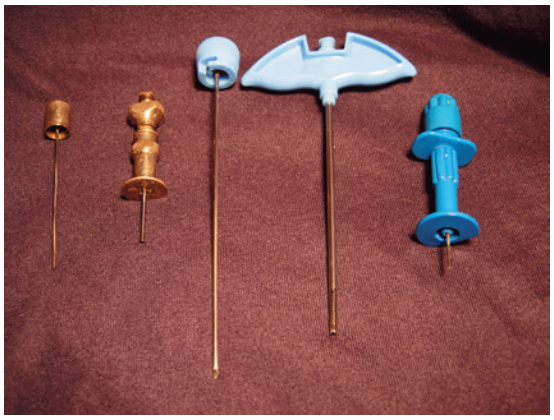
Some practitioners may prefer to reticulocyte produc tion ex. eticulocyte roduction ex ed dividing corrected reticulocyte percentage by maturation time of reticulocyte for observed patient's PCV. Maturation time values are based on patient's Table For previous example, dog's corrected reticulocyte count with eticulocyte roduction ex / which indicates patient producing reticulocytes ate reater rmal.

Patient Packed Cell Volume	Maturation Time

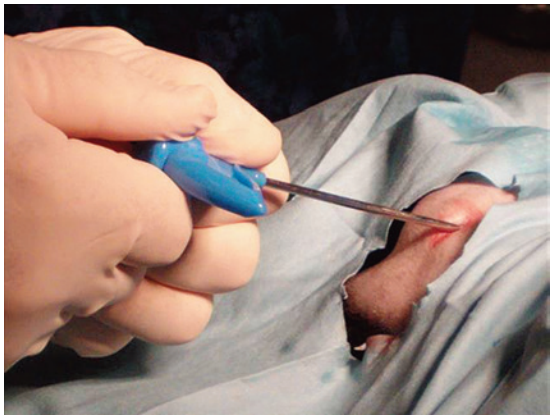
Bone row valuation aluable ool or prognosis ecific ed or one row valu ation ually etermined erential count rom eripheral lood ell ample. hen he ifferential leukocyte count from peripheral blood demonstrates ambiguous xplained normal esults, one row evaluation ually eded rovide ufficient etail or diagnostician. ecific ions or one row valuation include ersistent xplained topenia, utropenia thrombocytopenia, nd onregenerative nemia. ess-common indications for bone marrow evaluation include presence of cells ith normal rphologic eatures xplained presence f ure ells erential lood ell (e.g., nucleated left Bone marrow evaluation used to stage neoplastic to diagnose specific parasitic ., hrlichiosis). one row valu ation y e ontraindicated ticularly hen certain mostatic efects resent.

Samples y ollected ation emoval one marrow ore. roper estraint or iopsy one marrow ogs dation needed. General may be used, but complicated, because atients ndergoing one arrow valuation re sually compromised represent significant anesthetic risk. septic technique oughout rocedure.

All cessary quipment upplies athered before beginning procedure. To collect bone marrow aspirate repare ringes thylenediaminetetraacetic (EDTA) ution awing ringe ensing mpty lood ollection tube. ithdraw mixture, then repeat procedure; creates uted ringe one marrow edle. cessary line. sually, out repared. ther equipment eded ludes el lade, erile reparation upplies, uture erial, propriate. Special bone marrow needles are preferred, although 18-gauge hypodermic needle may be used for collection from puppies kittens. Bone marrow needles have stylet serves to prevent cclusion edle one urrounding issue rted row vity. eedle ypes used for bone marrow collection include **Rosenthal, Illinois sternal Jamshidi needles** Fig. Bone marrow needles are vailable osable roducts.



Rosenthal stylet, Rosenthal needle, Jamshidi needle, Jamshidi stylet, and Illinois needle.



Bone marrow aspiration technique.

Several sites be used for **aspiration biopsy** including head f umerus emoral aseptically repared aped. de site ith erile el lade, edle rted stylet place Fig. Slight pressure be placed on e f edle ylet void lockage needle with bony material. slight caudoventral angle introduces edle umerus. edle ylet are dvanced ortex umerus eached. needle otated orward ressure plied; allows needle to penetrate cortical bone, keeps edle ce. ylet emoved, ringe ched. ringe ually referred to low or reater gative ressure. ringe unger rapidly igorously ithdrawn ew ops lood enter ub edle. on ew ops erial are present hub of needle, pressure be released to e modilution deally, ylet e eplaced edle ce prepared o nsure dequate een btained. l uture eded one row aspirate apidly, de diately r ed

Even when smears are made immediately, placing small f ringe edle efore eginning ollection rocedure lpful. In situations, better-quality greater diagnostic information are obtained core of marrow collected ddition ation iopsy. lways erent for ch ollection hen ollecting ate ore o nsure rocedures roduce tifact into xt ore low chitecture issue o emain ct, rphologic eatures of vidual ells re aspiration resent, asites isible macrophages of bone marrow, will be more easily seen ith **core biopsy** he verall ellularity row ccurately etermined ore ecause uted lood. rocedure for ation iopsy. fter edle roduced cortical one, ylet emoved, edle dvanced approximately inch rotated back forth to cut piece of one om ortex. edle emoved stylet used to expel core through proximal (hub) end f edle. orcing ck ough row nd f edle roduces ressure tifact n imprint be made from before placed formalin. Never place smears from aspirate formalin-preserved specimens, because formalin interfere with tology ecimens

Smears f one row de diately they are mixed with EDTA time of collection. If EDTA een de ollec tion. arrow ate repared eripheral lood. wo are repared vailable dditional esting (e.g., immunofluorescence assay, special needed. ration edle with of pressure from syringe. Bone marrow are ker lood ontain ticles Excess lood emoved ilting e o low lternatively, expelled into Petri bone marrow removed with l ette lary ube. s, ompression be used for bone marrow modified compression technique rovide ompres sion rep thod een idely or ears or preparation tology ecimens echnique to used for coverslip preparation of peripheral blood smears. Bone marrow smears must be rapidly dried stained, ually omanowsky-type must e ncreased ccordance ith he ellularity nd hickness of mears eference oratories ill usually be stained with Prussian blue to identify iron particles row ematoxylin–eosin ften or core iopsy

Recent dvances tochemistry unochemistry ve rovided umber dditional echniques. These echniques volve ecial

with urface ecific ell ypes. They ve efinitive entification ells bone row.

stematic proach hen valuating one marrow one row ver valuated without results of differential white blood cell count from concurrent peripheral blood smear. Stained bone marrow smears must first be examined low-power magnification. This initial xamination is sed to valuate dequacy repa ration. If stained properly or cells are easily distinguished, another slide be obtained. ssuming reparation dequate, w-power xamination rovide ion verall ellularity one marrow. In adult normal bone marrow generally ontains approximately nucleated cells and Marrow samples from uvenile ually hereas eriatric animals ually have marrow onsists approximately 75% he row escribed cellular hypercellular yperplasia), ypocellular ypopla n roportion ucleated ells ersus f resent. dipocytes ved uring fixation f hich esults are n ther haracterized escribing ypes ells present ., ypoplasia, yeloid). eoplasia ossibility all of cells look alike. systematic approach to bone marrow evaluation ummarized rocedure

After overall cellularity determined, examined her nification o o etermine relative percentages of erythroid myeloid cells. method requires ounting lassifying ells. ature gmented neutrophils, eosinophils, basophils are present small numbers

Rubricytes metarubricytes usually comprise to of rythroid ells. etamyelocytes, gmented myeloid cells comprise approximately to of myeloid

200 cells, and calculate the myeloid and erythroid left shift

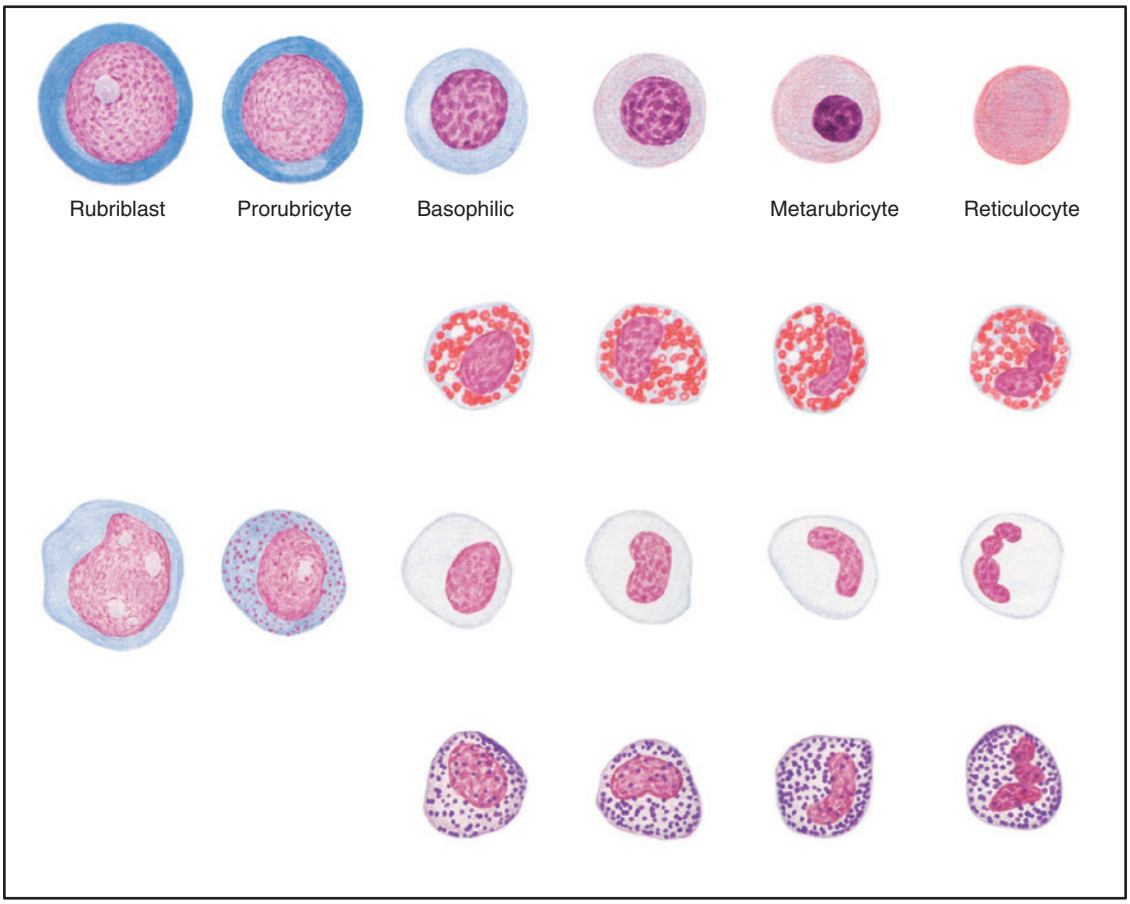
cells. he atio yeloid ells rythroid ells atio) determined by counting nucleated cells classifying them erythroid or myeloid. Normal ratios be between 0.75 0 and Normal alues for the fferential ounting of bone marrow cells vary considerably among different species. Because omplete erential row time onsuming, veral difications erential ell count e ome oratories stem cells o ne ight egories:

1. Immature yeloid
2. Mature yeloid
3. Immature rythroid
4. Mature rythroid
5. Eosinophilic
6. Monocytoid
7. Lymphocytic
8. Plasma ells

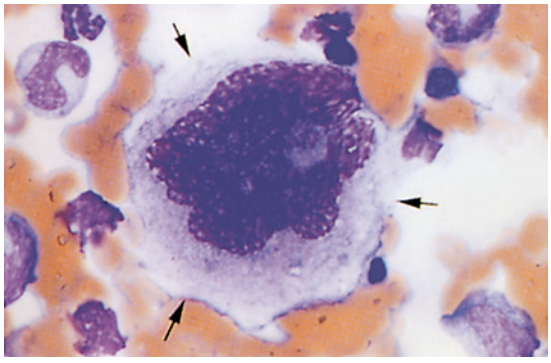
In lassification heme, ure ells lude myeloblasts, romyelocytes, ubriblasts, rorubricytes. Several lternate ystems or he valuation one arrow ells are d. stem volves lassifying ells roups then alculating rythroid aturation ndex yeloid maturation ex. dditional stem ft ex or yeloid rythroid ell erythroid ft ex volves ounting lassifying cells s ither mmature i.e., ubriblasts, rorubricytes, nd arly rubricytes) ure ubricytes tarubricytes) viding ercentage ure ure rythroid cells. he yeloid ex ed viding percentage of all immature granulocytes myeloblast through cell) by percentage of mature granulocytes 200-cell count erential.

Megakaryocytes are evenly distributed bone marrow aspirate. hese ery ells ontain ultiple nuclei ften en lusters, ticularly edges f egakaryocytes umber to er w-power er w-power re ommon. nerally, re er w-power would ell

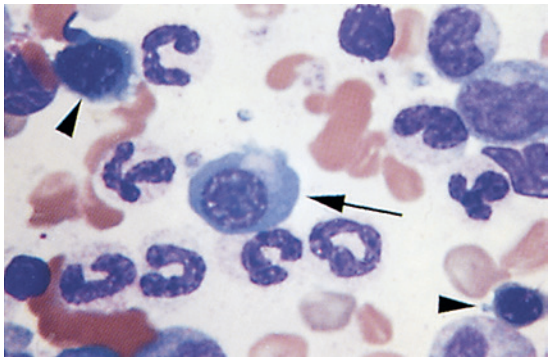
Other ell ypes resent one row include macrophages, lymphocytes, cells, cells, osteo blasts, osteoclasts. Osteoblasts appear to cells except uch er ontain r-appearing nuclear erial. hey end lusters hen they re een amples ollected spirate echniques. lasma cells are slightly larger lymphocytes, with greater nucleus-to-cytoplasm atio. ells ften ve erinuclear area ound ccentrically cated ucleus, ve distinctly basophilic cytoplasm Fig. Inclusions contain immunoglobulin ften resent. lusions om monly eferred ussell odies, ell ontains such lusions eferred ell. eoclasts ontain multiple uclei pear mewhat pearance gakaryocytes, xcept gakaryo cyte ucleus ultilobed. eoclasts en ften from young, actively growing The cytoplasm blue, ontain ranular erial ariable



Maturation of myeloid and erythroid cells. (Drawing by Dr. Perry Bain. From Meyer DJ, Harvey JW: *Veterinary laboratory medicine: interpretation and diagnosis,*



A megakaryocyte in a canine bone marrow aspirate sample.

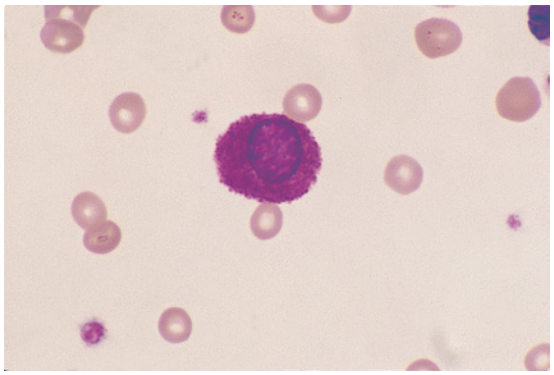


in a bone marrow aspirate from a normal

eep ed. acrophages one row ften contain phagocytized material may diagnosis. In core biopsy macrophages are seen center of clusters of erythropoietic rythropoietic ually disrupted uring ation iopsy. ymphocytes roduced in he one arrow, ut hey re sually resent ow umbers. Immature ymphoblasts rolymphocytes) to distinguish from rubriblasts prorubricytes. Reac tive ymphocytes rmal ure ymphocytes present appear they would they were seen peripheral blood ells haracterized resence abundant, small, metachromatic cytoplasmic granules Fig.

Hemosiderin present macrophages bone marrow, ound ee ells. entifiable ray to lack ranules hen raditional lood Prussian lue emonstrate res ence f on ore. emosiderin ways bone row reparations om ecrease nce hemosiderin ecies.

The esults one row valuation lude, verall ellularity atio, uration ex,



A mast cell in a feline blood smear. (From Valenciano AC,

or left index. If complete differential count of marrow cells ossible, ually escribed rative form, erns rphologic normalities are escribed. hen resent, on mosiderin); increased presence of mitotic figures; increased presence of eoblasts, eoclasts, ells; resence phagocytized erial tastatic ells om rgans ecorded. eported the oncurrent differential ount from eripheral lood smear.

The ate hich rythrocytes wn ill altered me erations ually result f hanges hemical ructure rythrocyte membrane, hich er hysiology mbrane. Generally, his esults endency or he rythrocytes ggre gate re eadily.

The **erythrocyte sedimentation rate** eter mined ariety ys. utomated yzers vailable to erform ommonly ncoun tered eterinary ractices eferral oratories. valu ation f endency or rythrocytes gregate accomplished ect urement roteins globulins, rinogen).

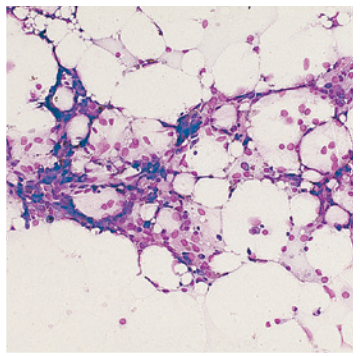
Manual thods or erforming esting volve f rated ube. epending ype ube pretreatment equired. everal ufac turers rovide ubes eded uents orm. The ube led A-anticoagulated lood upper calibration mark. It then placed rack been designed o ube erpendicular ube lowed o urbed ontrolled oom emperature. Manual ill ected ibrations ariations emperature oom. fter utes (or utes or quine vel op olumn ecorded.

The osmotic agility (OF) est provides asure of the RBCs' ability to withstand hemolysis varying concentrations of solution. he ures molysis occurs varying concentrations of solution compared with normal control solution. This test may provide some useful information for differentiating among some forms of anemia dog with diagnosis of hereditary spherocytosis. It outinely erformed eterinary xcept veterinary esearch eferral enters. ured by creating serial dilutions of ranging from to The EDTA-anticoagulated blood added to each dilution centrifuged. Each dilution then evaluated with ectrophotometer etermine ercentage molysis ch ution. esults ed eate rythrocyte fragility ve. xpressed oncentration of hich molysis ccurs. lthough xact relationship between result cell's ability to survive ivo ell haracterized, elationship etween abnormal esults ecreased urvival or ed cells. Increased resistance to hemolysis noted with variety of conditions increase surface-to-volume ratio of red cell (e.g., some forms of liver iron deficiency). Reticulocytes are more resistant to hemolysis because of their greater urface ecreased esistance molysis en ients toimmune molytic asitic infections uch mobartonellosis, ection with okworms.

Chapter eview uestions ppendix

- Reticulocytes ure equire upravital or entification.
- Bone marrow may be collected via core or aspiration echniques.
- A variety of techniques be used to prepare bone marrow with compression being common method.
- When examining bone marrow evaluate cellular mor phologic characteristics to etermine the relative ercentages of ucleated ells dipocytes, elative ercentages erythroid yeloid ells, atio.
- Prussian blue used to evaluate bone marrow for resence mosiderin.
- The results of bone marrow evaluation include, minimum, overall cellularity ratio, maturation index, or ft ex.
- Erythrocyte efers ility hemolysis arying oncentrations ution.
- The ure eed hich rythrocytes ir wn er ontrolled onditions.

Hematopoietic Disorders and Classification of Anemia



After studying this chapter, you will be able to:

- Describe ypes normalities en one row
- Use roper erminology escribe one row
- Describe rocedures or valuation one row
- Discuss lassification ccording erythrocyte
- Discuss lassification ccording tiology.
- Discuss lassification ccording one marrow esponse.

Disorders of the Bone Marrow,
Neoplasia,
Classification of Anemia,
Classification one arrow esponse,
Classification ed lood ell ize emoglobin Concentration,
Classification tiology,
Review Questions,
Key Points,

Aplastic
Chronic granulomatous inflammation
Chronic inflammation
Chronic pyogranulomatous inflammation
Fibrinous inflammation
Hypercellular
Hypocellular
Lymphoproliferative disease
Myeloproliferative disease
Nonregenerative anemia
Regenerative anemia

Hematologic abnormalities be primary or they may be secondary to other disorders. Specific blood cells or all blood cell ypes ected. alterations eripheral lood one row. general understanding of types of disorders diagnostic test results are characteristic of various disorders will help veterinary technician to provide diagnostic-quality test results. Disorders elated lood oagulation resented

Abnormalities en one row lassified hanges ell umbers ell rphologic eatures maturation. haracterized ither eased **hypercellular** or decreased **hypocellular** cellularity of all cell types r eased ecreased ellularity ell ype hen lood ell ypes ecreased marrow escribed **aplastic** n ddition, normal topoiesis hen ellularity rmal ccur. lossary of erms escribe normalities presented ox

Inflammatory onditions vident hen one row ate. onditions lassified according o he rimary ell ypes, hich resent our types: rinous, hronic, hronic ranulomatous, hronic pyogranulomatous. **Fibrinous inflammation** ypically volves infiltration of bone marrow with fibrin exudate without resence ory ells. **Chronic inflammation** hyperplastic condition characterized by increased numbers of ells, ure ymphocytes, ells. **Chronic granulomatous inflammation** haracterized eased numbers of macrophages. If both crophages neutrophils are resent, ondition escribed **chronic pyogranulomatous inflammation**

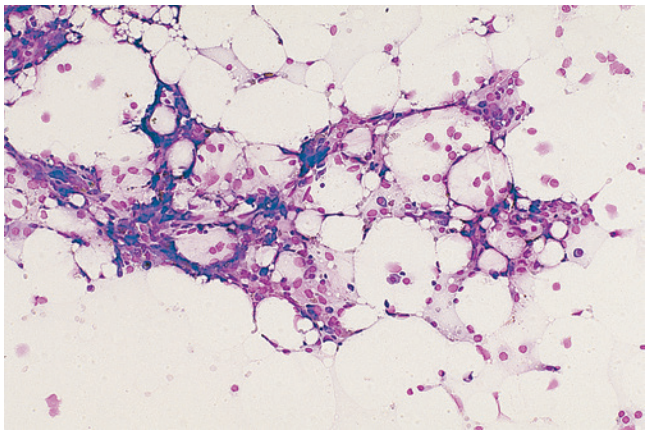


Fig. 13.1 Hypocellular bone marrow from a dog with chronic ehrlichiosis.

hematology of the dog and cat,

Erythroid hypoplasia: Normal or decreased cellularity with a normal or

Presence of intact, viable blood cells within

Megakaryocytic hyperplasia: Increase in numbers of megakaryocytes in

Increased presence of fibrous tissue that displaces hema

Neutrophilic hyperplasia, effective: Neutrophilia in bone marrow and

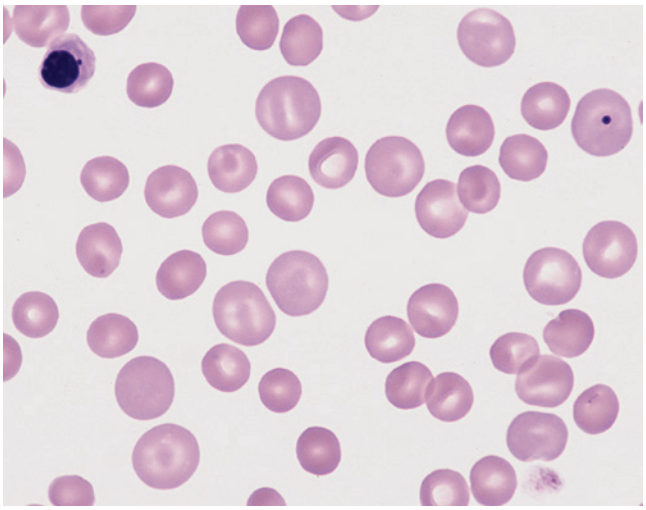
Neoplastic rders matopoiesis lassified ither **lymphoproliferative** or **myeloproliferative disease** The common term d o escribe resence oplastic lood ells bone row eripheral lood ukemia, acterized y redominance ells one row. omprehensive ncology ext onsulted or re details out lassification matopoietic oplasia.

The function of red blood cells to transport protect hemoglobin, hich xygen-carrying ment. production of erythrocytes equals daily from destruc tion f ed ells roduction decreased r estruction eased, esults. Anemia ondition volves educed xygen-carrying capacity f rythrocytes. esult om educed umber of culating educed cked ell olume reduced oncentration moglobin. nemia fied ccording one row esponse ither egenerative or nregenerative ccording moglobin concentration orpuscular olume corpuscular moglobin oncentration uch fications lp entify The eterinarian ill erpret esults oratory esting, other nostics ient's ory physical examination to identify of anemia to determine reatment eded. ecific oratory that re enerally valuated etermine ause nemia re reticulocyte count, erythrocyte indices, morphology, olor urbidity, rotein on centration. dditional laboratory diagnostics are sometimes required luded rum on urement, rum ilirubin measurement, one row valuation.

This ype f lassification ost linically pplicable ecause distinguishes between regenerative **nonregenerative anemia** For common domestic species other equine, bone marrow responds to anemia by increasing erythrocyte production releasing immature erythrocytes. These immature are polychromatophilic eticulocytes bserved on blood enumerated with reticulocyte count to provide indication marrow responsive or regenera tive. he ility one row espond of anemia probably either blood (hemorrhage) or blood destruction (hemolysis). In general, exhibit signs f egeneration out ys om rigger f dequate one row esponse ed hen ercentage eticulocytes qual greater expected percentage for corresponding Table ther ely resent hen iewing lood ell ive egenerative esponse include eased crocytosis, eased olychromasia, Howell-Jolly odies

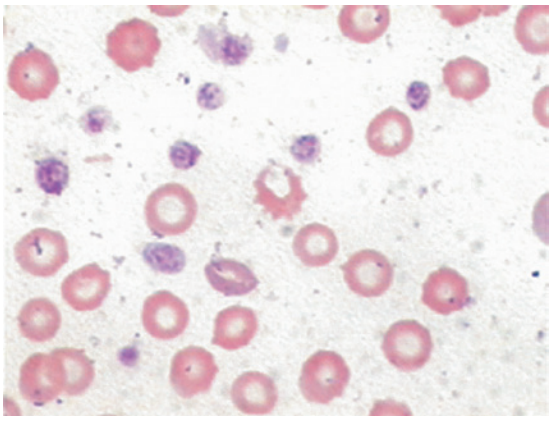
Count Relative to Packed Cell Volume in Dogs and Cats With Adequately Regenerative Anemias

From Cowell R:



Regenerative anemia in a dog with increased polychromasia and anisocytosis. A metarubricyte is present in the upper left of the image,

Veterinary laboratory



Because rses arely elease eticulocytes om one marrow, one row valuation enerally eded mia. one row eticulocyte reater e egenerative esponse rses.

In ients nregenerative one row le o espond eticulocytes absent n lood hich uggests one row ysfunc tion. one row ation iopsy ed er common ndocrine tabolic nregenerative anemia are excluded. Common of nonregenerative anemia include iron deficiency, ehrlichiosis, drug toxicity, histoplasmosis, hypothyroidism, enal ufficiency.

Erythrocyte indices be used to help classify anemia either normocytic rmal crocytic e er rmal), ocytic smaller rmal). ormocytic haracterized of normal size, occurs secondary to variety of acute hronic rders. omestic ommon

f crocytic ransitory size en ith **regenerative anemia** eticulocytosis).

Microcytic nemia lmost lways he esult ron eficiency. The vision ure rythrocytes ops hen itical concentration moglobin eached. dequate on for moglobin xtra vision ccur esult smaller erythrocytes. lthough chronic blood common on eficiency dult dequate dietary on esults on-deficiency ursing uch ens

Anemia y ypochromic educed globin oncentration) rmochromic rmal hemoglobin oncentration). yperchromic sible, ecause rythrocytes ve ed city or hemoglobin. Newly released polychromatophilic erythrocytes reticulocytes) are hypochromic, because full concentration of hemoglobin yet attained. Macrocytic hypochromic anemia suggests regeneration. Iron deficiency results hypochromic anemia, ut haracterized ocytosis Most r ypes rmochromic. ummary anemias lassified rythrocyte cated ox

Anemia lassified molytic mor rhagic, r esult ecreased efective production.

reticulocyte response is mild or if sufficient time has not

Endocrine deficiencies

in cats and dogs with persistent hypernatremia (may be

*The presence of a low MCHC with a low MCV strongly suggests

Hemolytic esult rythrocyte estruction within lood, ually egenerative. uring initial stages, type of anemia usually normocytic nor mochromic, ut ecomes crocytic esult one marrow's release of reticulocytes. Common of hemolytic anemias e cated able

Hemorrhagic esult om cute hronic lood he ient's ory ften etermination ommon of hemorrhagic anemia include trauma, parasites, coagulopathy, neoplasia, stitis, astrointestinal eration.

Iron deficiency be result of nutritionally deficient diet, or may result from chronic blood Erythrocytes are gener ally microcytic hypochromic. Low values are present. The efinitive on-deficiency equire valuation mosiderin one row.

Reduced ates of erythropoiesis or defective erythropoiesis (i.e., dyserythropoiesis) generally result normocytic anemia. Con ditions at result reduced defective erythropoiesis clude chronic enal ypothyroidism, ypoadrenocorticism, bracken ern oisoning, on opper eficiency, vovi rus, d oxicity.

Chapter eview uestions ppendix

- Abnormalities are seen bone marrow be classified changes cell numbers or cell morphologic features maturation.
- Inflammatory conditions evident on examination of bone marrow aspirate are classified fibrinous, chronic, chronic granulomatous, or chronic pyogranulomatous.
- Neoplastic disorders of hematopoiesis are classified either lymphoproliferative or myeloproliferative.
- Anemia generally considered regenerative when per centage of reticulocytes peripheral blood equal to or greater expected percentage for corresponding PCV.
- Blood from patients with regenerative anemias may show evidence of increased macrocytosis, increased polychromasia, Howell-Jolly bodies.
- Common of nonregenerative anemia include iron deficiency, ehrlichiosis, drug toxicity, histoplasmosis, hypo thyroidism, renal insufficiency.
- Anemia can be classified as normocytic, macrocytic, or micro cytic addition to being normochromic or hypochromic.
- Anemia be classified by hemolytic or hemorrhagic, or may be result of decreased or defective production.

Unit Outline

Chapter 14: Principles of Blood Coagulation,
Chapter 15: Sample Collection and Handling,
Chapter 16: Platelet Evaluation,
Chapter 17: Coagulation Testing,
Chapter 18: Disorders of Hemostasis,

Unit Objectives

Describe the processes and pathways that lead to the clotting of blood.
List the components of the blood-clotting systems.
Describe the proper collection and handling of samples for coagulation testing.
Discuss the methods for the evaluation of platelets.
List and describe the coagulation tests commonly performed in the veterinary practice laboratory.
List and describe the coagulation tests commonly performed in the veterinary reference laboratory.
List and describe common inherited coagulopathies.
List and describe common acquired coagulopathies.

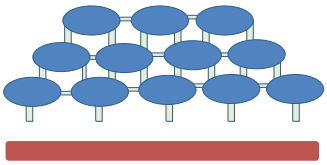
Hemostasis (i.e., blood clotting) involves multiple complex and interrelated processes. A variety of coagulation disorders can be seen in veterinary practice. A basic understanding of the processes involved in blood clotting is essential to ensure accurate test results.

A number of tests can be performed in the veterinary practice laboratory, and many of these tests do not require specialized equipment. Coagulation analyzers are also available for some tests and can be cost effective for the veterinary practice laboratory.

Normal values for blood coagulation tests are included in Appendix B

For additional sources for this unit see the Resources Appendix at the end of this textbook.

Principles of Blood Coagulation



After studying this chapter, you will be able to:

- Explain iew
- Describe ell-based del
- Explain elets iation oagulation.
- Describe on illebrand ctor lood coagulation.
- Discuss ormation oagulation omplexes.
- Describe ombin
- Discuss ormation rin egradation roducts D-dimers.

Overview of Blood Coagulation,
Coagulation Testing,

Review Questions,
Key Points,

D-dimers
Fibrin degradation products
Microparticles
Phosphatidylserine
Thrombin
von Willebrand factor

Hemostasis ility ody's stems integrity of blood blood vessels. It involves number of complex pathways, platelets, coagulation factors. ny altera tion parameters result bleeding disorder. In erms, oagulation lood roceeds ough mechanical hemical chanical initiated when blood vessel ruptured or torn. The exposed blood vessel subendothelium charged surface, platelets are attracted o urface. elets ongregate undergo rphologic hysiologic hanges. hanges cause the atelets to adhere each other well as to blood vessel endothelium. The adhesion of platelets to each other to ndothelium equires **von Willebrand factor** hich serves o ilize elet dhesion gregation elets elets elease iating ctor or hemical chemical hase eferred oagulation de, volves umber oagulation ctors Table classical iew parates hemical rinsic extrinsic pathways. Each factor participates chemical reaction rves o iate eaction way. result f oagulation de ormation fibrin rands orms involves egradation rin

It important to note coagulation pathways are inter related, interdependent, partly cell-based. The initial mechanical phase initiated by interactions of negatively charged phospholipid urfaces ells elets **microparticles** Microparticles are membrane-bound cytoplasmic fragments are released from platelets, leukocytes, endothelial cells serve to increase surface area on which coagulation complexes orm. issue ctor actor initiate coagulation reactions, Factors through serve to de. xtrinsic way ctually rves help initiate intrinsic pathway. small of **thrombin** enerated uring ecruits activates elets rinolysis. hen elets activated, **phosphatidylserine** (PS) is exposed outer surface of mbrane. elets elease esicles om surface during activation. These microparticles are enriched Phosphatidylserine acts binding site for complexes of coagulation de, hich ctivate actor rothrombin

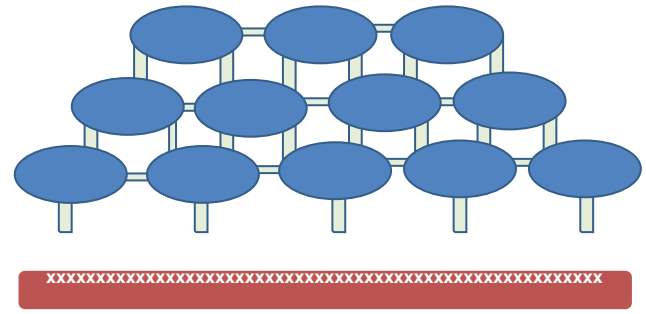
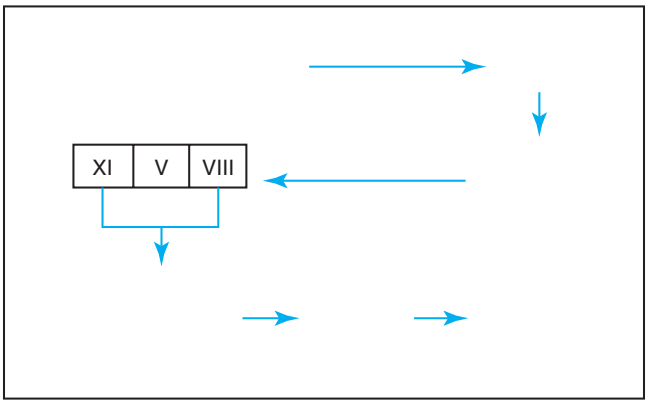
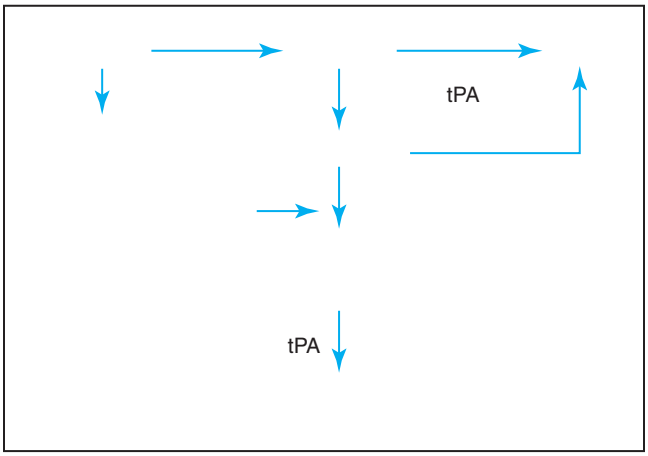


Diagram of a stabilized platelet plug.



The initial reactions of the chemical phase of hemostasis.



hemostasis and the breakdown of fibrin.

(Factor espectively. ctivation actor esults eneration ombin thrombin ontinues ecruit ctivate re elets triggers onversion rinogen rin.

Activated platelets expose phosphatidylserine on

The generation of fibrin proceeds through two phases, with soluble orm eing enerated ially, ollowed eation of n nsoluble orm hat onsists ross-linked brin trands. The oagulation rocess dulated esulting clots roken own ough ries errelated eactions. presence of tissue plasminogen activator (tPA) uble rin roken own **fibrin degradation products** (FDPs). Plasmin ct the insoluble fibrin to roduce oss-linked ersion **D-dimers** . lthough brief description given here seems complex, actual processes are great deal more complicated involve numerous dditional rum roteins. eader efer to ecommended eadings or re epth of mostasis. ummarizes iew chemical hase lood oagulation rocess.

Various coagulation tests have been developed to evaluate specific portions f mostatic chanisms. ome ure just chanical hereas rs measure pecific arts he hemical hase. ll atients hould be evaluated for coagulation defects before undergoing surgery. Most coagulation tests be completed with time equipment elatively xpensive.

Chapter eview uestions ppendix

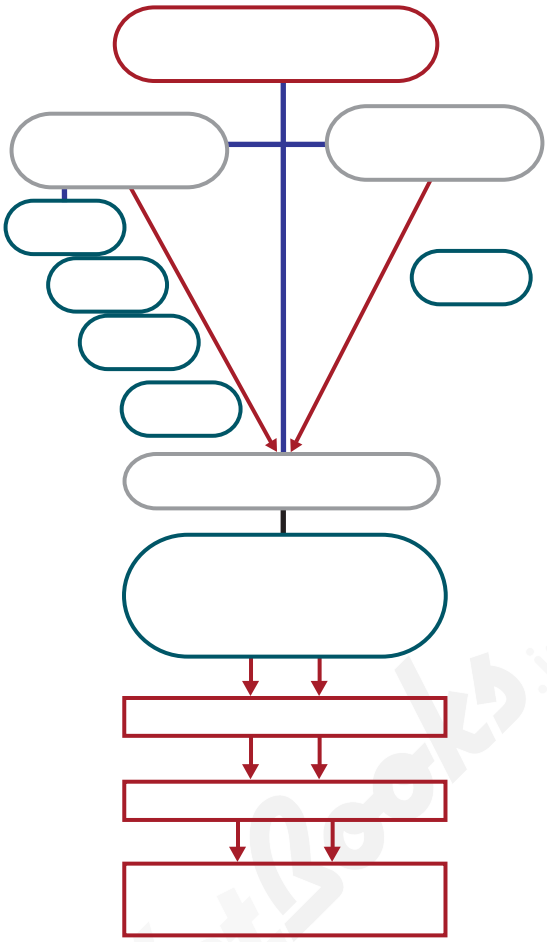
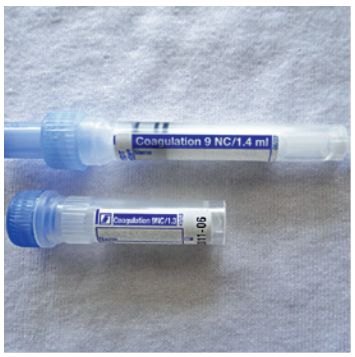


Fig. 14.4 A simplified summary of the chemical phase of hemostasis.

- Hemostasis requires platelets, number of coagulation factors, complex reaction pathways.
- Hemostasis proceeds through mechanical chemical pathways.
- The term mechanical hemostasis refers to aggregation adhesion of platelets to exposed blood vessel endothelium.
- The mechanical chemical phases of hemostasis are inter related interdependent.
- Activated platelets expose phosphatidylserine on their surface release microparticles contain phosphatidylserine.
- Coagulation complexes attach to phosphatidylserine on surfaces of microparticles platelets.
- Thrombin serves to enhance recruitment activation of platelets.
- Fibrinogen converted first to soluble form then to insoluble form.
- The breakdown of fibrin requires tPA.
- Fibrin broken down into soluble FDPs, insoluble FDPs, D-dimers.



Sample Collection and Handling

After studying this chapter, you will be able to:

- Describe roper ollection rocedures or coagulation esting.
- List icoagulants or lood oagulation tests.
- Describe thod etermine roper atio blood o rate icoagulant.
- Discuss roper or oagulation testing.
- Describe rumentation vailable or oagulation testing eterinary ractices eferral oratories.

Fibrometer
Hypercoagulable
Hypocoagulable
Monovette
Thromboelastography

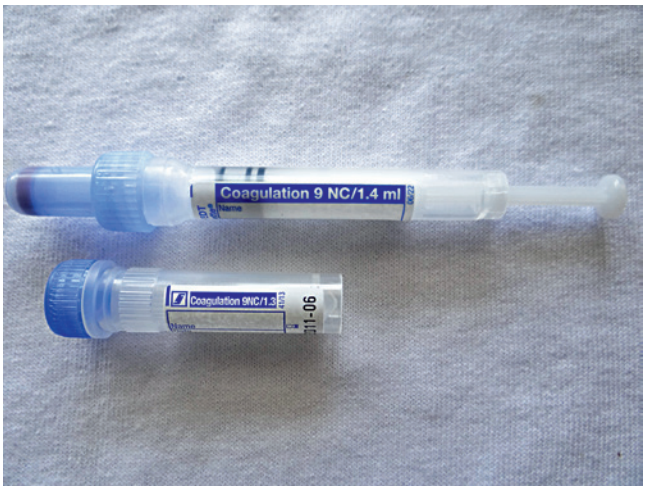
Blood or oagulation ollected fully, ith issue enous Patient xcitement elet ddition to ctivating elets. ncreased vels on illebrand ctor Factors occur. In addition, prolonged venous stasis can ctivate platelets and rigger rinolysis. Effects occur oth itro ivo.

Samples ver ollected ough welling eters, ecause rinogen, rin, elets are generally found around catheter. of best ways to eliminate elet ctivation acutainer or **Monovette** . ather ringe edle collect The preferred anticoagulant for coagulation ests dium rate. itrate upplied orm, ollected rate ution uted platelet ounts one rated corrected or ution. elet gregates orm eadily dog blood collected citrate. Ethylenediaminetetraacetic acid A) referred icoagulant or elet Citrate icoagulant ypically tions or lood ollection orage or ransfusions. for whole blood clotting time activated coagulation time do equire icoagulant.

Samples for coagulation testing are mixed with

Samples ust collected the roper order when multiple types f eing awn. eview hapter of ook or orrect rder aw. rate ube generally awn ontaminated el activators r icoagulants om ubes.

The proper ratio of citrate to blood part citrate to parts whole lood. itrate vailable oncentrations. hese icoagulants ill rovide erent lotting results. Samples be collected with citrate concentration used o oratory eference anges. roper atio of lood o icoagulant chieved acutainer



tube, rovided ube
patient is not anemic, polycythemic, or dehydrated. The volume of citrate to based on expected volume. Conse quently, lood dded atio, ill be ercitrated, hich ill esult rtened polycythemic ill vercitrated, hich ill esult prolonged clot times. Citrate volume be adjusted accord ingly or ients normalities ed ell he olume rate equired ed following quation:

%])

After een ollected, eled transported apidly oratory. ubes room emperature, emain ightly ped, ept right. Vibrational rauma voided. performed ollection. lternatively, entrifuged or utes. ithdrawn ithout urbing elet yer with he se oncontact ipette. lasma ay hen rozen ic ubes. ed ped they arrive frozen be thawed immediately before testing.

Automated yzers or oagulation esting vailable, some are relatively inexpensive. utomated analyzers are preferred over ual thods or erformance analyzers are designed to evaluate specific parts of coagulation process. ome erform ultiple yses hemical of mostasis, rs esigned ecifically valuate platelet tion.

Analyzers have been designed to evaluate chemical hemo ve chanisms everse icoagulant hich collected. Some make of liquid reagents are added to the sample. Others ontain eagents in cartridges to which dded. yzers nitor or formation ither chanical ptical stems.

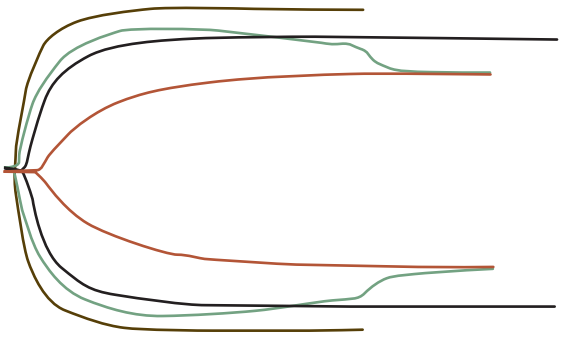
The oag nalyzer dexx oratories, estrbook, erforming ariety oagulation involve lood rate-anticoagulated blood . yzer tridges ontain required eagents or he ests. vailable ests nclude rothrom bin ime ctivated tial omboplastin ifferent versions of test cartridges are used for citrated for hole lood

Sample dded tridge, yzer back forth through internal channel. Light-emitting diode ptical etectors valuate ate lood flow, hich ill ecrease hen orms. yzer been alidated or eline prothrombin een alidated or quine

Although longer widespread **fibrometer** may still be encountered some veterinary referral practices laborato ries. he miautomated yzer perform umber oagulation rated ient ced propriate eagent awn o ette ched hen reagent ensed om ette, imer riggered, ops ires The ires ve ck orth ough lot etected.

variety of automated analyzers are available to perform **throm boelastography** Fig. They may vary considerably design specific reagents needed. Some make of fresh whole blood, others involve of citrated In general, yzers ontain hich eagent added. he yzer valuates ntire lotting rocess,

Thromboelastography machines. (Haemonetics Corp., Braintree,



from ormation ough rinolysis. results e ecorded rovide ure required or ormation, valuation rength lot, equired or reakdown

The results are usually provided graphically used to identify whether ient **hypercoagulable** **hypocoagulable** . here idespread reement eterinary practitioners egarding ysis. hrom bocytopenia ecreased ohematocrit emonstrate hypercoaguability. urrent esearch rovided efinitive evidence egarding hether itro ivo Heparin used either for anticoagulation or patient treatment may erfere esults. ariations esults e vident hen diately yzed. Specific protocols for veterinary are well described. Each oratory evelop wn rotocol xpected normal anges ollection rocedure elapsed ime erform esting.

Several yzers vailable or elet adhesion aggregation. The PFA-100 analyzer (Siemens Palo lto, een alidated or

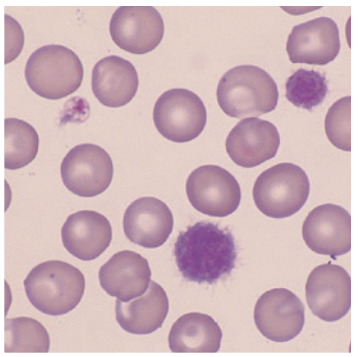
The yzer osable tridge ontains ollagen-coated mbrane erture. lood drawn ough erture, elets dhere brane. hen ufficient umber elets ve dhered aggregated, blood longer flow through aperture. The time equired ecorded.

Other analyzers are used to evaluate platelet aggregation cretion elet ctors. here ide ariety principles ew ve een alidated eteri nary ecies.

Several dditional yzers vailable or coagulation esting, ut ve een alidated or eterinary ecies. hey equently mer gency departments and physician offices. Some are available for uman ients eceiving icoagulant rapy monitor ir oagulation

Chapter eview uestions ppendix

- Blood or oagulation esting ollected efully ith rauma void riggering lotting mechanisms.
- Patient excitement excessive venous alter coagu lation est esults.
- Most oagulation volve dium-citrate–anticoagulated
- EDTA-anticoagulated are preferred for evaluation of elet umbers.
- For oagulation esting, roper atio rate lood t rate lood.
- A variety of automated analyzers are available to monitor for clot ormation ither chanical ptical stems.



After studying this chapter, you will be able to:

- Describe thods or ounting elets.
- Describe elet imation thods.
- List escribe elet
- Define ombocytopenia, ombocytosis, thrombopathia.

Mean platelet volume
Platelet distribution width
Platelet–large cell ratio
Plateletcrit
Thrombocrit
Thrombocytopenia
Thrombocytosis
Thrombopathia

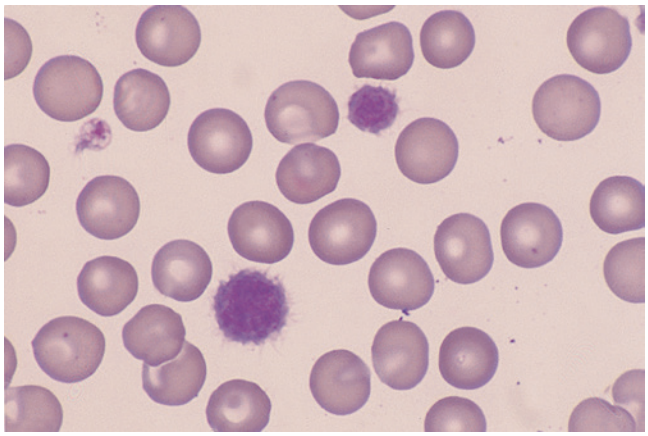
Platelets are small cytoplasmic fragments are shed from mega karyocytes the bone marrow. Methods for platelet valuation include he latelet ount, latelet ndices, nd latelet unction testing. The majority of platelet evaluations are completed with automated yzers. **Thrombocytopenia** ecrease circulating elet **Thrombocytosis** ease circulating elet **Thrombopathia** efers o normal platelet tion.

Platelets ounts erformed tomated matology ers. Some automated counts be highly inaccurate result of platelet clumping platelet/red blood cell overlap. freshly collected ethylenediaminetetraacetic acid (EDTA)–anticoagulated blood hen erforming elet Results from automated analyzers must be verified by viewing peripheral blood cell The previously used manual counting system from Becton–Dickinson Unopette system) longer manufactured, but several alternative products are available. hese hamber ube ontains premeasured olume uent hich dded. Platelets e ounted mocytometer o escribed or eukopet hapter

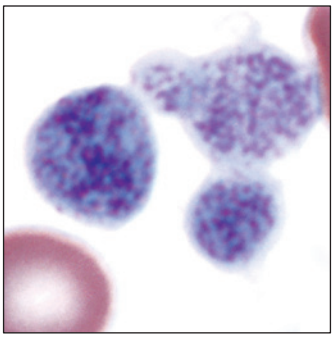
Unit orphologic hanges elets lude gregation iant elets normalities ill evident with automated analyzers must therefore be detected erential lood

Indirect urements elet umbers imates) performed with of differential blood described Chapter of Unit Platelet numbers be evaluated monolayer area of blood The numbers of platelets minimum of microscopic fields be counted. Gener ally, o elets er il-immersion en rmal patients. owever, umber ary reatly epending ld f iew oscope ultiplying mated atelet umber veraged over 10 lds) 15,000 or ect ure elet

Patients ith ombocytopenia ve er rmal platelets gaplatelets). owever, rmal ogs



A giant and a slightly enlarged platelet in a canine patient.



The intense basophilia of these macrothrombocytes suggests

routinely ound ave egaplatelets hen ample iewed on lood or erential lood ell These e ften ounted rythrocytes tomated ers. ll lood valuated or resence platelet lumps. eticulated elets epresent wly eleased platelets, ontain vels hey are gous eticulocytes emonstrate one marrow esponsiveness. ecialized entify numerated tometry.

Some tomated yzers rovide valuation elet vidual elet **platelet distribution width** (PDW). The methods differ depending on type of analyzer. In addition to PDW, platelet indices include measurement of **plateletcrit** (PCT) **mean platelet volume** (MPV). Some analyzers may provide **platelet–large cell ratio** (P-LCR). Depending yzer, ect urements, or they may be calculated from other reported values. Note although yzers eport ameters, usefulness as een ell ocumented or eterinary pecies.

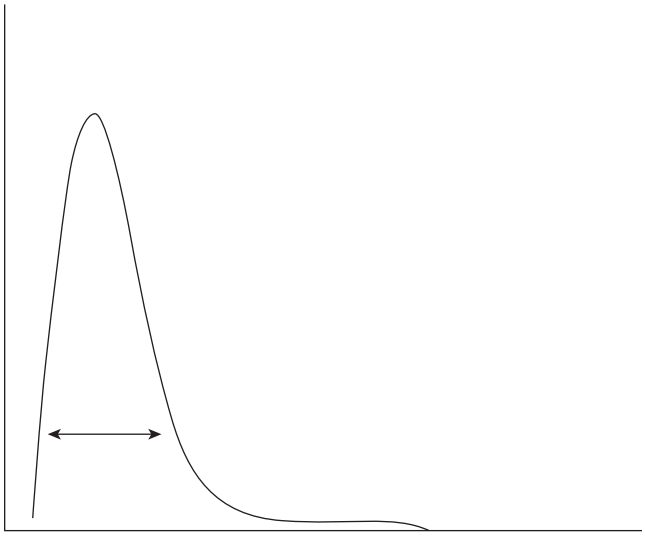
The , hich ured emtoliters, mati verage vidual elets ounted analyzer. n eased xpected uations which eased estruction, onsumption lets accompanied by megakaryocytic hyperplasia. ccelerated thrombopoiesis ends esult elease er elets; however, end ve er elets, alue may be useful for feline patients. Certain breeds, such Cavalier King Charles Spaniels, have larger platelets other breeds. Some automated analyzers may count larger platelets white lood ells.

h ogs dequate one row response. owever, ormal ow PV hrombocytopenic dogs does predict inadequate bone marrow response. Dif ferent results may occur depending on anticoagulant specific analyzer used. Exposure to EDTA been demonstrated to elets ell. ome ve emonstrated much olume uring er ollected. owever, ve een agreement. Similar results have been seen with citrated Studies f edance thods ve emonstrated increases with time, whereas laser flow cytometry methods ve een wn ecord ecreased When erforming rial valuations, elapsed ime er enipuncture.

The eletcrit, hich eferred **thrombocrit** measure f ercentage lood olume comprised of platelets. It comparable to packed cell volume that is ecorded for ed lood ells. This ue generally eter mined y ultiplying elet or alue ypically

PDW ariations elets. any to mated yzers rovide ogram rovides isual evaluation f er elets en patients ith ombocytopenia. elet ered depending ecently elet eleased om bone row. elet eased hen elets are ctivated. owever, ariations elet ccur normal ients, een ell orrelated bone arrow esponsiveness ypercoagulable tates. imilarly, some yzers eport alue or elet–large ell atio, which ure ercentage elets er rmal.

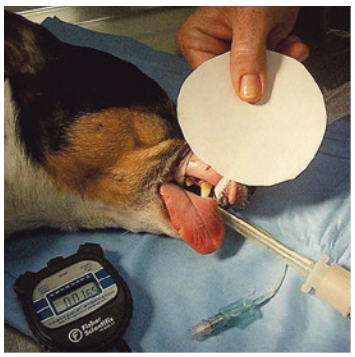
Thrombopathia, eration elet tion, assessed ariety ys. utomated yzers hapter provide evaluation of ability of platelets to aggregate crete elet ctors. ther ures elet tion are ect hapter



Veterinary eference eferral oratories ffer ariety platelet valuations enerally ractical or erformance ractice oratory. lude iplatelet antibody assays. ntiplatelet antibody assays are immunoassays designed o entify ibodies ve dhered urface of elets.

Chapter eview uestions ppendix

- Platelet valuation ludes elet elet ests f elet tion.
- Platelet estimates be performed variety of ways lood om erential lood ell
- Specialized tests for reticulated platelets antiplatelet antibodies e erformed eference oratories.
- Platelet ndices nclude lateletcrit, latelet istribution idth, elet olume.



After studying this chapter, you will be able to:

- List ommonly erformed lood oagulation.
- Describe rocedure or erforming uccal ucosa bleeding ime
- Describe rocedure or erforming ctivated clotting ime
- Describe rinciples ctivated tial thromboplastin rothrombin
- Describe rocedure or erforming precipitation rinogen

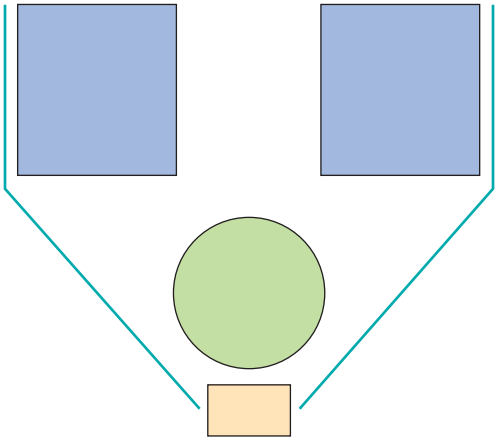
Buccal Mucosa Bleeding Time,
Activated Clotting Time,
Whole Blood Clotting Time,
Activated Partial Thromboplastin Time,
Prothrombin Time Test,
Clot Retraction Test,
Fibrinogen Determination,
PIVKA,
D-Dimer and Fibrin Degradation Products,
Von Willebrand Factor,
Coagulation Factor Assays,
Review Questions,
Key Points,

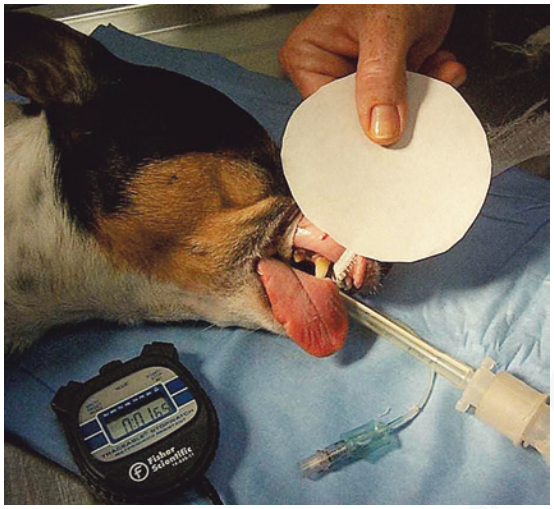
Activated clotting time
Activated partial thromboplastin time
Buccal mucosa bleeding time
Clot retraction
D-Dimer
PIVKA
Prothrombin time tests

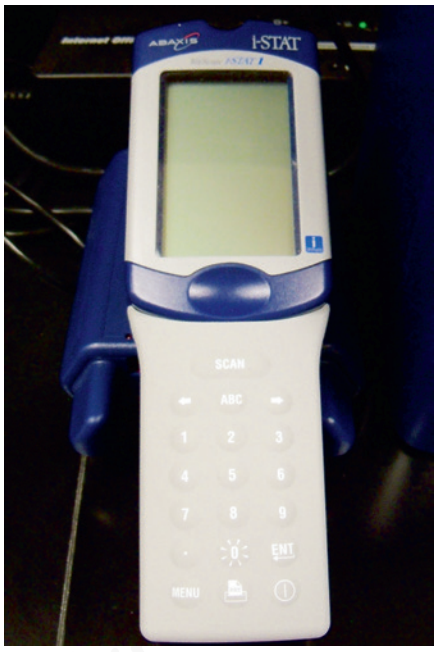
Tests f he lood oagulation echanisms re esigned valu ate ecific oagulation rocess ome require specialized instrumentation, whereas others are performed manually. he thods referable esult variability erformance. hen thods ital onsistent proach eference anges e lished eterinary oratory f ecific upplies rocedures any tests ill e ered hen elet umbers w. elet count erformed entify resence om bocytopenia, hich validate esults.

Tests of coagulation are designed to evaluate specific

The **buccal mucosa bleeding time** T) rimary for etection normalities elet tion. requires spring-loaded lancet (e.g., Surgicutt, SimPlate), blotting paper r er er, opwatch, ourniquet rocedure lways evice hen erforming because evices er egard epth, number f de. ient dated anesthetized ced eral ecumbency. rip auze d o ie per ck xpose ucosal urface. An incision made with lancet, standard blotting paper or er er one y htly ouching er op lood allowing lood rbed ithout ouching sion. he lood ill icked way om lotting epeated very conds leeding opped. normal leeding or omestic utes. prolonged leeding ccurs elet ysfunction syndromes well with deficiencies von illebrand factor. It ill e rolonged ients ombocytopenia, elet ount erformed. ome ients ill olerate est hen tized. owever, ient







The i-STAT analyzer has cartridges that are used for the per formance of coagulation tests.

method or erforming equires acutainer ube contains dditive uch omaceous triggers ctivation oagulation ways. ube must e rewarmed er block. enipuncture performed, of blood is ollected directly o ube. imer ted on lood enters ube. ube ed nce entle version placed or er ube bserved conds cond ervals or resence clot. Normal values are approximately to seconds. Severe thrombocytopenia platelets/mL) abnormalities associated rinsic oagulation de rolong activated lotting utomated yzers vailable for erformance esting

must e lowed urb ongue, sedation ually equired.

The **activated clotting time** (ACT) test can valuate every clini cally nificant lotting ctor xcept ctor

The hole lood lotting hich erformed ee–White thod, er rinsic ting chanism. lood lotting om monly erformed, ecause re nsitive. erformed ollecting lood ringe, noting ime lood pears ringe stopwatch. Immediately, of blood placed each of ee ubes ve een inse The ubes ced er first n cond ubes ilted cond ervals until oagulation ccurs. ube ilted manner. he lapsed etween pearance lood ringe ormation ube lotting time. he rmal lood lotting or ogs minutes; or rses, utes, or to utes.

The **activated partial thromboplastin time** T) valuates rinsic ommon lotting chanisms. epending on ecific yzer thods equire ultiple reagents. he oag yzer analyzer erforms rothrombin T est. itrated ed ctiva tor f ctor elet ubstitute ephaloplastin). fter ddition orm rin determined. ariety cquired reditary rders, addition to administration of heparin, reduce one or more factors cessary or rmal rinsic oagulation cascade.

Many coagulation assays are performed with auto

This analyzer is capable of performing fibrinogen assays as well as other coagulation tests, chemical analyses, and electrolyte evaluations.

Prothrombin time (PT) **tests** which are referred to one-stage rothrombin ually erformed by tomated yzers. valuates xtrinsic common oagulation ways. volves f rated hich issue omboplastin reagent added. reagent been designed to recalcify dded. nder rmal onditions, form ithin conds. rmal ange or ogs conds. rolonged rothrombin ciated with severe liver disseminated intravascular coagulation r reditary cquired eficiencies ctors extrinsic coagulation cascade. The test sensitive to vitamin eficiency onism uch farin oxicity.

f ne ube etermined, ually efractometer. The cond ube ed or utes. second ube ecentrifuged, ured. The otal rams er eciliter ultiplied obtain concentration milligrams per deciliter. The fibrino gen imate ed ollowing equation, ith alues iven ligrams er eciliter:

/

The **clot retraction** rocedure s lder est hat llows or he evaluation of platelet number function of intrinsic extrinsic ways. thod volves awing lood erile ube ube ube xamined utes eexamined eriodically over eriod. vident utes, retracted approximately hours, markedly compact hours. bnormalities test result do provide information regarding ce oagulopathy.

Automated thods or rinogen etermination volve photometric analysis are commonly performed veterinary practice. utomated electrochemical methods are available for veterinary ractice oratory thod y e or rinogen imation volves two matocrit ubes. ubes entrifuged be for determination of packed cell volume, total

The acronym **PIVKA** refers to proteins are induced (invoked) by nce equired ctivate coagulation actors hen cient, recursor roteins actors e etected IVKA esting hrombotest (Axis-Shield oC, slo, orway). he est ay elp ifferenti ate rodenticide toxicity from primary hemophilia when activated clotting time prolonged. It slightly more sensitive test rothrombin hen ependent ctors are epleted. IVKA esting ecome rolonged hours f estion icoagulant odenticides, hereas prothrombin rolonged ctivated partial hromboplastin ime rolonged ithin ours. ome controversy exists regarding usefulness of test, many practitioners refer rothrombin ro longed, IVKA rovides dditional ormation clinician. f reatment ient volves istration of vitamin PIVKA test must be performed prior to iation reatment.

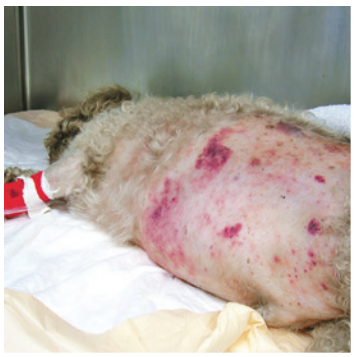
Both D-dimer fibrin degradation product tests are used to evaluate tertiary hemostasis fibrinolysis). **D-Dimers** fibrin degradation products (or fibrin products) are formed lot egraded. refore identifying presence of to provide diagnostic infor mation ver ure, rauma, mangiosarcoma. vailable or ysis. rin degradation roducts ys. Several ypes vailable, ut volve ex agglutination methods (see Unit The D-dimer test consid ered more specific sensitive for evaluation of fibrinolysis, because rin egradation roducts roduced efore lot orms.

von illebrand ctor equired or elet dhesion. When platelet function defects are evident, assay gener ally erformed. here veral ypes unoassays vail able eference oratories uantify Additional tests are available for evaluation of function.

Deficiencies clotting factors occur with variety of hereditary cquired onditions er ariety oagulation test esults. ssays entify ecific ctor deficiencies erformed eference oratories, are enerally erformed entify ecific reditary ctor deficiencies. he ajority hese ssays nvolve he se ho tometric rinciples.

Chapter eview uestions ppendix

- Coagulation valuate ecific oagulation pathways.
- Most oagulation ys erformed tomated analyzers.
- Buccal mucosa bleeding time provides evaluation of platelet number tion.
- Coagulation factor assays are performed reference labora tories hen ecific ctor eficiency entified.
- vWF valuations erformed eference oratories immunologic thods.



After studying this chapter, you will be able to:

- Describe ypes mostatic rders en veterinary ecies.
- Differentiate etween reditary cquired efects hemostasis.
- List escribe ommon mostatic disorders.
- List ommon rited rders oagulation ecies reeds ually ected.
- Describe chanisms volved minated intravascular oagulation.

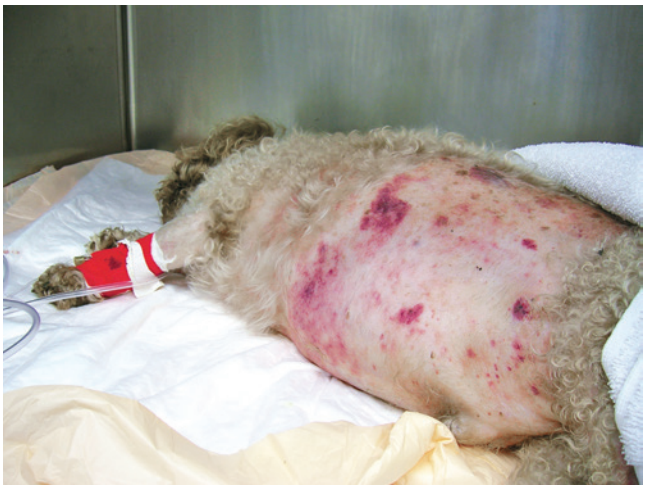
Disseminated intravascular coagulation
Hemophilia
von Willebrand disease

Bleeding sorders ay aused ongenital cquired efects coagulation proteins, platelets, or vasculature. Most bleeding disorders ound eterinary pecies re econdary ome ther process. Primary coagulation disorders are rare, they are ually esult rited efect roduction of oagulation ctors. igns ongenital cquired eficien oagulation roteins ually volve elayed eep-tissue hemorrhage hematoma formation. Clinical signs associated with ongenital cquired efects eficiencies elets include uperficial etechial cchymotic morrhages pistaxis lena, rolonged leeding injection nd ncision ites. ith unctional efects eficien oncentration oagulation roteins, usually pear efore eaches majority of congenital coagulation factor disorders veterinary species volve eficiency normality ctor.

TECHNICIAN NOTE Clinical signs associated with defects or deficiencies of platelets include superficial petechial and ecchymotic hemorrhages, epistaxis,

Coagulopathies rited lude ariety ctor deficiencies. ome ommon rited oagulation ctor ders f eterinary ecies ed able lthough diagnosis of specific factor deficiency or defect requires testing completed eference oratory, umber oagulation tests e erformed eterinary ractice oratory diagnosis. Factors are components of intrinsic coagulation pathway. Tests designed to evaluate intrinsic way ctivated lotting ctivated tial thromboplastin ill ely emonstrate normal esults.

Hemophilia common inherited coagulation factor



Petechiae may signify a coagulation abnormality. (From Sirois

deficiency ogs, actor eficiency. Hemophilia hich led hristmas esults from actor eficiency. emophilia volve X-linked ecessive raits.

The ommon rited oagulation rder omestic **von Willebrand disease** he esults from ecreased eficient roduction on illebrand factor. on illebrand ctor lycoprotein circulates ith actor tions elet aggregation iation oagulation ways. occurs with relative frequency Dobermans, been eported ozens reeds ell rabbits swine. Several distinct forms of have been identified n erns ritance Table ype ws tosomal-dominant ritance pattern ith omplete enetrance, haracterized low vels f culating rmal ructure. affected y ype ve culating vels their normal ructure tion. ype vWD s haracterized he ear bsence ny WF. ype inherited ominant rait; ype rited tosomal-recessive raits. ogs ith ypes nd WD end xperience severe bleeding. Prolonged bleeding during estrus, after venipuncture, er urgery ommon buccal ucosa leeding ime rolonged. pecific enetic ests are vailable or ype ype on illebrand Affected or reeding.

Plasma Von Willebrand

With Known Mutations

Enzyme-linked immunosorbent assay results: normal

From Battaglia A: *Small animal emergency and critical care for*

Coagulation Disorders of Dogs

Great Pyrenees, English Springer spaniel

Coagulation disorders result from decreased production or increased destruction of platelets well from nutritional deficiencies, ver estion ertain tions r oxic ubstances. ver rimary or production oagulation ctors. ny ers ver function esult oagulopathy.

Thrombocytopenia efers ecreased umber elets, ommon oagulation rder en veterinary practice. The of platelet deficiencies are often unknown. However, infection with certain bacterial, viral, and arasitic gents an esult hrombocytopenia Table 8.3). Thrombocytopenia ccur esult one row depression educes roduction elets, result from autoimmune increases rate of platelet destruction. riety edications ave lso een mplicated ombocytopenia ox spirin cetaminophen are common toxins encountered small patients. These medications estroy ermanently elets, esolve ed platelets egin eleased om one row.

Vitamin equired or ctivation coagulation ctors. itamin-K–dependent ctors lude Factors eficiency ccur esult f tary ufficiency uct bstruction. ny ers ctivity leeding orders. he estion oxic ubstances farin, sweet lover) eate leeding rders. nticoagulant rodenticide oxicity ignificant ause econdary emostasis l eterinary ractice. arfarin veral compounds are found rodenticides have anticoagu roperties. pear or veral ys after estion lude thargy, rexia, yspnea result of bleeding into thoracic cavity. Ecchymosis, petechiae, marthroses ccur. leeding rain

Continued

*Refer to Greene CE: *Infectious diseases of the dog and cat,* ed 3, for more information on diagnostic tests, available test kits, specimens, and commercial diagnostic laboratories.
Available at the North Carolina State University, Tick-Borne Disease Laboratory, Raleigh, NC.

Thrombocytopenia in Dogs and Cats

ord esult urologic rothrombin time enerally oagulation ollowed by ctivated tial omboplastin ctivated lotting time. The PIVKA est has also been suggested as agnostic Patients e ually econtaminated hen estion wn to e ecent. rapy metimes iated require veral eeks or uccessful reatment.

Although entity on own, **disseminated intravascular coagulation** associated with many pathologic conditions. ften en rauma ell

Disseminated Intravascular Coagulation

Viremia (infectious canine hepatitis, feline infectious peritonitis, African swine

Protozoal parasites (babesiosis, trypanosomiasis, sarcocystosis, leishmaniasis,

many ectious umber vents rigger ox ontains ummary ommon ondi tions associated with The resulting hemostatic disorder may manifest stemic morrhage ovascular ombosis. Microthrombi esult issue ypoxia, ormation of ombi onsumes elets oagulation ctors, hich leads o eased endency or morrhage. rinolysis microthrombi leads to formation of excess fibrin degra dation roducts rs. ck ccur.

Because riggering vent esulting rder diverse, oratory hly ariable. here single test be used for diagnosis, all tests exhibit abnormal esults ients. ients ve prolonged activated partial thromboplastin time prothrombin time ell ombocytopenia. histocytes often resent lood rinogen rmal decreased. Buccal mucosal bleeding time prolonged, fibrin degradation roducts rs enerally eased. Table contains summary of laboratory seen with ell ommon mostatic rders.

Chapter eview uestions ppendix

Expected Laboratory Test Results for Common Bleeding Disorders

Activated clotting time; activated partial thromboplastin time; buccal mucosa bleeding time; fibrin degradation products;

Kirk & Bistner's handbook of veterinary procedures and emergency treatment

- Bleeding disorders may be caused by congenital or acquired defects coagulation proteins, platelets, or vasculature.
- Clinical signs of bleeding disorders include delayed deep-tissue hemorrhage, hematoma formation, superficial petechial and ecchymotic hemorrhages, epistaxis, melena, prolonged bleeding injection incision sites.
- The most common inherited coagulation disorder of domestic vWD.
- Thrombocytopenia refers to decreased number of platelets, common coagulation disorder seen small veterinary practice.
- Vitamin-K–dependent factors include Factors
- Thrombocytopenia be result of wide variety of conditions, including infection with certain bacterial, viral, parasitic agents. It may be caused by bone marrow depression or autoimmune
- DIC consumptive coagulopathy occurs secondary to other conditions.
- Clinical signs laboratory results are highly variable for patients with

Unit Outline

Chapter 19: Basic Principles of Immunity,
Chapter 20: Common Immunologic Laboratory Tests,
Chapter 21: Blood Groups and Immunity,
Chapter 22: Intradermal Testing,
Chapter 23: Reference Laboratory Immunoassays,
Chapter 24: Disorders of the Immune System,

The objectives for this unit are:

Describe the physiology of the immune system.
List the components of the immune system.
Describe the functions of the various immune system components.
Describe commonly performed tests that are used to evaluate the immune system.
Discuss disorders of the immune system.

The science of the detection and measurement of antibodies or antigens is called serology or immunology. Detection depends on the binding of antibodies and antigens. Unfortunately, this binding phenomenon is ordinarily invisible. Visualization—and thus detection—of the antigen–antibody reaction depends on sec ondary events by which the union is easily detected and therefore of diagnostic use in the veterinary practice.

The commercial production of monoclonal antibodies to many different antigens has resulted in a variety of test kits for use in the veterinary laboratory. These specific antibodies to many different antigens can be produced and used in the laboratory for the rapid identification of disease-producing organisms.

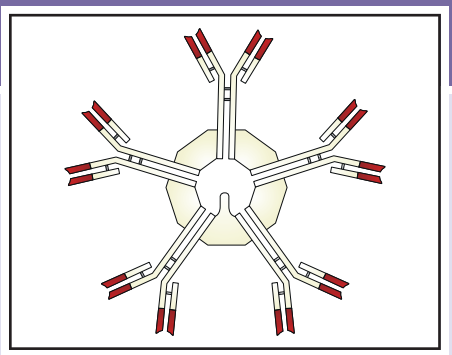
Immunization with viruses, bacteria, or other entities stimulates antibody production in an animal. The antibody-secreting, transformed lymphocytes (plasma cells) may be isolated from the animal and chemically fused with a type of "immortal" cell that propagates indefinitely, such as mouse myeloma cells. The antibodies that these hybrid cells produce, which are called monoclonal antibodies, are collected. Because each monoclonal antibody attaches to only one specific part of one type of molecule (antigen), the use of these antibodies in diagnostic kits makes the tests specific and greatly reduces interpretation problems of the results. For example, the feline leukemia virus antigen only reacts with the feline leukemia virus antibody. A specific reaction is diagnostically significant for this complicated disease. In addition to their specificity, these procedures allow for the rapid identification of the pathogen.

Many serologic tests involve the use of monoclonal antibodies. Enzyme immunoassay, latex agglutination, immunodiffusion, and rapid immunomigration are methods that are used in veterinary laboratories. Methods such as complement fixation, immunofluorescence, immunoelectron microscopy, virus neutralization, and polymerase chain reaction DNA amplification are used in veterinary reference laboratories and research facilities.

Reference laboratories offer myriad serologic tests specifically developed for veterinary samples. Tests for blood typing, allergies, bovine leukemia virus, reproductive hormones, Lyme disease, and brucellosis just are a few of the diagnostic tests currently available.

For additional sources for this unit see the Resources Appendix at the end of this textbook.

Basic Principles of Immunity



After studying this chapter, you will be able to:

- Differentiate etween stem adaptive stem.
- Describe omponents stem.
- Describe quence vents omprise system ory esponse.
- Discuss tokines esponse.
- Differentiate etween umoral unity cell-mediated unity.
- List unoglobulins, structure rimary ch.
- Define *immunologic tolerance.*
- Describe arious opulations ymphocytes lymphocytes, ch system.
- Differentiate etween ve ctive unity.

Innate Immune System,
Adaptive Immune System,
Humoral mmune ystem,
Cell-Mediated mmune ystem,
Immunologic olerance,
Passive Immunity,
Immunization,
Key Points,

Active immunity
Antigen
Avidity
Cell-mediated immune system
Complement system
Humoral immunity
Immunoglobulin
Immunologic tolerance
Inflammatory response
Interferons
Natural killer (NK) cells
Opsonization
Passive immunity
Phagocytosis
Vaccination

Vertebrate ecies ve ernal efense stems: innate, or nonspecific, immune system adaptive, or spe cific, mmune ystem also alled cquired mmunity). **Antigens** are y ubstances enerating esponse from stem.

Foreign odies uch cteria, iruses, asites, first ncounter riers stem. barriers lude hysical iochemical ompo nents pharynx, ungs, enitourinary ract; opulations ommensal cteria ompete invading gens ody's **inflammatory response** The ory esponse esponse ection issue injury. lerted by chemicals released from infected site, blood vessels e low utrophils issue, here they phagocytize bacteria kill pathogens with chemicals stored toplasm. ion edness, elling, tion. ch elated o hysiologic chanisms ce uring inflammatory process. Inflammation protective mechanism of e stem, ut verreact ctually issue

Monocytes follow neutrophils to inflammatory sites. Here, like utrophils, estroy rt ticles, iruses, bacteria, cellular debris by **phagocytosis** In blood, they are led nocytes, ut hen rate arious issues rgans eract ecific tokines, ecome macrophages. acrophages erived om tissues. hey cated onnective issue, ver, rain, lung, spleen, bone marrow, lymph nodes, together they make nonuclear hagocytic stem.

In ddition hagocytic ells, **natural killer (NK) cells** **interferons** **complement system** e ortant om ponents f stem. ells ymphocytes, ut ather, ubset ympho cytes ound lood eripheral ymphoid rgans. ells ecognize estroy ells ected microbes, uch iruses. hey ctivate hagocytes releasing erferon- nterferons tokines uble ro teins creted ells diate esponses) other ellular eactions, uch revention iral eplica tion, nce ctions ells. hey active daptive esponse.

The complement system consists of group of proteins found blood. Collectively they are referred to complement, they are integral both innate adaptive systems. hen activated, series of chemical reactions known complement cascade occurs. The system be activated through one of three pathways, ut er eps or ways. components of complement system are numbered through ith me ving veral ubunits esignated tters.

The lassical way, hich chanism daptive immune stem, ctivated hen igen–antibody complex. The other pathways of complement activation are part of innate immune system, they are triggered by microbial surfaces lectins bind to microbes Fig. ll ee ways yze ries eactions other omplement ve umerous hysiologic effects. These include **opsonization** of microbes to promote phagocytosis. psonization efers omple ment o igen. omplement ctivation esult imulation ion ell ysis ormation of mbrane ck omplex urface igen.

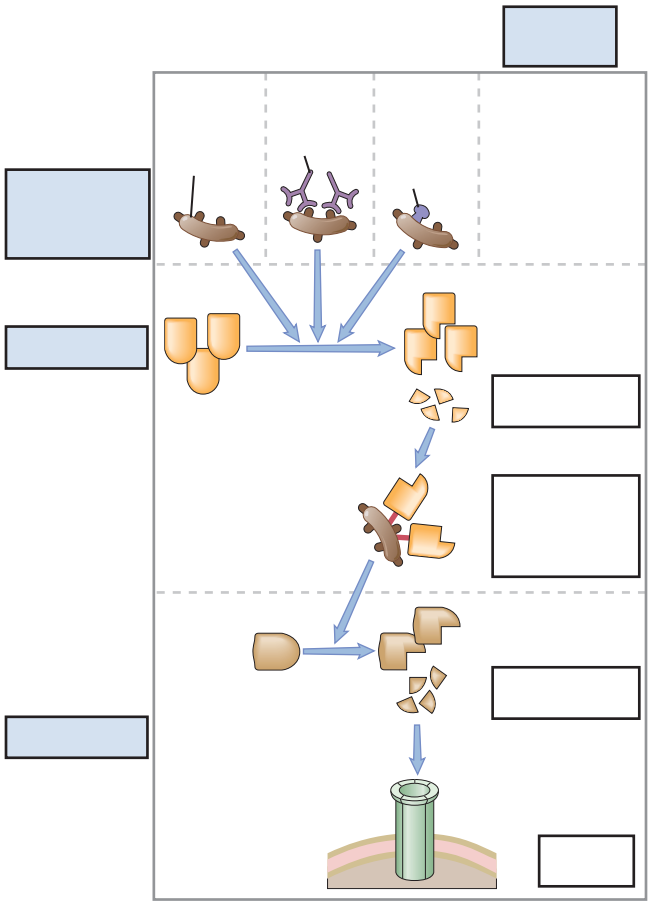
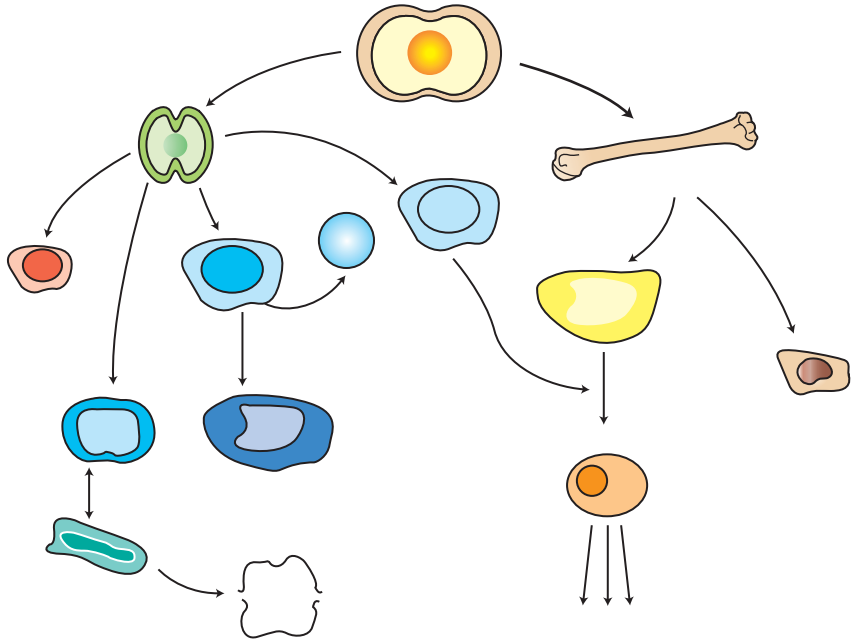


Fig. 19.1 Pathways of complement activation. The activation of the

If oreign odies vade stem, encounter daptive stem, hich re phis ticated. he daptive stem vided om ponents: umoral stem **cell-mediated immune system** he daptive stem ility to espond ecifically oreign ubstances. ubstances, which e led igens, cterial, iral, asitic, or al, r ered ndogenous ells 's body. heir resence iates umoral ellular esponses utralize, etoxify, liminate oreign erials from

Lymphocytes rogeny ell ypes ely responsible for adaptive immune system. However, of defense s ot ivorced rom he nnate mmune ystem. acro phages process antigens present them to antigen-committed lymphocytes. ther ords, hey ct ntigen-presenting ells.

Lymphoid stem cells develop first yolk sac then fetal liver. The bone marrow assumes responsibility parturition rves ce ells oughout postnatal e. ymphoid em ells estined ther mature one of two places: bone marrow or thymus. lymphocytes mature bone marrow, whereas lymphocytes mature ymus

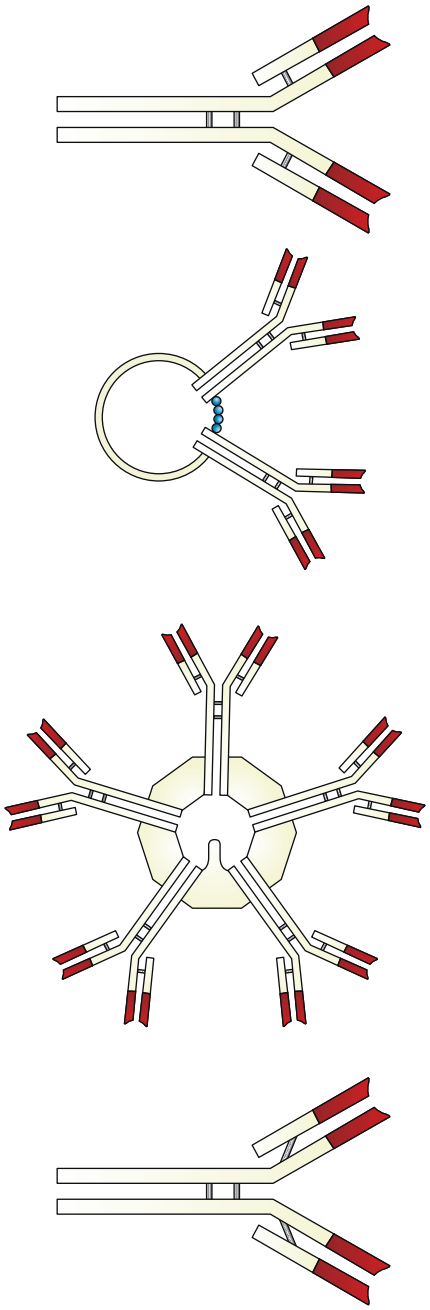


Lymphocytes mature bone marrow cells) are con cerned hiefly roduction cretion **immuno globulin** g) cules, hich wn ibodies. This referred to **humoral immunity** because antibodies are secreted into body's or humors." Their maturation process onsists ee ymphoblast, rolym phocyte, ure ymphocyte. ure ells ve one row ed condary ymphoid rgans en ymph des), here ncounter igens. The umoral stem ecognize illions er ent igens ecause, ells ure, ch ell evelops specific receptor molecule to specific antigen. hen antigen enters ody, ure ell ommitted ticular igen ill eact imulation ell to produce antibodies complex process requires help of specialized lymphocytes called helper cells. Helper cells produce roteins led tokines ctivate ells. antigen-stimulated cell then quickly divides differentiates, thereby producing clone of identical cells all produce type of antigen-specific antibody. These antibody-secreting ells e led ells, hich ype ffec tor ell. n ffector ell ell stem performs ecific tions estroy oreign igens. ome of igen-stimulated ells erentiate mory cells, hich espond er cond xposure igen (see .

Antibodies unoglobulins) rotein consist of two pairs of polypeptide chains configured shape Fig. ch ontains ariable egions one onstant egion. ariable egions egions) igen, onstant egion egion) esponsible for ue tions erent ibody

Five distinct classes of immunoglobulins are produced: IgM, IgG, gE, gA, gD. ibody ype roduced response o igen entameric contains five monomers), comprises approximately f culating unoglobulin. elatively therefore nter issue ces. ibody produced esponse igen, ften escribed high-titer, low-avidity antibody. It abundant early stages following xposure igen wer er es. **Avidity** efers o rength antigen ibody tly esult gM or ecific igen. ing mmunoglobulin gG. omprises pproximately 5% circulating unoglobulin, emains culation for ngest elatively nomer capable of entering tissue spaces, usually produced during condary esponse. w-titer, h-avidity antibody. gE ually resent ery structure to IgG. IgA comprises approximately of circulating antibody, IgD monomer when present, ery w undance.

TECHNICIAN NOTE The most abundant circulating immunoglobulin is IgG.



Immunology and immunopathology of domestic animals,

Table ummarizes tions immunoglobulins.

Antibodies interact with antigens different ways to prevent antigenic chment vasion ody ells. utralization antibody reaction occurs when antibody binds directly with antigen. For example, foreign microbe or microbial toxin bound y ibody, ect ody ells. essentially utralizes otential ffect igen. ome times antibodies coat microbes. The Fab region of antibody attaches o eceptors obial urface. egion antibody then binds to macrophages or neutrophils, microbe hagocytized. igenic erial parasite, gE ibodies psonize orms; wever, ead of hagocytosis, hich ffectual worms, osinophils estroy asites. hen complement ctivated ibodies, esult antigenic ell ysis.

Precipitation eactions ccur hen igens bodies orm uble omplex. recipitate orms on urfaces, recipitant gy. or example, recipitation cterial agments merular mbrane esult lomerular phritis, hich described hapter ype ypersensitivity eaction.

Lymphoid stem cells mature thymus develop into T-cell lymphocytes. ells, uration rocess onsists three morphologically distinct stages: lymphoblast, prolympho cyte, ymphocyte. ells ure, evelop receptors to specific antigens become immunocompetent or antigen-committed ymphocytes. ome eferences efer cells stage naive lymphocytes. Then, after contact with their specific antigens, cells proliferate differenti ate o ither lones mory ells lones ffector ells against igens.

Memory ells ecognize igens hich ve revi ously een xposed. ubsequent ncounter, more apid esponse.

Different ypes -effector ells uch lper ells cells) cytolytic cells cells). The terms efer o urface kers on helper cytolytic lymphocytes, respectively. Cytolytic lymphocytes are called cytotoxic lymphocytes. Human immunodeficiency irus hich irus acquired immunodeficiency syndrome special affinity or lper ymphocytes.

Helper lymphocytes recognize antigen been phago cytized y igen-presenting ell uch cro phage. he ys ortion igen urface resents lper ymphocyte. imulates helper ymphocyte elease tokines. tokines urn, e hemical ther phagocytize ested obe. elper ells, hen imu lated, elease tokines ells erentiate antibody-producing ells.

Cytolytic ymphocytes ecognize igen ticles on urface ected ody ells yse l ected ells. icrobe-infected ells, umor ells, ells f oreign issue raft liminated manner e

f ortant eatures system oes estroy wn ells. em obvious, ut ctually pen. aturing ymphocytes develop igen eceptors or oreign igens ut or animal's igens wn ells. herefore, lf-reactive lymphocytes could attack self-antigens. However, healthy animal, echanisms ormally re lace hat revent his elf-destruction. stem iminate etween n-self, hich esults **immunologic tolerance**

Invading microbes typically immunogenic; they will inter act ith ir ecific ve ymphocytes, hich rolifer ate erentiate ffector ells estroy oreign microbes. owever, olerate lf-antigens, elies on mechanisms such antigenic tolerance ignorance. Self-antigens e rmally olerogenic; ymphocytes ither unable o espond hen ncounter lf-antigens rgy), or y hen ncounter lf-antigens optosis). elf-antigens y nored ve ymphocytes, hich lf-antigens led nimmunogenic.

These mechanisms are elaborate. hen naive lymphocytes are destroyed by apoptosis, immune system effect selecting for eneficial ymphocytes ve eceptors or oreign antigens eliminating self-lymphocytes would self-destruction. This called negative selection, takes place one row, ymus, eripheral ymphoid issues.

Another mechanism of immunologic tolerance occurs through ctivity egulatory ymphocytes. ome ymphocytes, formerly led uppressor ells, ecome egulatory ympho cytes. Regulatory cells prevent self-reactive lymphocytes from differentiating ffector ells. hey estroy self-antigens.

This oversimplification of intricacies involved maintenance of immunologic tolerance. hen mechanisms autoimmune results, animal's immune system ected

Animals gain **passive immunity** to by receiving maternal antibodies colostrum or by receiving preformed antibodies by ction. ibodies ve een roduced onor onor accinated gen. hen rum ibodies each oncentration, bled, lobulin ortion ontains ibodies separated ified. rotection eceives from ction unoglobulin rt ved ut immediate.

Animals become actively resistant to by having developing antibodies or by being vaccinated or immunized, hich evelop wn ibodies. referred to **active immunity** Immunization, or **vaccination** accomplished by injecting suspension of microorganisms into for purpose of eliciting antibody response but The microorganisms may be either attenuated (weakened but still alive) or inactivated (killed). ttenuated vac normally longer-lasting more potent immune response. nactivated accines enerally er ve ability to although vaccine-associated sarcomas have been issue. adjuvant may be added to vaccine to enhance normal immune response. Some adjuvants do by simply slowing rate of antigen elimination from body igen resent nger imulate ibody ro duction. Killed vaccines require more adjuvant, increased adjuvant been implicated one of potential of sarcomas.

Effective DNA vaccines are now being developed with of cular enetics. hey xpected er re stable raditional accines, de re quickly. he accines volve ect roduction ody issues quence epresenting igen to hich stem esponse esired.

Vaccines iven ubcutaneously ramuscularly, depending accine. ther accines erosolized given intranasally, some vaccines are put feed or drinking water. eterinary echnicians ork cheries vaccinate tting accine er.

- Vertebrate species have two major internal defense systems: innate or nonspecific immune system adaptive, or specific immune system called acquired immunity).
- The innate immune system includes physical biochemical components nasopharynx, gut, lungs, genitourinary tract; populations of commensal bacteria compete with invading pathogens body's inflammatory response.
- Cytokines are chemical messengers produced by variety of cells interact with components of immune system.
- Five classes of immunoglobulins are produced by cells. Each class specific role immunity.
- The complement system made of series of chemicals interact with cells of immune system.
- Passive immunity involves maternal antibodies colos trum or injection of preformed antibodies.
- Active immunity involves introduction of vaccines to stimulate immune response to specific antigen.

Common Immunologic Laboratory Tests

After studying this chapter, you will be able to:

- Discuss nsitivity ecificity elate immunologic
- Describe ollection rotocols or immunology esting.
- List ypes nostic vailable or eterinary ractice oratory.
- Describe rinciple esting.
- Describe rinciple ex glutination esting.
- Describe rinciple unomigration esting.

Sample Collection and Handling,
Handling Serologic Samples,
Tests of Humoral Immunity,
Enzyme-Linked mmunosorbent ssay,
Competitive me-Linked mmunosorbent ssay,
Latex gglutination,
Lateral w mmunoassay mmunomigration
Immunochromatography,
Immunology Analyzers,
Chemiluminescence,
Key Points,

Chemiluminescence
Competitive ELISA
Enzyme-linked immunosorbent assay
Immunochromatography
Immunodiffusion
Lateral flow immunoassay
Latex agglutination
Rapid immunomigration
Sensitivity
Specificity

Immunologic tests performed veterinary practice labora tory are designed to detect specific infectious agents. The tests are provided n it orm hat ontains ll he eagents, ipettes, reaction chambers needed to complete evaluation rapidly ith ffort. owever, eful ention to uality ontrol nsuring ccuracy esults.

Tests e valuated or **sensitivity** **specificity** Sensitivity refers to ability of test to correctly identify all ruly ositive or iven eaction rocedure. large umber gatives tivity. ecificity ure umbers ositives produced with given reaction procedure. No test provide nsitivity ecificity.

rectly identify all animals that are truly positive for a given reaction procedure.

Nearly all serologic tests require serum or Whole lood nostic oratory when serum or specified. The practical method of collection acutainer System (Becton Dickinson, Franklin Lakes, hich ommonly vailable om eterinary dical upply ompanies. ed-topped acuum ube used when serum required, lavender-topped tube used to collect heparinized specifically requested, reen-topped ube

Reference oratories ve rict equirements oncerning specimen ype, uality, ling. ertainty oratory ontacted or ecific etails. or ch

test, he equirements hould ead arefully, nd xactly hat requested be submitted. If blood to be col lected ringe, ringe auge edle combination hould sed, ecause auses he east emolysis.

When rum ubmitted, lood lowed clot or o utes oom emperature, centrifuged for minutes speed of faster pm. If rum parated er entrifuging, rimming" tube with wooden applicator stick to loosen clot may help; however, may hemolysis. If desired, y entrifuged diately er ollection.

After entrifugation, ette ate serum or upper layer) off of packed erythro cytes. The aspirate placed into transfer tube or another able est ube learly eled. rum tested diately ozen efrigerated or er thawed, e-frozen or er esting ithout compromising

Samples for serologic tests do need to be frozen, but they be shipped cold, especially during weather. The major problem with shipping tubes breakage. The tubes must be cked mly ce cking erial move around when package jarred. Each must be learly orrectly eled ertinent erwork enclosed o cilitate roper eporting esults om laboratory.

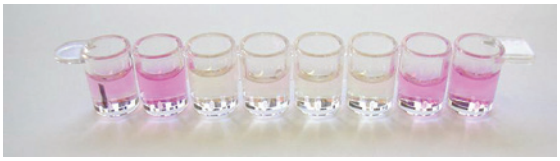
Enzyme mmunoassay, **latex agglutination, immunodiffusion, rapid immunomigration** e thods vailable or veterinary ractice oratories. thods ve een dated or esting entify umber ecific igens. Additional unoassays orporate thods detect ertain lood omponents, uch oagulation ctors,

The **enzyme-linked immunosorbent assay** een adapted o ents ommonly ested or eterinary laboratory Figs. to monoclonal antibodies, specificity of meaning cross-reactivity occurs with r ents. henomenon ccurate way o etect ecific igens iruses, cteria, asites, hormones) rum. or antibody rum, hich ontains specific igen. ome vailable etect worms, eline ukemia irus, eline unodeficiency irus, vovirus, rogesterone oxes or igen-detection stem, noclonal ibody bound o ells ray, mbrane, or o ic ntigen, resent ibody cond nzyme-labeled ibody added to help with detection of antigen. This followed by insing. itical ep rocess. ells

A critical step in the Microwell enzyme immunoassay is washing away the unbound enzyme-labeled antibodies.

Fig. 20.2 A kit used to detect feline leukemia antigens in cat serum

(Courtesy Zoetis Inc, Florham Park, New Jersey.)



With the enzyme-linked immunosorbent assay wells format for the determination of progesterone in canine serum (Ovuchek Premate),

chromogen to the membrane. This reacts with the enzyme on the

he bulbous ends of plastic wands come precoated with antibodies specific

reagent reacts with the enzyme-labeled antibodies that are bound to

fter incubation, the wells are rinsed to wash away excess enzyme-labeled

bound to the antibodies on the test wells is antigen from the patient, and

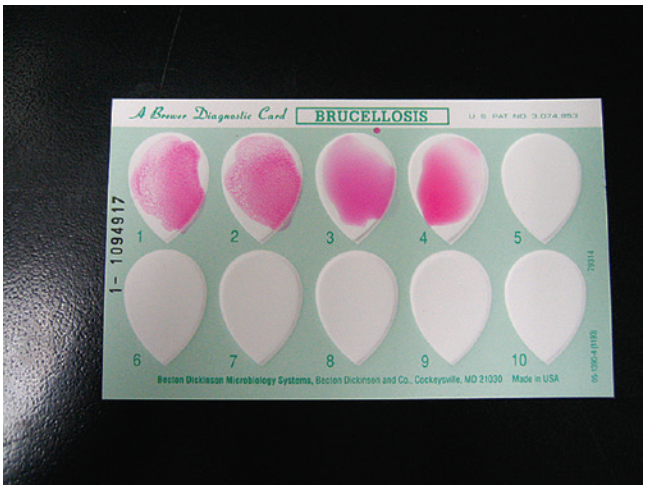
the antibodies being tested) and the patient sera (with possible antibodies)

roughly ositive esults ccur. hen chromogenic (color-producing) substrate added to mixture, eacts ith nzyme evelop ecific olor, reby indicating resence igen contained antigen, entire enzyme-labeled antibody would be d way uring rocess, olor eac tion ould evelop.

rocedure or ibody etection. During this procedure, antigen bound to wells, membrane, or ient yed or resence ecific ibody.

The **competitive ELISA** when used to test for patient antigen, volves nzyme-labeled igen ell noclonal ibodies. atient igen, resent, ompetes with nzyme-labeled igens or ibodies oat test ells. olor eveloper eacts nzyme roduce olor. he ensity olor roduced aries concentration ient igen ox quine ec tious mia ibodies etected rse rum est.

The ex glutination herical, ex particles are coated with antigen suspended water. If



plexes. Samples 1 and 2 indicate a positive reaction. Samples 3 and 4, which show no clumping, indicate a negative reaction.

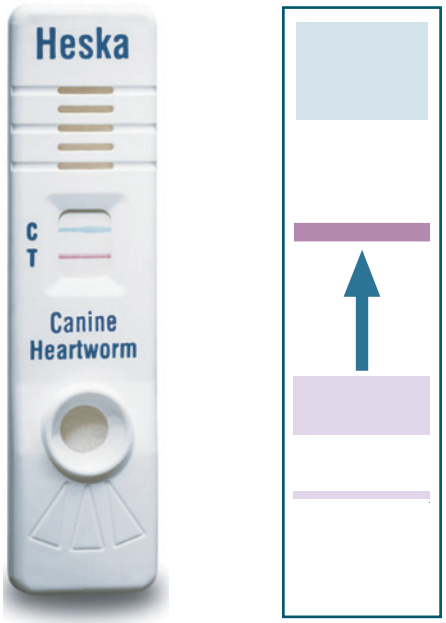
serum contains corresponding antibody added to mixture, ormation ibody–antigen omplexes agglutination (clumping). gglutination changes appearance of latex suspension from smooth milky to clumpy because ex ticles ve lustered oss-linked ogether. ibody resent ure ex serum emains venly ersed. rength eaction be raded 1, 2, r o rovide ion igen resent. ommon method or ovine rucellosis ibodies ox

It ortant gatives ccur hen excessive igen ibody resent n resence xcess ibody, ossible each ibody nly ibodies oes glutination oes ccur. This wn rozone henomenon. xcess igen result lack of cross when excess antigen surrounds any small clumps may form. This known postzone phenomenon. It important to note prozone postzone phenomena ccur haracteristics ient r ccur rrors erformance uch roper etting echniques esult propriate quantities f eagent dded stem.

leukemia virus. (Courtesy Zoetis Inc, Florham Park, New Jersey.)

Lateral flow immunoassay een escribed immunomigration **immunochromatography.** he signal-generating omponents olloidal enzymes, olor eagent glutinated ex ticles. ll three ypes omponents eate ositive esult.

In ype rocedure, nal-generating omponent onjugated ibodies ecific or igen eing ested. These onjugated ibodies resent mbrane est tte here ient plied. igens are resent ient onjugated antibodies, ibody–antigen omplexes rate mbrane tte, here esults are read. Buffer may be added to help with the grational of antibody–antigen complexes. In reading area, second antibody present membrane. If antigen captured—along with first antibody conjugate—by cond ibody. ccumulation onjugate area olor hange. nsure uality esults, ontrol antigen resent mbrane rip. conjugated ibody igen ontrol area. ts ccumulation olor hange ccurs whether r igen resent ient tive ient esult ws olor hange: or patient or uality ontrol igen. ontrol antigen ea oes hange olor, onsidered invalid, egardless olor hange ient



Immunochromatography. A sample that contains antigen flows through a porous strip. Positive reactions are shown by the appearance

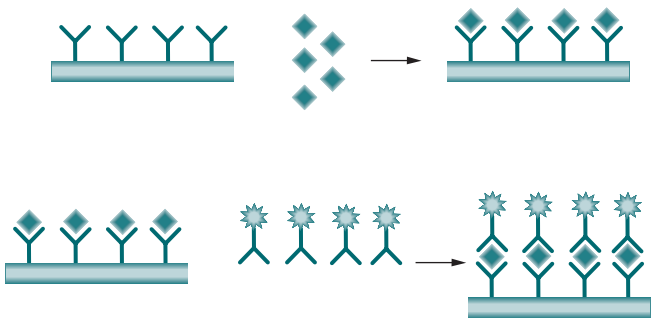
ox

Automated analyzers are available for veterinary practice laboratory. In some analyzer required to run certain tests. Large reference laboratories often have automated analyzers for performance of immunoassays. These analyzers are capable of performing tests on numerous ients simultaneously. Most are ompletely utomated nd equire nly he atient nforma tion ntered yzer's omputer ient loaded. mall unology yzers enerally ype nly ead esults. echnician repares test device and places evice lyzer. The esults ead yzer propriate

antigen is present in the patient sample, it binds to the conjugated anti

reading area of the membrane contains capture antibodies to the test

Many tomated yzers tilize rinciples **chemilumi nescence** to detect quantify specific antigens. The principle to method except test substrate reacts to produce light rather enzyme reacts to produce olor roduced etected by hotomultiplier, roduced be uantified. hemiluminescent unoassay y plications tries. n ddition unology esting, een for etection uantification ubstances, luding thyroid hormones, cortisol, pancreatic progesterone, testosterone.



Format for chemiluminescent immunoassays. (From Turgeon

Chapter eview uestions ppendix

- Sensitivity efers ility orrectly en tify l ruly ositive or iven eaction procedure.
- Specificity measure of numbers of positives pro duced ith iven eaction rocedure.
- Serum or are required for immunoassays.
- Many f unoassays eterinary ractice laboratory tilize thod.
- The ex glutination herical latex ticles oated igen uspended er to etect ibody
- Lateral ow mmunoassay lso eferred mmunochro matography
- With eral thods, ontains antigen ws ough orous rip, ositive eactions are wn y pearance olored

Blood Groups and Immunity

After studying this chapter, you will be able to:

- Describe arious lood roup igens large
- State lood roups ogs.
- State lood roup
- Discuss ects lood yping elated
- Describe ube thod lood yping.
- Describe glutination thod lood yping.
- Describe unochromatographic thod lood typing.
- Describe rocedures or crossmatching.

Alloantibodies
Blood group antigens
Crossmatching
Dog erythrocyte antigen
Neonatal isoerythrolysis

Red lood ell igens ructures urfaces ne eact ibodies another ecific urface kers vidual e enetically etermined eferred **blood group antigens** he umber lood roups aries species. ntigen–antibody reactions occur with blood trans fusions result of variations blood group antigens between ecipient onor. eactions ually esult lumping glutination clinically ysis.

Some species of domestic (e.g., sheep, pigs) have naturally occurring antibodies **alloantibodies** against igens nce antibodies, mismatched transfusion given to results in ntibodies orming gainst ntigen ransfused ibodies). reeding emales ways be iven roperly ched lood otential or roduction ibodies esult estruction of onate's

The eased vailability lood omponents uch packed elet-rich roved reatment for me ients mergency itical ttings. et erinary lood rovide lood omponents, perform blood typing **crossmatching** These procedures e erformed eterinary ractice oratories. Veterinary echnicians erstand oncepts lood component transfusion related procedures to help ensure ransfusion rapy

More ozen erent lood roups ve een described. omenclature or lood roup stems esignated

with (which for **dog erythrocyte antigen** followed by umber. or stems, rythrocytes esignated as ositive r egative or he pecific ntigen. he EA roup once considered to have three subgroups, but recent research documented reflected varying degrees of expression of ene. esignate other r lood roups. lood roups onsidered clinically nificant greatest antigen response serious transfusion reactions. pproximately ogs ositive or Transfusion eactions he ther lood roups re ess ikely linical igen, een described. ecause urally ccurring ibodies are wn ransfusion ositive blood o gative ecipient esult immediate reaction. However, antibodies develop result elayed ransfusion eaction eek riginal mismatched ransfusion. gative og reviously received ositive lood, vere eaction ccur dog subsequently transfused with 1–positive lood.

TECHNICIAN NOTE Administration of mismatched DEA 1 blood elicits the

lood roup stem een entified been esignated stem. lood roups B, . ew ve roup lood. rity nited ve roup lood, hich robably accounts or ence ransfusion eactions Type lood ertain ebred reeds evon Rex, ritish rthair) ertain eographic tralia). nlike ogs, ve urally ccurring ibodies rythrocyte igen ck. ype ve rong anti-A ibodies, hereas ype ve bodies. ransfusing ype ype lood esult rious ransfusion eaction hus, lood or transfusions ebred lected yping crossmatching. hich lood ell igen, een escribed **Neonatal isoerythrolysis** een documented ype ype ens ype ueens with urally ccurring ibodies.

Eleven lood roups ve een escribed have een esignated roup olymorphic, re erent igens. Anti-J ibodies nly ommon ural ibodies gative onors ransfusion reactions.

Seven lood roup stems ve een entified ep, ve een esignated stem hly olymorphic. aturally ccurring antibodies may be present. Neonatal isoerythrolysis may occur are administered bovine colostrum. This caused by resence ibodies ep rythrocytes ovine colostrum. Five major systems have been identified goats designated aturally ccurring ibodies may e resent.

More lood roups ve een escribed ight blood roup stems in horses; the major groups ave been es ignated D, P, T, U. Naturally occurring antibodies do xist, ut ibodies resent esult accina tions that contain quine tissue or transplacental immunization. Crossmatching one efore ransfusion horse, ecause ransfusion eactions orses re ommonly atal.

The mare–foal incompatibility test crossmatching proce dure etects resence ibodies rum colostrum) oal rythrocytes onfirm revent onatal isoerythrolysis.

Methods of identifying some feline blood groups are available or eterinary ractice. thods lude an mmunochromatography ssay nd ard/slide gglutination assay. he ube thod or lood yping, but rimarily eference oratories.

The ube thod or etermining lood ype equires of antisera, which consist of antibodies specific for each possible blood ype iven ecies. ommercial isera or and eline roup esting re vailable or ew anine nd eline blood groups Box The tube method requires collection of hole lood thylenediaminetetraacetic (EDTA), parin, cid–citrate–dextrose icoagulant. blood centrifuged for minutes. fter removal of oat, rythrocytes ee

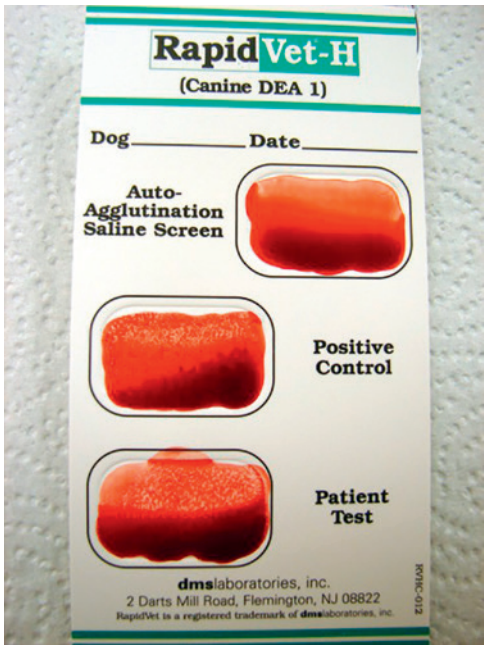
*Typing antisera are available for these blood types.

times n aline olution, entrifuged, nd esuspended. he BC suspension ributed ubes equired or number of blood type antisera being tested. small (usually isera dded propriately labeled ube. ubes ed or utes oom temperature ecentrifuged or conds Each tube examined macroscopically microscopically for evidence f molysis glutination. ositive esults may equire epeat esting.

The lood yping ractical or outine analysis before transfusion. Literally of different anti sera would be required because of large number of different blood roups ep, ow, rse.

Blood erform d-based already w vidence toagglutination, hich ually visible lumps lood phosphate-buffered vage showing vidence gglutination. idVet-H anine Laboratories) blood-typing test card used to classify ogs ositive gative or typing d ontains noclonal ibody ecific ch d isually efined ells or ient controls. drop of EDTA-anticoagulated whole blood one drop f hosphate-buffered ed yophilized reagents ithin ch ell. ositive ell, monoclonal ibody orms iserum, hich ed with hole lood om ient. resent, ositive erythrocytes eact iserum glutination. The iserum ient ell oes eact 1–negative rythrocytes.

RapidVet-H eline) lood-typing or classifying eline lood ype

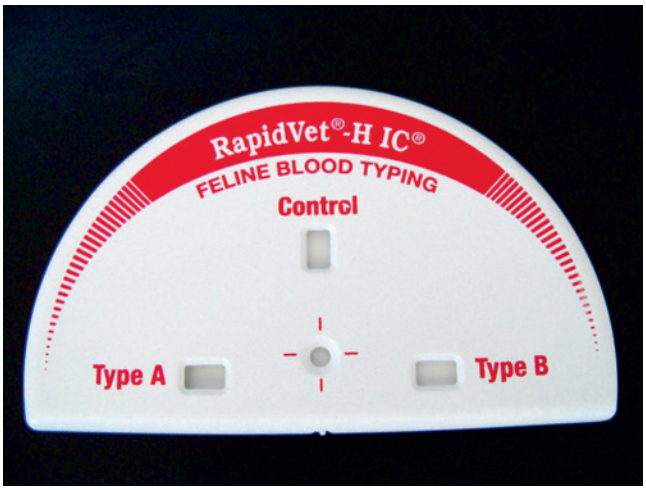


wells contain lyophilized reagent, which represents anti body o igen, igen, hich onsists ctin. ythrocytes om ype glutinate monoclonal antibodies well on card), erythrocytes from type agglutinate with anti-B solution well on card). Erythrocytes from type agglutinate with both anti-A eagents. ell rves toagglutination een gative or results o e eened or toagglutination. f toagglutination resent, with phosphate-buffered autoagglutination screen repeated. f toagglutination esult gative, yping test y e erformed.

The card agglutination and immunochromatographic

Several commercial test make of immunochromatographic est rinciple ather glutination The control detects separate antigen on The er rip regnated noclo i–DEA ibody cond ibody versal antigen control. solution strip, ells xpress oncentrate antibody regnation. ells oncentrate of ontrol igen, reby emonstrating ells ve successfully rip. eline works y; wever, ontains anti-A noclonal ibody, ontains monoclonal ibody, ontrol ibody or ommon feline igen, reby lowing or entification blood ype

In nce ommercial isera, ossmatching lood donor ecipient educes ossibility ransfusion



An immunochromatographic test for feline blood typing.

Blood typing tests. (Courtesy Alvedia.)

Label a plain tube with the donor name and the word

two drops of the donor plasma and two drops of the recipient cell

reaction. he o-part rocedure matching) equires rum lood Procedure uspensions, hich ollected or blood yping, repared. or ossmatching, ew drops f rum om ecipient dded ew ops of d cked om onor. ure bated entrifuged. croscopic oscopic presence f molysis glutination lood-type mismatch. ossmatch xcept onor serum ecipient rocedures performed for all with unknown blood types require transfusion. wo ontrols or hich onsists of onor ells onor rum ell ecipient ells with ecipient rum. ommercial or ossmatching are vailable

Critically ill patients should undergo blood typing

Agglutination eactions metimes raded. everal sification hemes or pose. able ws one ype f rading etermines hether evidence of agglutination constitutes unsuitable transfusion.

Chapter eview uestions ppendix

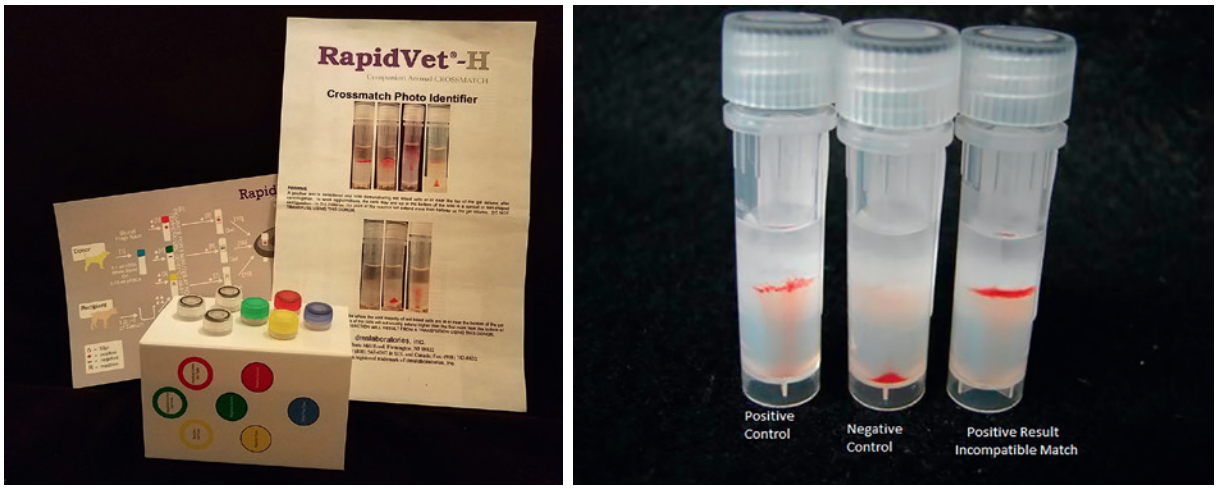
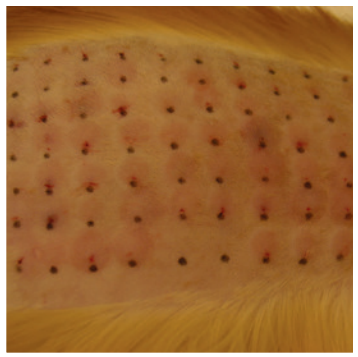


Fig. 21.4 RapidVet-H test kits are available for in-house canine and feline blood crossmatching. (Courtesy

- Proper blood typing crossmatching minimize prob lems critically ill patients.
- Dogs have more dozen different blood groups.
- Cats have one blood group antigen system.
- The clinically significant blood group dogs
- The majority of have type blood.
- Type have naturally occurring antibodies to type antigens; type have naturally occurring antibodies to type antigens.
- Dozens of blood groups have been identified large species.
- Blood typing with tube method performed refer ence laboratory.
- Blood typing of dogs be performed veteri nary practice laboratory with either agglutination or immu nochromatographic methods.
- The crossmatching of blood before transfusion helps to mize potential reactions.
- Both major minor crossmatching are needed before transfusion.



After studying this chapter, you will be able to:

- Describe ions or radermal esting.
- Describe rocedure or erforming radermal testing o etect lergens.
- List ommon lergens eterinary ecies.
- Describe uberculin
- Describe ells phils lergic reactions.

Tests of Cell-Mediated Immunity,
Intradermal
Tuberculin

Key Points,

Allergen
Angioedema
Histamine
Tuberculin skin test
Urticaria
Wheals

Tests f umoral unity volve etection culating antibodies; valuation ell-mediated unity uch more ommonly erformed radermal used to evaluate patients with allergic (hypersensitivity) reactions o etect resence uberculosis igen.

Skin ests e arious lergies **allergens** nvironment. llergies diated unoglobulin (IgE) ibody hey etected ler genic extracts of grasses, trees, weed pollens, dust, insects, r ossibly ffending igens. xtracts cted intradermally, ction nitored or lergic reactions. positive reaction appears raised welt lergic igen.

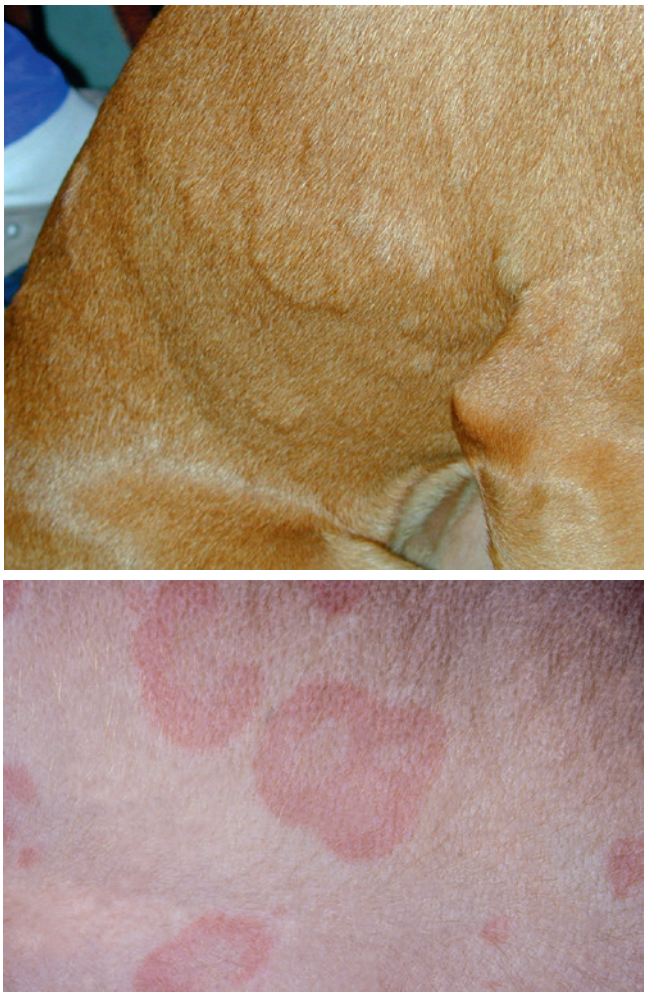
Patients ith ypersensitivity eactions **urticaria** (hives), **wheals** or **angioedema** (edema of dermis ubcutaneous issues). eactions riggered hen basophils or cells release their histamine-containing gran rigger ory esponse. any ubstances environmental parameters have been demonstrated to urticaria ox ioedema.

Allergic dogs frequently have reactions to more

Allergens are chosen on of patient's history geographic ommon lergens lude dust, uman er, eathers, eeds, rasses, rees. Intradermal esting or ood lergens een ell validated. ogs equently lergic re stance. alse-positive gative eactions ccur or ariety f easons oxes

When erforming ient ced eral recumbency, ved om eral rax. scrubbed. felt-tip marker used to mark injec tion es, hich auge edle used o ct olume enerally uspected individual lergens. ients olerate ctions ell o equire dation.

An intradermal injection of used negative control, ction roduct ositive control. The injection sites are then evaluated minutes after he njection, nd he eactions, ny, re raded. he aline injection graded positive control graded 4. The test sites are scored relation to two controls. Each test



Erythematous wheals. (From Miller WH, Griffin CE, Campbell KL: *Muller and Kirk's small animal dermatology,*

site valuated or resence rythema. ter each heal ured

An enzyme-linked immunosorbent assay test available for determination of allergen-specific IgE antibodies ogs, rses CEPT, high-affinity eceptor vailable or esting ozens grasses, rees, eeds, cts,

The **tuberculin skin test** correlates with specific cell-mediated immune eaction. ected *Mycobacterium* pp. bacteria develop characteristic delayed hypersensitivity reactions when exposed to purified derivatives of organism called tuber he est ommonly erformed rimates. For he uberculin kin est, uberculin njected ntradermally e ervical egion

Caused Urticaria and Angioedema in

efa shampoo,

From Miller WH, Griffin CE, Campbell KL: *Muller and Kirk's small*

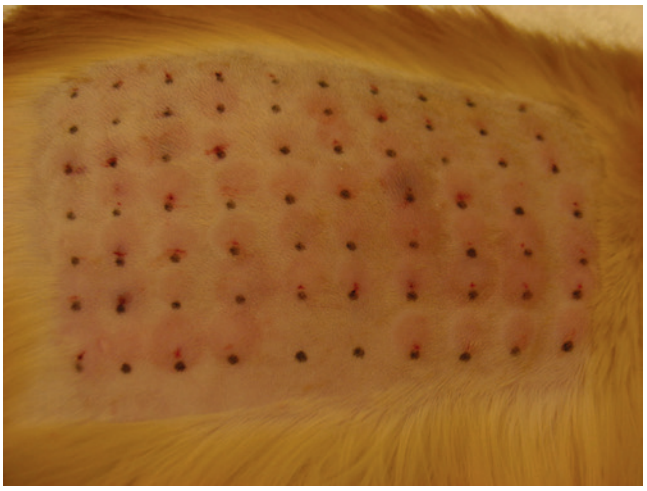
Intradermal Skin Test Reactions

From Miller WH, Griffin CE, Campbell KL: *Muller and Kirk's small*

Intradermal Skin Test Reactions

(traumatic placement of needle, dull or burred needle, too

From Miller WH, Griffin CE, Campbell KL: *Muller and Kirk's small*

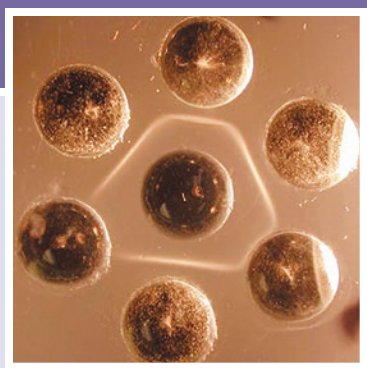


wheal and flare reactions. Unlike what is commonly reported in cats, this

e elayed ory eaction observed f he nimal as een xposed *Mycobacterium* he reaction o ction elayed ecause re before lymphocytes migrate to foreign antigen injected into ermis.

Chapter eview uestions ppendix

- Intradermal esting erformed entify gE-mediated allergic responses to detect presence of *Mycobacterium* antigens.
- Intradermal testing for allergies requires extracts of common antigens.
- Lesions result from intradermal testing are evaluated for erythema rade eaction.
- Allergic eactions esult hen phils ells elease their ontaining ranules rigger matory esponse.



Reference Laboratory Immunoassays

After studying this chapter, you will be able to:

- Describe rinciples oombs
- Describe rescent ibody esting.
- Describe rinciples unodiffusion radioimmunoassay.
- Describe olymerase eaction, usefulness nostic esting.
- Describe eneral eps olymerase reaction.
- Explain ibody iters, ive ion or

Coombs Testing,
Immunodiffusion,
Radioimmunoassay,
Fluorescent Antibody Testing,
Antibody Titers,
Molecular Diagnostics,
Reverse ranscriptase olymerase eaction,
Real-Time olymerase eaction,
Polymerase eaction,
Key Points,

Antibody titer
Coombs test
Fluorescent antibody
Immunodiffusion
Polymerase chain reaction
Radioimmunoassay

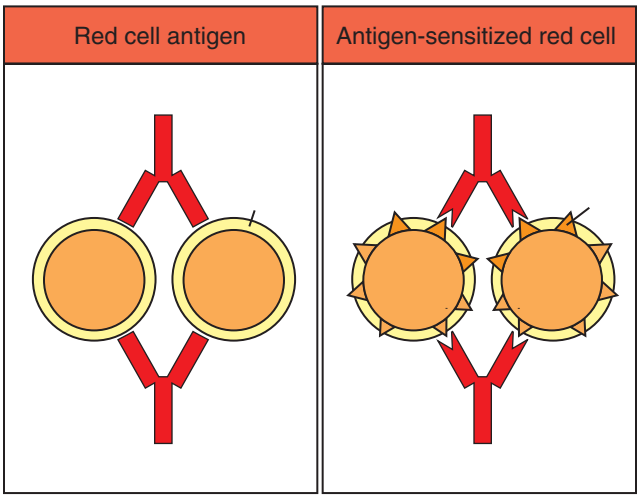
The presence of inappropriate antibodies antibodies against ody's wn issues) etected **Coombs test** .

The direct Coombs reaction used to detect antibody attacked body's own erythrocytes. There are commercially available oombs erformed practice oratory, ut re ommonly erformed eference

positive direct Coombs test provides evidence of immune-mediated hemolytic sease. The rocedure involves incubating uspect isera, hich eacts ecies' immunoglobulins. rythrocytes oated with unoglobulin lf-antibody), isera immunoglobulin rythrocytes ill eact esult visible glutination rythrocytes.

Indirect Coombs testing detects circulating antibody. positive indirect Coombs test result indicates presence of circulating antibodies ody's wn issues. isualize eac tion, patient serum incubated with erythrocytes from normal f ecies. ibody resent ient serum, ill rythrocytes own. he ubsequent ddition i–gamma lobulin or ecies eing ested esults magglutination.

For **immunodiffusion** ient rum ossibly contain antibodies antigen to antibodies, which supplied test are placed separate wells agar gel plate. oth omponents orm isible band f recipitation hen hey ombine. and orms, antibody xists ient's rum ient's antibody vels ufficient recipitation el. Diseases etected unodiffusion quine infectious ox



Indirect Coombs test.

trols with antibodies in alternating surrounding wells. The control samples

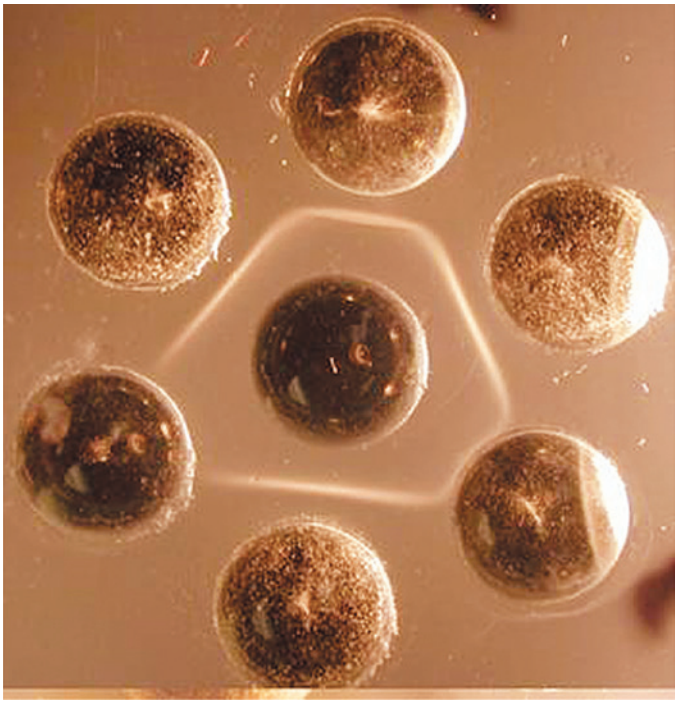
center well and antibodies from the surrounding wells

here the antigens and antibodies meet, a line of visible precipitation forms.

ompetitive orm **radioimmunoassay** rimarily een used research diagnostic laboratories for many years. The test rinciple imilar he ompetitive nzyme-linked mmu nosorbent echnique xcept adioisotope is sed n lace nzyme. ssay ypically onsists antigen labeled with radioisotope antibody. hen combined ient rum ontains igen, both antigens compete for antibody. increasing of ient igen, re eled igen ced om antibody. he emaining adioactivity ured ompared ve etermine oncen tration f igen ient's rum.

erinary practice laboratory. (Courtesy Alvedia.)

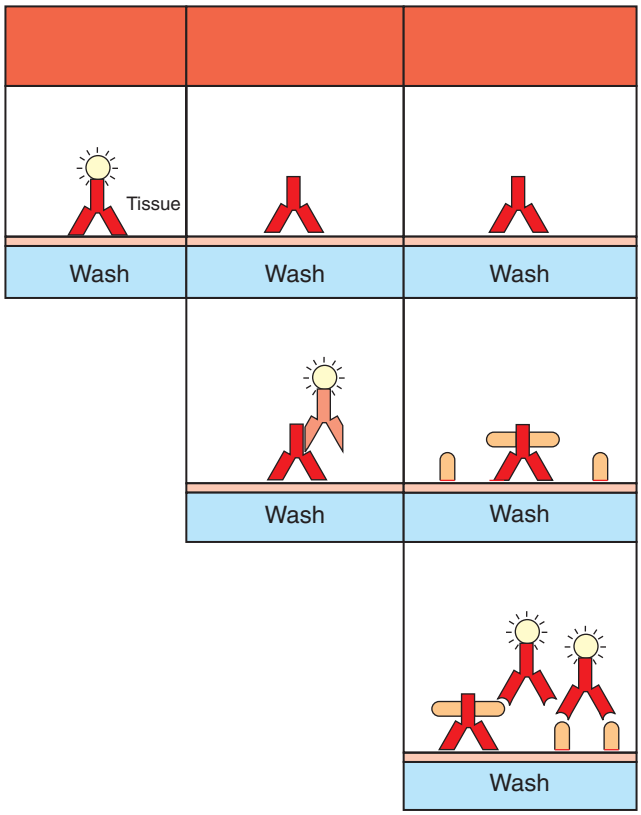
Although ommonly erformed eterinary rac tices, fluorescent testing available veterinary reference laboratories. rocedures equently erify entative een de eterinarian. Two thods vailable: ect ibody esting ect antibody esting. etect resence ecific antibody ect rocedure, patient dded een recoated with rescent-dye–conjugated igen. ye ombines with ecific ibody resent ient The e xamined ecial oscope been designed for fluorescent microscopy. For cellular antigens, ell ill pear tlined rescent erial. With ect **fluorescent antibody** A) echnique, patient ed ontains ecific test igen. emove antibody. Fluorescent-labeled antiantibody added to system, slide then microscopically examined. ny fluorescence indicates ositive esult. uorescent echniques or antigen etection ell.



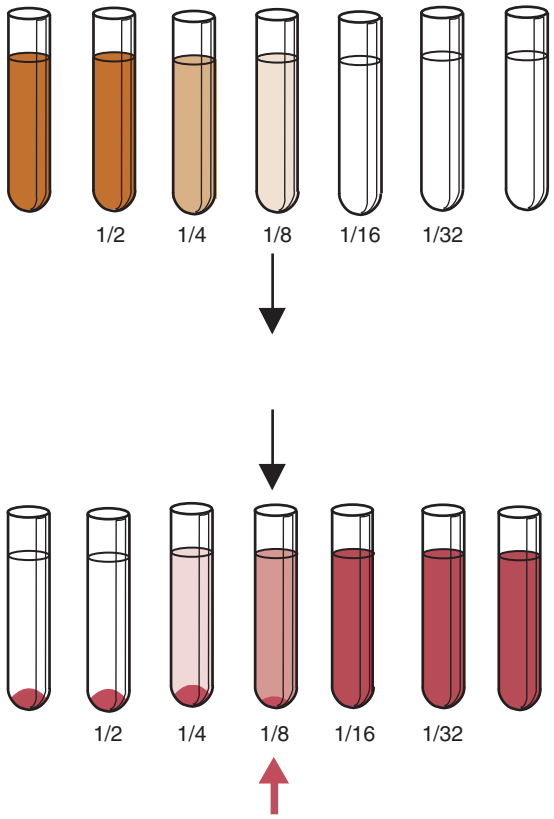
Agar plate showing lines of precipitation. No lines of precipitation

Courtesy Zoetis Inc, Florham Park, New Jersey.)

Although outinely erformed eterinary practice oratories, **antibody titer** ests y eded linician etween ctive ection rior exposure to certain antigens. This particularly important when



Fluorescent antibody technique.



eliable igen vailable. iter efers reatest dilution hich ient nger ields ositive result or resence ecific ibody.

the last tube in which a reaction has occurred is identified. In this example, agglutination has occurred in all tubes up to a serum dilution of 1 . The agglutination titer of the serum is said to be 8. (From Tizard

Antibody titers are performed to differentiate active

The test performed reference laboratory requires of serial dilutions of Each dilution then examined for presence of antibody Fig. The recip rocal of greatest dilution still elicits positive test result iter. iter ften ctive ection. ow titers ually revious xposure ecific igen.

Recently, umber iter ve een de vailable for eterinary ractice oratory. primarily nzyme-linked unosorbent technology, rovide ccurate esults. ome clinicians ill equest hen etermining ed revaccinate ient.

to e ested rt thods oo sophisticated or eterinary ractices. any veterinary nostic oratories ffer veral tests. The obvious for veterinarian to identify pres ence f gens uch iruses, cteria, ut re are y or echnology Tables

The branches of medicine science types of DNA tests include microbiology, genetics, immunology, pharma cology, forensics, biology, food science, agriculture, archaeology, cology. vailable ers, etect genetic defects, verify pedigrees, determine bacterial contaminants food science applications, to but few

The advantages of of tests are increased sensitivity eased ecificity. ecimen eded or est xceedingly factors nce rocedures—such condition f rowth equirements, viability of organism—are crucial with molecular diagnostic tests. The newer techniques have faster turnaround times. hereas traditional identification of bacterium may take r ys re, nostic esting accomplished er epending

Disadvantages lude ontamination positive esults, vel echnical xpertise eded

Leptospira p., hich w-growing cteria ure plate, e ne cteria entified of molecular diagnostic testing. The DNA molecule of bacteria, which contains genetic information, molecule of erest or olecular nostic esting analysis of DNA or RNA. eterinarians send out

driving along a highway in northern California one night when he had a sudden

to un ests, ed or re oom hich to erform any roblems are eing ved, ommercial tomated ru ments e vailable nostic laboratories.

Many arieties nostic vailable, but perhaps widely used—is **polymerase chain reaction** his etects segment f erest ecimen ubmitted ox

Sometimes ucleic or test, uch hen esting or iruses. rocess called everse ranscriptase ut single-stranded RNA must first be converted to double-stranded DNA efore rocess ontinue.

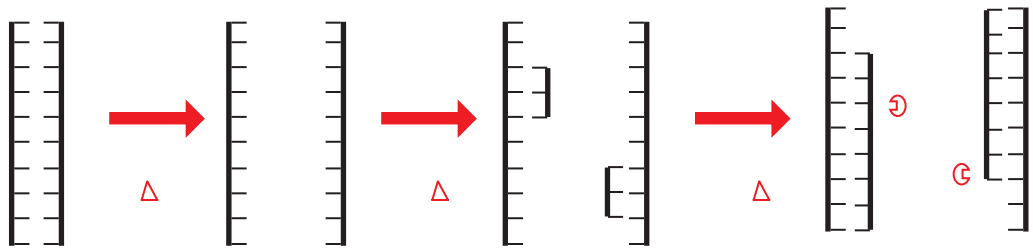
Another nificant eal-time ompared thod ecreases ontamination, re automated, generally faster easier to run. fluorescent probe dded robe ches DNA gments; uantity gments rescence rescence, onsidered ositive.

led ion ecause A gment etected test etter etermine esults. ords, test roduces opies lect egion molecule. Before performing test, nucleotide sequence of section of DNA must be known proper reagents are d. he egion ill entify irus bacterium redetermined.

The amplification process consists of three steps: dena turation, annealing, extension. fter amplification, DNA segments are separated on electrophoretic gel for identification. The ure ontains ecimen riginal DNA uestion resent), rimers, ucleotides, DNA olymerase

The ed reak ouble-stranded molecule into two separate strands. Each strand serves tem plate o hich ucleotides ill ch.

The temperature lowered to primers to bind to parated rands. rimers eginning end f ction opied. ill nly pen A resent omplementary primers.



New DNA segments

The emperature aised nce re, oly merase nzyme ead ode mble the ucleotide ases orm ew omplementary trands) auses new DNA segments to be produced (extended). Portions of two DNA molecules have been obtained, each with two strands. They are omplete ut ontain desired gment.

This process repeated to times automated thermal cycler Fig. The timing, temperature, number of cycles are regulated by instrument. The of DNA segments produced reater riginal ecimen. hy or etecting ute quantities f wn ed ecimen.

Finally, o obe resent ecimen, agarose el electrophoresis is used. The segments are ga tively harged ticles ill ve el oward positive lectrode hen urrent pplied. he egments epa rate ccording pear parate gel. ontrols knowing entity ontrol be ompared entified.

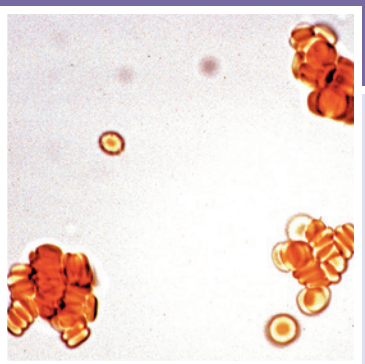
The erpretation one efully. microbe may be present but may be of ient's oratory esults must e valuated ormation om linical

Thermal cycler for polymerase chain reaction. The instrument

Laboratories, Inc., ©2019. Hercules, Calif.)

Chapter eview uestions ppendix

- Molecular nostic esting or RNA analysis to identify pathogens, classify cancers, detect genetic defects, verify pedigrees, determine bacte rial ontaminants ood nce plications.
- Coombs esting erformed entify toantibodies.
- Fluorescent ibody esting erformed ect or ect thod.
- Antibody titers are performed to differentiate active infection from prior exposure to evaluate need for revaccination.
- During unodiffusion ient rum ough el eact antigen om ells ibody resent.



After studying this chapter, you will be able to:

- Describe ypes ypersensitivity eactions.
- List onditions ype ypersensitivity.
- List onditions ype ypersensitivity.
- List onditions ype ypersensitivity.
- List onditions ype ypersensitivity.

Hypersensitivity,
Key Points,

Anaphylactic shock
Atopy
Hypersensitivity
Immune-complex disease
Immune-mediated hemolytic anemia
Lymphoma

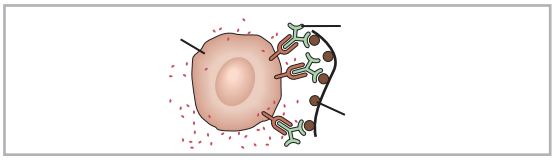
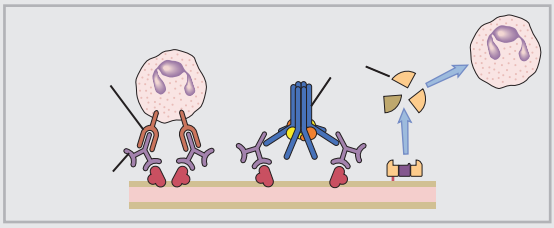
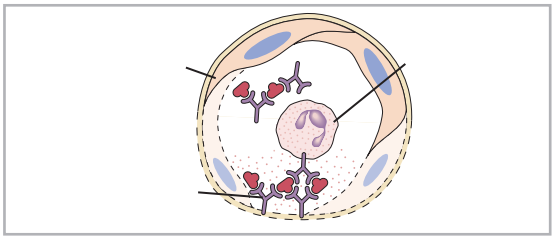
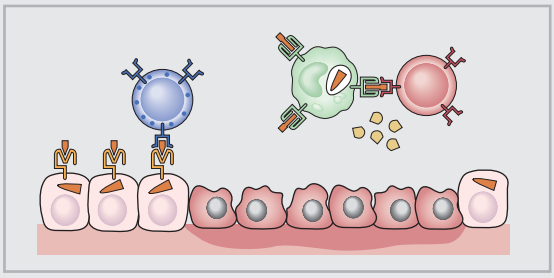
Some une esponses ve dverse ffect
If immune responses are uncontrolled or hypersensitive, they issue ury. mmune esponses hich ody's own tissues are attacked by body's own antibodies occur. In ddition **hypersensitivity** eactions, stem may show deficiencies. deficiency may exist phagocytes or immunoglobulins. condition called combined immuno deficiency ects uring ly er rum vels maternally derived antibodies have declined. rabian foals with ften om pportunistic ection esults from nce eficiency unoglobulins.

Four types of hypersensitivity have been categorized on f unologic chanism volved Type ypersensitivity diate ypersensitivity occurs hen hemical diators om ells eleased. Allergies **(atopy)** **anaphylactic shock** severe reaction may ccur conds er igen nters tion) e ype ypersensitivity rders ccur when immunoglobulin (IgE) antibodies are formed response to reviously ncountered igen. hen igen encountered, eceptors ells, hich results elease ell mediators. Mast cell mediators smooth muscle contraction increase permeability of vasculature within minutes. Mast ell diators tokines ract ells inflammatory esponse utrophils osinophils) ea. ariety vident, epending cation ypersensitivity eaction

TECHNICIAN NOTE

Antibody-mediated hich ibodies directed against animal's own cells or components of extra cellular rix ype ypersensitivity rders. **Immune-mediated hemolytic anemia** which condition estruction ed lood ells immune-mediated ombocytopenia hich esults platelet estruction, ype ypersensitivity rders. ype ypersensitivity rders diated receptors on cell surfaces. IgM may be involved, resulting immune omplexes ill serve to activate the complement system. he ctivation omplement iation inflammatory response. In of antibodies bind o veral urface eceptors ed lood ell brane, here ergo psonization ubsequently phagocytized. chanism ccurs

Neonatal erythrolysis IMHA of neonates occurs often foals The disorder results from inges tion of colostrum contains maternal antibodies against

Type of		
(Type I)	2 cells, IgE antibody, mast cells, eosinophils 	
(Type II)		
(Type III)		
(Type IV)	CTLs (T cell-mediated cytolysis) 	

functions and disorders of the immune system, ed 3, Philadelphia, 2011, Saunders.)

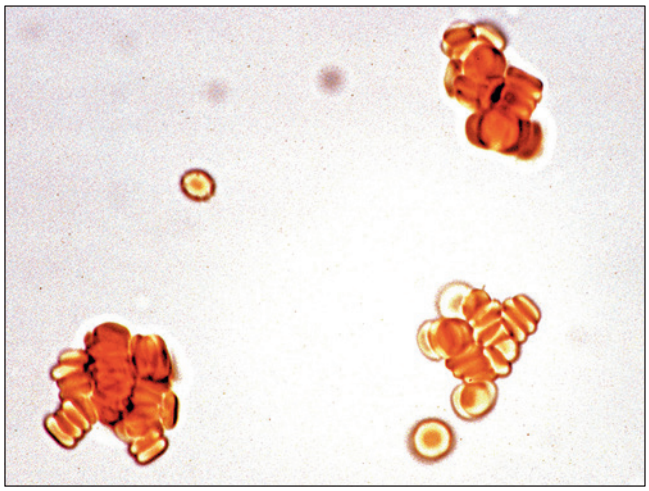
fetal erythrocytes Fig. Transfusion reactions are medi ated y ibodies.

Immune-complex disease or type hypersensitivity occurs when antibodies antigens form complexes are deposited various blood vessels. Glomerulonephritis, which caused by eposition ibody–antigen omplexes y, xample ype ypersensitivity. ystemic rythe matosus une-complex rder haracterized production toantibodies verse population ells issues. lear.

Type hypersensitivity T-cell–mediated caused by eaction ymphocytes lf-antigens issues. Contact hypersensitivity reactions, such may occur dogs after contact with plastic food collars or human eings er ontact oison vy, issue ury with elayed esponse. hemicals om ubstances react with proteins, immune system recognizes chemical–protein complex foreign, thereby resulting derma titis. ype iabetes, heumatoid rthritis, nd hronic nfections such tuberculosis are all T-cell–mediated autoimmune

Clinical manifestations of type I hypersensitivity reactions. (From

ed 3, Philadelphia, 2011, Saunders.)



Microscopic agglutination in an unstained wet mount preparation of saline-washed erythrocytes from a foal with neonatal isoerythrolysis.

of immune system. The immune system normally recognizes estroys er ells efore ecome lished body, ut metimes er ems ecome escapes efense chanisms.

Lymphoma hich ype umor haracterized uncontrolled proliferation of lymphocytes, another abnormality

Chapter eview uestions ppendix

- Immune esponses issue ury led yper sensitivity eactions.
- Type hypersensitivity called immediate hypersensitivity.
- Atopy ype ypersensitivity rder.
- Type ypersensitivity ludes umber ibody-mediated
- IMHA, IMT, neonatal isoerythrolysis, transfusion reactions are ibody-mediated ype ypersensitivity eactions.
- Immune-complex disorders are type hypersensitivities result eposition omplexes arious tissues.

Unit Outline

Chapter 25: Anatomy and Physiology of the Urinary System,
Chapter 26: Sample Collection and Handling,
Chapter 27: Physical Examination of Urine,
Chapter 28: Chemical Evaluation,
Chapter 29: Urine Sediment Analysis,

The objectives for this unit are:

Describe the formation of urine.
List and describe a variety of urine sample collection methods.
List and describe physical and chemical evaluations performed with urine samples.
Describe the formed elements that may be encountered in urine samples.
Describe the procedure for preparing urine for microscopic examination.
Describe the procedure for evaluating the formed elements in a urine sample.

Urinalysis is a relatively simple, rapid, and inexpensive laboratory procedure. It evaluates the physical and chemical properties of urine as well as the urine sediment. A urinalysis provides information to the veterinarian about the status of the urinary system, the metabolic and endocrine systems, and the electrolyte and hydration status. Therefore, the veterinarian may request that the owner bring a urine sample for initial testing. Samples may also be collected in-house using a variety of techniques.

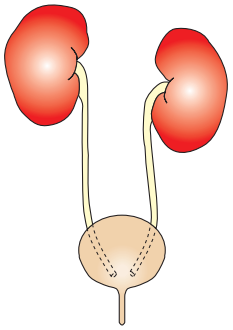
Abnormalities in the urine may reflect a variety of disease processes involving several different organs. The basic equipment needed to perform a urinalysis is minimal and readily available in most veterinary clinics.

Quality assurance begins with proper specimen identification and handling. All samples should be labeled immediately after collection, and urinalysis should be performed as soon as possible. Reagent strips and tablets must be kept in tightly sealed bottles, and outdated reagents must be replaced with fresh reagents. Reactions for most constituents in the urine may be checked against available controls (e.g., Chek-Stix, Bayer Corpora tion, Leverkusen, Germany; Uritrol, YD Diagnostics, Seoul, Korea; Liquid Urine Control, Kenlor Industries, Inc., Santa Ana, CA). In addition, urine samples with distinct reactions for certain constituents sometimes may be preserved and used as positive controls. The results obtained from control samples and made-up controls should be plotted to determine whether observer drift or reagent decomposition is occurring. The urinalysis laboratory report should include patient information, collection technique, date and time collected, method of preservation (if used), and complete urinalysis results, including the results of microscopic examination results. A standard protocol for reporting results must be followed. See Appendix F for an example. Precision and accuracy need to be maintained by the veterinary technician for the proper interpretation of results.

Appendix B contains reference ranges for urinalysis tests of common domestic species.

For additional sources for this unit see the Resources Appendix at the end of this textbook.

Anatomy and Physiology of



After studying this chapter, you will be able to:

- List escribe omponents inary stem.
- Explain ormation
- Describe ructure phron.
- Name rmones volved egulation volume.

Formation of Urine,
Ureters,
Urinary ladder,
Urethra,
Urine Volume Regulation,
Key Points,

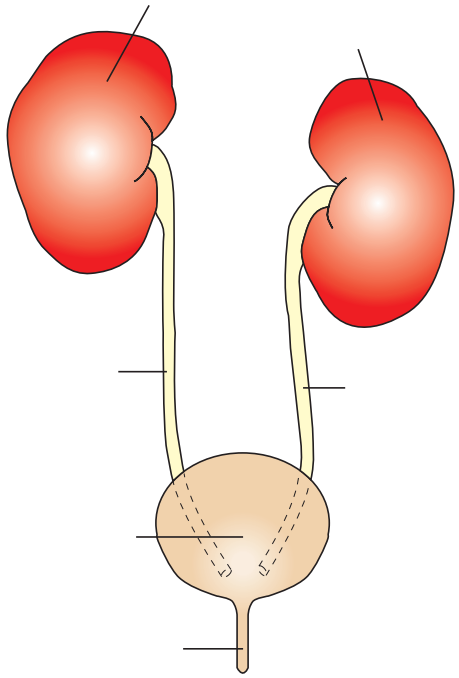
Anuria
Glomerulus
Nephron
Oliguria
Polyuria
Renal threshold

The many metabolic reactions take place body's cells generate ariety hemical y-products. ome stances e ill ody ecycled, ut rs would e ere lowed ccumulate body. hese roducts liminated. urinary stem rimary hich roducts are emoved om lood. onsists ys, ureters, urinary bladder, urethra Fig. The left right kidneys are located dorsal part of abdominal cavity, ust entral ertebrae. ve ean-shaped ys. owever, ight kidney f rse t-shaped, ovine ys ve lobulated pearance.

Blood ymph essels, rves, eter nter leave ough ented led rough-appearing ter ortex rapped appearing inner medulla. The area deep to hilus region renal pelvis, which funnel-like beginning of ureter. The work of kidneys done within **nephrons** Depending on animal's size, each kidney may contain from several hundred o veral lion phrons. ch phron ube composed of epithelial cells with several bends. Each portion of the ephron haracterized ifferent ype pithelial ell. l umber pithelial ells egularly hed off. ncreased umbers ecific ypes pithelial ells etermining enal ysfunction. The phron ollowing enal orpuscle, roximal convoluted ubule, op enle, onvoluted ubule, collecting ubule Fig. 5.2). ach enal orpuscle omposed **glomerulus** surrounded by Bowman's capsule. The glomerulus tuft of capillaries between arterioles enter leave enal orpuscle.

TECHNICIAN NOTE

When blood enters renal corpuscles, portion of along ith ered ough lomerulus roximal onvoluted ubule. roteins ells nter ubule re lomerulus. The ered wly ough phron dified ves ng. ubstances uch er lucose esorbed ck lood lary network. he phron ecific or esorption specific ubstances wn **renal threshold** filtrate ontain xtremely oncentrations substances, xcess esorbed ead xcreted

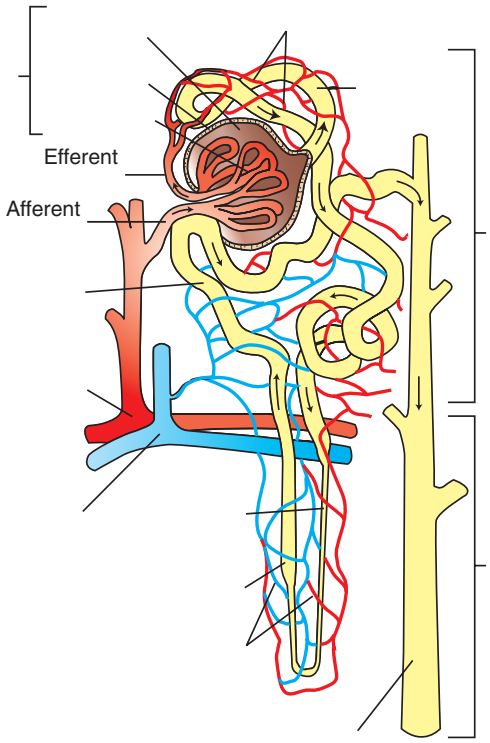


Parts of the urinary system. The urinary system is made up of

ine. phron eaches collecting ubules, ecome ollecting ubules all phrons enal elvis pening eter

From each renal pelvis, urine transported to urinary bladder by ureters, which are muscular tubes conduct urine via oth-muscle ontractions. eters nter ladder blique hich orm alvelike penings revent ckflow eters ladder

The inary ladder ransitional epithelial cells stores urine releases periodically to outside during process called urination. The kidneys constantly produce ine. ccumulates inary ladder, ladder nlarges, retch eceptors ladder are ctivated hen olume eaches ertain oint. reflex hen nitiates he ontraction he mooth uscle he bladder l. oluntarily ontrolled hincter uscle ck f inary ladder nables onscious ontrol of ination.

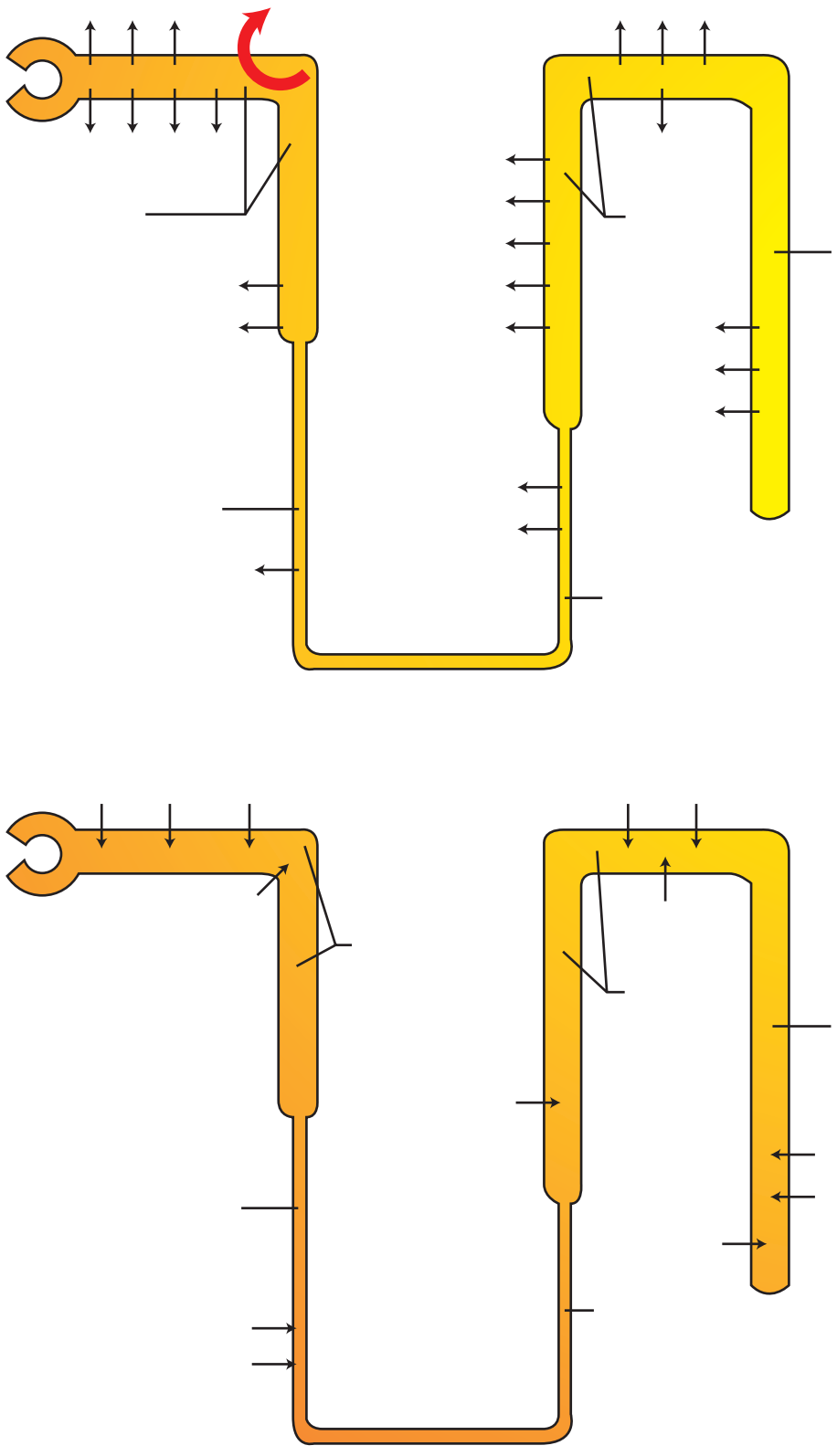


Clinical anatomy and physiology for veterinary technicians, ed 2, St Louis, 2008, Mosby.)

The urethra tube carries urine from urinary bladder to he utside he ody. emales, elatively hort, traight, ide, rictly inary tion. relatively ng, ved, row, rves inary eproductive tions. rmal ra (bacteria) e ommonly ethra l umber hite lood ells.

The egulation olume rine roduced ontrolled two hormones: antidiuretic hormone, which released from posterior uitary osterone, hich creted drenal ortex. Antidiuretic rmone cts ollecting ducts to promote reabsorption of water. lterations water volume ay nvolve ecreased roduction **oliguria**), ncreased production **polyuria** r nce **anuria**

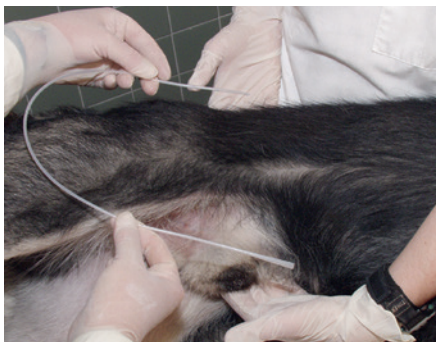
Chapter eview uestions ppendix



cal anatomy and physiology for veterinary technicians,

- The urinary system consists of two kidneys, two ureters, urinary bladder, urethra.
- The functional of kidney nephron.
- Parts of nephron include renal corpuscle glomerulus Bowman's capsule), proximal convoluted tubule, loop of Henle, convoluted tubule, collecting tubule.
- The term renal threshold refers to maximum absorptive capabilities of nephron for specific substances.
- Antidiuretic hormone aldosterone are involved regulation of urine volume.

Sample Collection and Handling



After studying this chapter, you will be able to:

- List thods btain or inalysis.
- Discuss ects ollection ee ch.
- State quipment eded or terization cystocentesis.
- State rocedures or ollection catheterization stocentesis.
- Describe roper

Voided or Free Catch Samples,
Bladder Expression,
Catheterization,
Cystocentesis,
Specimen Storage and Handling,
Key Points,

Bladder expression
Catheterization
Cystocentesis
Free catch
Tom cat catheter

The st ep erforming inalysis roper ollec tion f hich efully btained ensure ccurate esults. ysis be erformed nly en efore dministration of therapeutic agents. Urine specimens may be obtained via natural oiding **bladder expression** **catheterization** **cystocentesis** he o referred thods stocentesis terization. thods rovide ptimal for l ects inalysis voiding ontamination om enital ract xternal ollecting by oiding xpression ladder r, ut urine collected ways may be of limited diagnostic value. Except or tologic xamination, erforming inalysis preprandial morning best, although always prac tical, eterinary ients. orning end oncentrated ected tary ctors, reby increasing hances ormed lements.

The to obtain voided **free-catch** which ollected inates. ollected r isfactory or cteriologic xamination, because often contaminated during urination. Occasionally voided ontain eased hite lood ell esult f ontamination om rmal ra ory lesions f enital ract. esults valuations are ually ected.

Voided samples often contain increased white blood

voided collected clean dry (although necessarily erile) ontainer. ossible, ulva repuce be washed to decrease contamination of before collection. nimals' wners ay sked ollect ample hen ontaneously inates. urthermore, leansed tissue external orifice area does remain clean for long. ination ream) ecause likely o e ontaminated. owever, ially oided metimes ollected recaution eing collect ufficient olume ination Dogs y egin inate op hen ollection empted. hances uccessfully btaining increased y ching er ollect ithout urbing oided from btain. casionally ill inate mpty er ox, ut eterinarians refer ive

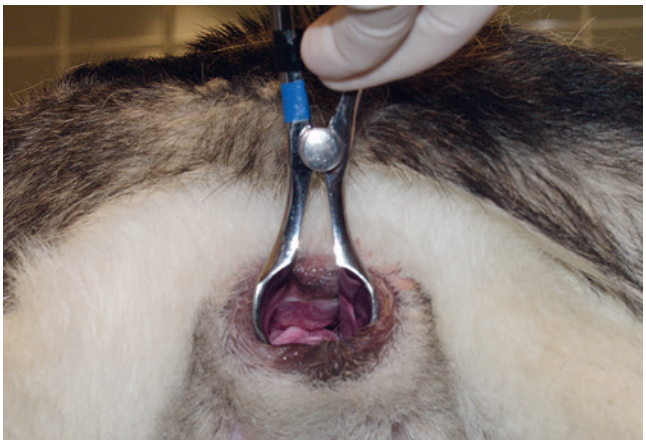
Fig. 26.1 Tom cat catheter. This is a 3.5-Fr polypropylene catheter

Veterinary instruments and equipment,

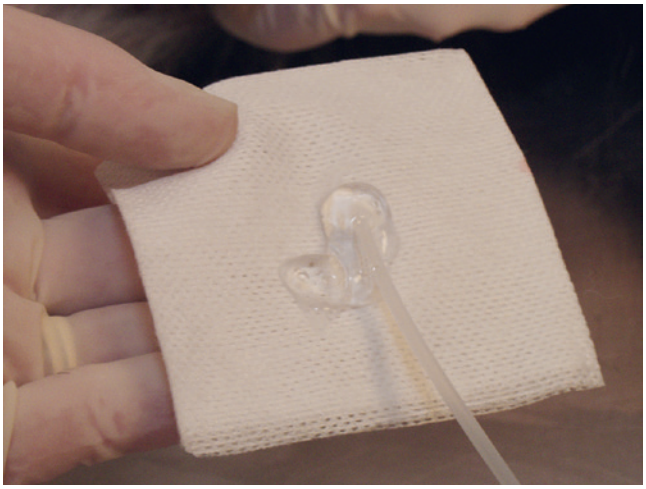
owners nonabsorbent granules to litter box. Cows may be imulated inate ubbing entral on ulva ep imulated urinate by occluding their nostrils. Horses may be stimulated to urinate by rubbing warm, wet cloth on their ventral abdomen or y cing y.

Urine ay e ollected rom mall nimals ia anual ompres sion f ladder. owever, btained are isfactory or cteriologic uring. collection f oided xternal enitalia cleansed efore ladder xpression. or eral ecumbency, ladder ed abdomen, entle, eady ressure plied. are taken o xert oo uch ressure, hich ure rupture ladder. elaxation ladder hincters ften takes ew utes. casionally ed lood ells will e ound esult ressure plied ladder, ease hite lood ells esult om ontamination riginates enital ract. cteria urine, ys ecome ected cteria. method ver ethras may e bstructed ladder agile, because xcessive ressure ladder upture. In e ination imulated pressure n ladder ough ectal erform ectal ion.

Catheterization rtion olypropylene ubber catheter o ladder ethra. ariety catheters xist or erent ecies enders with previous two methods of collection, external genitalia e leansed efore rocedure, dation times equired. terile atheters hould lways sed, nd terile gloves ust orn. are en erility o revent rauma inary ract. thod be d or ure nsitivity stocentesis performed. For female speculum improves visualiza tion of urethral orifice thus facilitates catheterization Fig. ter ough ethra. l erile er-soluble ubricating lly, uch



A vaginal speculum is used to facilitate visualization of the urethral orifice in female patients. (From Taylor S: *Small animal clinical*



elly Johnson ohnson, rlington, placed n ter are en to void rauma he ensitive rethral ucosa. he istal of many catheters designed for attachment to syringe urine e ollected entle ation. ollection sterile syringe especially advantageous bacteriologic culture icipated. en ortion btained ded ecause ossible ontamination ccur catheter advanced through urethra. Occasionally increase ed lood ells pithelial ells seen esult ethral ucosa om ter. rocedures ummarize eterization rocedures or emale ogs

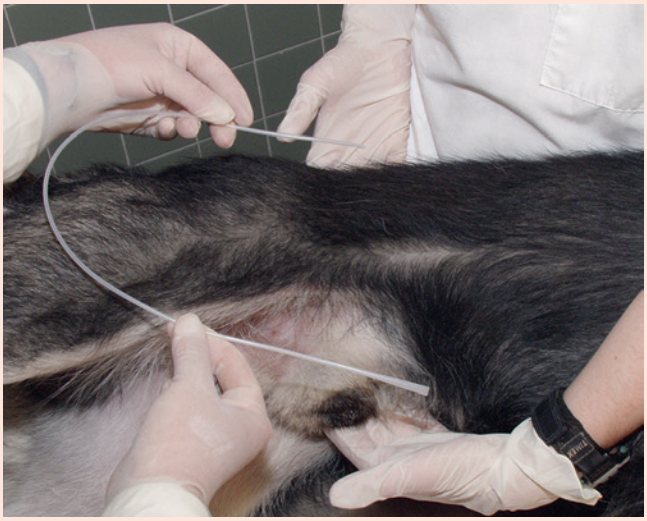
A 4- to 10-Fr polypropylene catheter is usually used

Cystocentesis ften ollect erile om dogs but only when bladder sufficiently distended

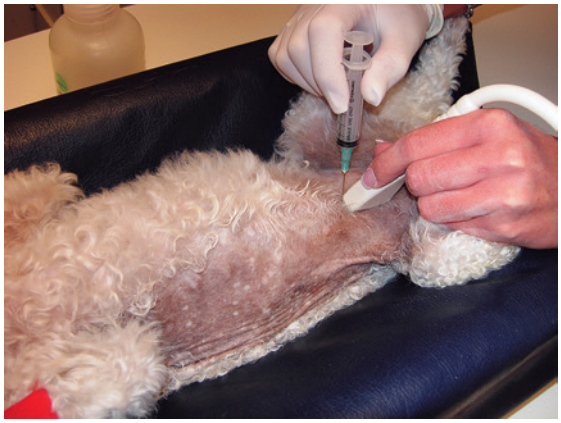
Catheterization: Male Cat

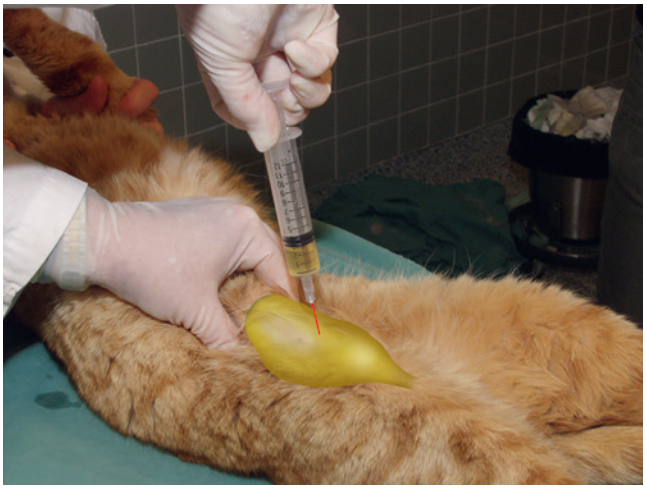
the penis by pushing the penis caudally and pulling the prepuce

Catheterization: Male Dog



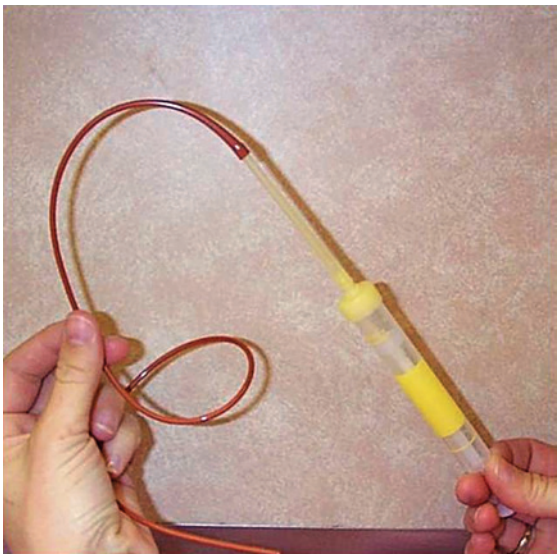
Catheterization: Female Dog





Direct the needle dorsocaudally for cystocentesis. (From Taylor

ed. rocedure er formed nly estrained ients ultrasound-guided stocentesis erformed. bladder must be palpated before procedure to avoid damage to other internal organs. hen performing cystocentesis, or 20-gauge needle to inches long syringe e edle ough never be redirected because of potential for damage to other internal organs. lateral or ventral recumbency or standing, bladder gently palpated immobilized, needle inserted into abdomen directed dor socaudally Fig. For dogs, insert needle to umbilicus to side of For female dogs for rt edle entral umbilicus. ntly ate ringe, roperly label ringe ient ormation. ransferring om ringe ollection ube, emove



Urine S-Monovette system. (Courtesy B. Mitzner, DVM.)

needle om ringe efore ransferring deally, ill ransferred ectly erile ube. lter nately, larger-bore needle be placed on syringe then transferred by puncturing stopper of collec tion ube. ollected stocentesis for culture sensitivity testing. Occasionally contain ease esult ladder rauma. S-Monovette system (Sarstedt Co., Nümbrecht, Germany) ommercially vailable ine-collection evice onsists of sterile, individually wrapped syringe with disposable tube Fig. This system cystocentesis collection, es otential or ontamination hen transferred etween ringe ollection ube, ecause awn ectly ube. ube be entrifuged or diment xamination. ecial luded ollect ter ecimens. echnique or cystocentesis ummarized rocedure

Cystocentesis is preferred for sample collection

All of aforementioned methods of collection are satisfac tory or ualitative ysis. owever, or uantitative ysis, ollected. atios ertain constituents rotein/creatinine) ve een btain erpreted urinary xcretions.

Ideally be analyzed within minutes to of ollection void ostcollection tifacts egenerative changes. If immediate analysis possible, refrigeration pre serves onstituents or dditional

bladder to assess its size and location, and clean the skin

dorsocaudally so that, as the bladder shrinks, the needle tip remains

Refrigeration ve ct ecific ravity, est one efore efrigeration. oing o efrigerated, ve ight-fitting prevent evaporation contamination. Crystals may form when urine cools. Decreased glucose bilirubin concentrations, increased result of bacterial breakdown of urea into ammonia, crystal formation with increased turbidity, breakdown ecially ute urine), bacterial proliferation may occur are allowed o or eriods oom emperature. any crystals y orm efrigerated efrigerated e med oom emperature efore valuation, ut crystals ormed uring ooling ve hen brought to room temperature. The urine be ed y entle version efore valuation ormed elements are evenly distributed. Cells tend to break down rapidly urine, cytologic evaluation to be performed, urine e entrifuged on er ollection, ops ient's rum ovine dded sediment o reserve ell rphologic haracteristics.

Urine samples should be analyzed within 30 minutes

Samples o ransported tside oratory for re reserved ollowing: op ormalin oluene sufficient o orm yer op ymol crystal; r ne henol ormalin d reservative, hemical erformed before addition of formalin, because formalin interferes with some hemical yses ecially or lucose). owever, formalin reservative or ormed lements

Chapter eview uestions ppendix

- The best samples for urinalysis are morning samples or samples collected after several hours of water deprivation.
- Preferred methods of urine collection are cystocentesis catheterization.
- All be labeled immediately after collection.
- Urine be analyzed within minutes to of collection.
- Always note method of urine collection on urinalysis report.
- If be examined within of collection, be refrigerated or preserved.
- A refrigerated be allowed to warm to room temperature before evaluation.

Physical Examination of Urine

After studying this chapter, you will be able to:

- List hysical valuations ompleted
- Describe nificance ariation olor.
- Describe ossible urbid
- Describe ossible or dor ariations.
- List escribe thods or valuating ecific gravity.

Urine Volume,
Color,
Clarity/Transparency,
Odor,
Specific Gravity,
Refractometer,
Causes f ltered rine ecific ravity,
Key Points,

Anuria
Flocculent
Hematuria
Hemoglobinuria
Hypersthenuria
Hyposthenuria
Isosthenuria
Ketones
Myoglobinuria
Oliguria
Pollakiuria
Polydipsia
Polyuria
Specific gravity
Urease
Urinometer
Urochromes

Physical roperties lude bservations y e de ithout oscope hemical reagents. he olume, olor, dor, ransparency, ecific gravity of urine are evaluated. Procedure describes pro cedure or outine inalysis.

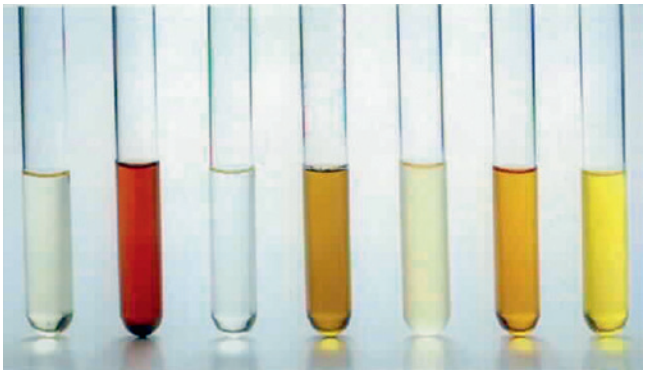
The s wner ften rovides ormation oncerning of urine passed. However, owners may mistake frequent urination **pollakiuria** for increased urine production **polyuria** Therefore, btaining imate roducing ortant. any ctors elated nce roduced. ome factors lude xternal ecially ough espiratory stem estinal ract), nvironmental temperature umidity, ype ood, vel physical ctivity, ecies bserv le ination ufficient or imating output f deally, olume be determined, although often impractical. Observing tdoors rovide ough imate the olume of urine eing produced. Table 27.1 lists the pproxi mate y roduction ommon omestic ecies. f roduced er ariable. ormal output or dult ogs proximately body eight er y.

An ease tput roduction ermed polyuria, usually accompanied by **polydipsia** Polydipsia efined er onsumption. olyuria, ine ually ellow olor, specific ravity. olyuria ccurs luding nephritis, diabetes mellitus, diabetes insipidus, pyometra dogs liver It seen after administration of uretics, orticosteroids,

Production for Domestic Species

a laboratory sheet with the patient's information and the date,

the pad's color at the appropriate time intervals as stated by the



Urine color is best assessed in good lighting against a white background. (From Little S:

Oliguria which decrease daily urine output, may occur when estricted ccess er. vere when environmental temperature increases excess water ss through espiratory stem. ith iguria, the urine ually oncentrated ecific ravity. liguria ccurs cute phritis, ever, ck, dehydration. **Anuria** which absence of urine production, may be seen with complete urethral obstruction, urinary bladder rupture, enal utdown.

Normal ine olor ellow er esult presence f ments led **urochromes** he nitude ellow color ine aries egree oncentration

dilution Table olorless ually ecific gravity, ften ciated olyuria. ellow yellow-brown enerally ecific ravity, may be associated with oliguria. ellow-brown or green urine produces greenish-yellow foam when shaken likely to contain bile pigments. Red or red-brown urine indicates presence of red blood cells **hematuria** or hemoglobin **hemoglobinuria** Urine that brown when oided contain yoglobin **myoglobinuria** yoglobin xcreted hen onditions uscle ell ysis resent, uch habdomyolysis horses. ome ugs er olor ed, reen, blue urine may be observed. hen observing urine, be lear ontainer hite ckground to e roperly valuated or olor

In ecies, eshly oided ransparent lear. Normal quine rine loudy esult he igh oncentration of calcium carbonate crystals mucus secreted by glands renal pelvis. Normal rabbit urine high concentrations f bonate ystals, pears y. hen observing or egree ransparency, placed ainst tter-print ckground. ransparency ed clear, slightly cloudy, cloudy, or turbid **flocculent** depending on w ell tters ead ough ually ve uch diment entrifugation.

Cloudy ually ontain ticles ften ield nificant diment entrifugation. rine become loudy esult cterial ultipli cation r ystal ormation. ubstances cloudy include white blood cells (WBCs), epithelial cells, ystals, cteria. ther urbid ity lude ontaminants om ollection ontainer surface contamination with feces. Flocculent contain suspended ticles metimes nough en with ed ye.

The dor f hly nostic, ut times be helpful. Normal urine distinctive odor varies among ecies. oats, strong dor. dor ccur stitis by cteria roduce **urease** *Proteus* pp. *Staphylococ* p.) ve tabolized left oom emperature ccasionally evelop ammonia dor esult cterial rowth. haracteristic sweet r uity dor **ketones** commonly ound etes llitus, etosis ows, pregnancy wes.

Specific gravity efined eight ensity) tity f uid ompared qual distilled er. umber eight ved solutes etermine ecific ravity ecific ravity may e etermined efore er entrifugation, ecause particles settle during centrifugation have or effect on ecific ravity. hichever thod erform evaluation ecific ravity ecific hether valuation ccurs efore er entrifugation, method ust onsistently erformed erson nel. f urbid, entrifuged upernatant etermine ecific ravity. specific ravity om olyuric ients ends w, ine om ients ends ecific gravity of normal urine depends on eating drinking habits, nvironmental emperature, hen lected. n ly rning ination ends oncentrated. erpretation ecific ravity yields ormation out ient's ydration ability f ient's ys oncentrate ute The ecific ravity rmal xtremely ariable, tuates oughout y. able specific ravity anges or rmal omestic rmal dogs, ine ecific ravity ange om rmal ange om

To etermine ecific ravity efractometer, **urinometer** r eagent strips be sed. Reagent strip pecific gravity reliable method for determining urine specific gravity Urine specific gravity frequently deter mined ith inometer. rument equires large proximately enerally provides eproducible esults efractometer.

Specific gravity commonly determined by refractometer. More ormation out rinciple efractometer presented Procedure which be found Unit Chapter rine ontains ubstances hat bsorb rious avelengths light. he ves end ough dium, end ured efractometer. efractive index of influenced by factors determine specific ravity, refore rovides imate specific gravity. It important to note refractometers are rated or eterinary ients rovide ccurate results, especially with feline Research demonstrated eline entical ecific ravities have erent efractive exes. efractometers rated or veterinary idely vailable, rovide ccu rate valuations ecific ravity. ommon or ecific ravity eading her ange be ead y rument esigned or eterinary species. In must be diluted reevaluated by equal volumes of with distilled water. Use op f ure etermine ecific ravity, then ultiply umbers er ecimal oint or example, ution ontains qual illed er urine ecific ravity ution actual ecific ravity

Increased ecific ravity eferred **hypersthenu ria** h erm ommonly en decreased water intake, increased through sources other urination (e.g., sweating, panting, diarrhea), increased excretion of urine solutes. Decreased water intake with

normal enal tion apidly eased ecific gravity. ncreased ecific ravity ccur cute renal ure, ehydration, ck.

Decreased urine specific gravity referred to **hyposthenuria** t en ith hich ys esorb water eased uch olydipsia or xcessive dministration. yometra, etes psychogenic polydipsia, some liver certain types of renal uretic rapy ecreased specific ravity.

Isosthenuria occurs when urine specific gravity approaches of glomerular filtrate to In other words, urine with ecific ravity ange een oncentrated diluted y ys. hronic enal quently roduce nuric disease, he loser pecific ravity sosthenuric, reater tion een hen are deprived of water, their urine specific gravity usually remains isosthenuric range. with decreased renal function e ften htly derately ehydrated ve urine ecific ravity htly reater nuric o

Chapter eview uestions ppendix

- Physical roperties lude olume, olor, dor, transparency, ecific ravity.
- Normal rine utput or dult ogs nd ats pproximately o ody eight er y.
- Normal olor ellow er aries egree f oncentration ution.
- In ecies, eshly oided ransparent lear.
- Substances urine to be cloudy include epithelial ells, ystals, cteria.
- Urine specific gravity measure of the dissolved utes urine.
- Urine ecific ravity rmally valuated efractometer.

After studying this chapter, you will be able to:

- Describe rocedure or erforming hemical analysis f
- List hemical ommonly erformed urine
- Discuss nificance roteinuria.
- Describe thods erentiate etween maturia moglobinuria.
- Discuss nificance lucosuria.
- List onditions haracterized etonuria.
- List onditions haracterized ilirubinuria.

pH,
Protein,
Protein etermination eagent rips,
Protein etermination ulfosalicylic urbidity Test,
Urine rotein/Creatinine atio,
Interpretation rotein rine,
Glucose,
Ketones,
Measurement rine etone ontent,
Bile Pigments,
Blood (Hemoprotein),
Hematuria,
Hemoglobinuria,
Myoglobinuria,
Leukocytes,
Urinalysis Analyzers,
Key Points,

Bence Jones protein
Bilirubinuria
Glucosuria
Hematuria
Hemoglobinuria
Ketonuria
Proteinuria

Testing for various chemical constituents urine usually per formed with reagent strips are impregnated with appropriate chemicals r eagent lets. here tomated analyzers are used for serum chemistry be used for urine testing, although modifications of procedures may be required. Many of tests are performed concurrent with electrolyte testing, they are discussed further Unit The container f eagent rips ored oom emperature with ightly losed, xpiration be noted. Some reagent strips simultaneously test for numerous constituents, hereas rips or vidual reagent rip ped ly immersed; then be removed long edge tilted on paper owel llow xcess rine icked way. lternatively, urine e dded eagent rip om ette, sure ch eagent ly urated. olor hanges each eagent ed ecific ervals. on centration f arious onstituents etermined omparing olors n rip olor hart el strip ontainer ufacturer's ections be carefully followed. It important to note large number of onditions dications, tary ctors, nvironmental factors) ect inalysis esults Table

The xpresses ydrogen oncentration. tially, ure egree cidity urine. re hereas roper echnique btain

Reagent strip test container and combination dipstick strip.

accurate esults. ccu
rate esults btained.
open oom emperature ends esult
of bon xide, hereas elays eading eaction
lead to color changes readings. If contain
urease-producing cteria *Proteus* pp. *Staphylococcus* spp.) e ft ually eased.

The ys egulation
body. he ys ary ompensate
for ient's roducts tabolism.
of y epends ely
urine usually found consume plant-based diets,
whereas high-protein cereal diets or diets of origin
acidic urine. Therefore, herbivores normally have urine,
carnivores have acidic urine, omnivores have either acidic or urine, depending on what been ingested. Many dog
foods ontain ubstantial erial
ine htly ursing rbivores ve
acidic ine om onsumption ther ctors uch
ress xcitement ecially
y eate ransient **glucosuria** able
normal urine constituents characteristics, including for common omestic ecies.

> Herbivores normally have alkaline urine, carnivores have acidic urine, and omnivores have either acidic or alkaline urine, depending

Urine ually ured eagent rips
meter. actors ecrease cidity) lude ever,
starvation, h-protein diet, acidosis, excessive muscular activity,
administration of certain drugs. Increased (alkalinity)
may be caused by high-fiber diets infection of urinary tract with urease bacteria, the of certain drugs, or
urine retention such which occurs with urethral obstruc
tion r ladder alysis. oo
too ecific ystals orm.
be ed ve revent
them om orming.

Protein ually resent nly race
normal ine btained terization stocentesis.
healthy roteins lomerular
filtrate are resorbed renal tubules before filtrate reaches
enal elvis. owever, oided btained
expressing ladder ontain rotein
from cretions ontaminate uring
along inary ract. rauma inary ract esults
from stocentesis, terization, ladder xpression
occasionally ufficient leeding esults race
protein urine. Urine protein measurements are interpreted
light of collection method, urine specific gravity,
rate of urine formation, and contributions from hemorrhage
or ion ed ysis. rotein vels
urine y e ured veral thods, luding eagent
test rips ulfosalicylic urbidity

Urine icks low or miquantitative urement
protein rogressive olor hanges eaction
pad. eagent rip ysis onvenient, eason
ably accurate method of determining urinary protein levels. The
accuracy f thods ariable. eagent rips rimarily
detect albumin (protein soluble water), they are much
sensitive o globulins (proteins insoluble water). False-positive
results y ccur epending ctors uch
t, inary ract ection, etention ethral
obstruction). Protein measurements are considered excessive
or pathologic be confirmed by sulfosalicylic acid turbid
ity test or specific biochemical analysis. Microalbuminuria
presence of albumin urine detected by reagent
strip thod. eagent rip thod etects rotein
concentrations reater
capture enzyme-linked immunosorbent assay method
used o ure vels ee
Unit for more information about principles of tests.

Sulfosalicylic urbidity etermines rotein vels
acid precipitation. The resultant turbidity proportional to
concentration rotein. esults ompared vels
prepared tandards hus ay eported emiquantitative
The advantage of method equally sensitive
to umin lobulins, uite onfirm rip
methods, ecially ures
Bence Jones proteins hich e roteins
through glomerulus. Components of extremely

Effect of Drugs and Other Factors on the Measurement of Constituents in Urine

Drug or Variable	

, Value increased because of physiologic change; , value decreased because of physiologic change; interference with method; , value decreased because of interference with method or collection changes; sulfosalicylic acid

urine may interact with acid decrease of protein precipitated.

This test used to help confirm significant of protein urine. To determine significance, urine protein concentration be compared with of creatinine. The centrifuged to separate particulate matter (cells) from dissolved substances (protein), creatinine protein concentrations of supernatant are used. The ratio obtained by dividing the protein concentration by the creatinine concentration. The ratio affected by urine concentration volume therefore accurate assessment of urine protein patients with low specific gravity.

Urine Values for Common Domestic Species

The presence of protein urine **proteinuria** usually abnormal, primarily attributable to of urinary tract or possibly of genital system. Occasionally small of protein found urine of normal Transient proteinuria may result from temporary increase glomerular permeability allows excessive protein to enter filtrate. This condition caused by increased pressure glomerular capillaries, may be found with muscle exertion, emotional stress, or convulsions. Occasionally small of urine protein found after parturition, during first few days of life, during estrus.

Very dilute urine may yield false-negative result, because protein concentration may be below sensitivity of testing method. trace of protein very dilute may be clinically significant, because dilute urine often occurs when large volume of urine being produced, such patient with chronic renal failure. Trace protein very dilute indicates higher degree of proteinuria trace present normally concentrated

In proteinuria indicates of urinary tract, especially of kidneys. Both acute chronic renal lead to proteinuria. Acute nephritis characterized by marked proteinuria with white blood cells (WBCs) urine, whereas with chronic renal degree of proteinuria qualitatively However, with chronic renal urine output usually excessive with low specific gravity; therefore total protein excreted actually quite significant. The ratio of urine protein to creatinine used to determine degree of protein patient with chronic renal

Multiple myeloma, which cancer of cells, may produce large quantities of light chain proteins (Bence Jones proteins) may through glomerulus. In patients with myeloma, proteins may be passed in the urine because they have damaged glomerulus or because they are "light chains" freely through glomerulus. Because proteins do react with protein pads on reagent strips, sul fosalicylic acid method is necessary to detect and quantify them.

Mild proteinuria seen with passive congestion of kidneys, occurs with congestive heart failure or any other impediment of blood flow from kidneys. Proteinuria of renal origin may be caused by trauma, tumors, renal infarcts, or necrosis results from drugs chemicals such sulfonamides, lead, mercury, arsenic, ether.

Inflammation of urinary or genital tract may pro teinuria of postrenal origin. Proteinuria may be seen with traumatic catheterization or bladder expression.

The presence of glucose urine known glucosuria or glycos uria. Glucose filtered through glomerulus resorbed by the kidney tubules. The amount of glucose in the urine depends on blood glucose levels on rates of glomerular filtra tion tubular resorption. Glucosuria usually does occur normal blood glucose level exceeds renal threshold (approximately to for dogs). At concentration, tubular resorption keep with glomerular filtration of glucose, glucose into urine.

Glucosuria occurs patients with diabetes mellitus result of deficiency of insulin or inability of insulin to function. Insulin necessary to transport glucose into body cells, deficiency hyperglycemia spilling of glucose into urine. high-carbohydrate may lead to blood glucose levels exceed renal threshold thus glucosuria. Because of period of fasting recommended before urine glucose concentration determined. Fear, excitement, or restraint (especially often hyperglycemia glucosuria result of epinephrine release. Glucosuria often occurs after intravenous administration of contain glucose occasionally after general Rarely, glucosuria found with hyperthyroidism, Cushing's chronic liver rare condition called renal glucosuria may occur when blood glucose concentration normal range. Renal glucosuria caused by reduced resorption of glucose renal tubules. Glucosuria may occur some with chronic possibly result of altered proximal renal tubular function.

False-positive results for glucose may be seen after of various drugs, including ascorbic acid (vitamin morphine, salicylates (e.g., aspirin), cephalosporins, penicillin.

Clinitest Reagent Tablets for the detection of sugars in the urine.

Various eagent rips vailable etect lucose urine. Clinitest Reagent Tablets (Bayer Corporation, Leverkusen, Germany) Fig. are available. These tablets detect any sugar hereas eagent rips etect nly glucose.

Ketones include acetone, acetoacetic acid, -hydroxybutyric acid. etone odies ormed uring omplete f y ormal ve ketones blood. Conditions are characterized by altered carbohydrate metabolism may result excessive of catabolism rovide nergy. hen tabolism accompanied by sufficient carbohydrate metabolism, excess ketones e resent hich ondition led **ketonuria**

ommon etonuria etonemia, etosis, lactating cows pregnant ewes cows. Ketosis usually occurs early uring ctation eeks er eshening), hen nergy or roduction xceeds city ow to ingest sufficient feed meet energy requirements. In ewes, ondition led regnancy oxemia, en hen we rying riplets. etosis ciated hypoglycemia, bohydrate insufficient o eet nergy equirements. ody at hen apidly metabolized, hich esults etonemia etonuria.

Ketonuria frequently occurs with diabetes mellitus. Because lacks insulin necessary for carbohydrate metabolism, roken own et nergy needs, xcess etones xcreted etones important ces nergy, rmally roduced during at etabolism. owever, roblems evelop hen xces sive etones roduced. etones oxic entral nervous stem epression cidosis. cidosis esults from etonemia ermed etoacidosis.

Ketonemia etonuria ccurs starvation, fasting, long-term anorexia, impaired liver tion. ith bohydrates et elatively percentage nergy eds, reat meet nergy eds. ing, ved, rexic body used to meet energy needs, thus producing greater-than-normal etones. ver ed carbohydrate metabolism leads to serving energy source, especially when damaged liver store adequate f lycogen.

Urinary ketones are detected by urinary reagent strips with ketone reagent pad. The color intensity roughly proportional to oncentration etones. thods sensitive to acetoacetic acid; they are sensitive to acetone, they do detect -hydroxybutyric acid. -Hydroxybutyric acid st etone roduced ody ondition causes etosis. rine eagent est trips ay ot dequately dentify ients etosis een resent or

Bile pigments are commonly detected urine are bilirubin obilinogen. onjugated ilirubin er uble) found ine, ecause onjugated ilirubin oes through lomerulus enal rate; albumin, er uble. ormal ogs ecially males) ccasionally ave ilirubin heir rine **bilirubinuria**). because of low enal threshold for onjugated ilirubin ability of their kidneys to conjugate bilirubin. Many normal ve ilirubin ilirubin usually ound ep, rses. enal ogs; f ilirubin onsidered normal suggests

Bilirubinuria seen with number of including obstruction om ver estine well liver disease. Bilirubinuria results from the accumulation patic ells onjugated ilirubin eleased blood xcreted onditions iliary obstruction lude uct, umors of ile uct, cute nteritis, eatitis, bstruction upper intestinal tract. hen conjugated, bilirubin enters bloodstream er eing eleased om ed ver ells then

Hemolytic ilirubinuria, ecially ogs. n ients molytic ver's ility metabolize excess bilirubin may be exceeded, thereby result elease onjugated ilirubin lood ultimately ilirubinuria. ogs, onjugated ilirubin om

hemoglobin nonuclear hagocytic stem e onjugated

Bilirubinuria detected with Ictotest (Bayer Corporation). o ompound eagent let eacts ilirubin to roduce lue olor. eed hich color hange ccurs egree olor hange of bilirubin present. Reagent strips are sensitive Ictotest tablets. Ictotest tablet tests be performed to confirm ilirubinuria een etected ick Urine o e ested or ilirubin xposed because ilirubin roken own rt-wave negative esults or ilirubin ccur xposed sunlight r tificial

In intestines, bacteria convert bilirubin into stercobilino gen obilinogen. roducts xcreted eces, ut esorbed loodstream excreted y ver estinal ract. of esorbed obilinogen xcreted ys urine. Urobilinogen urine considered normal. The reliability f eening or etection obilinogen questionable esult ility obilinogen.

Tests for blood urine detect **hematuria** which presence of intact red blood cells urine; **hemoglobinuria** which presence of free hemoglobin urine; myoglobinuria, which resence yoglobin ematuria, hemoglobinuria, yoglobinuria may occur simultaneously; presence of one does rule out others. The urine sedi ment xamined or ct

Hematuria ually leeding somewhere ogenital ract, hereas moglobinuria usually indicates intravascular hemolysis. Some systemic condi tions may hematuria. In very dilute or highly urine, BCs ften yse ield emoglobin. herefore, ilute or hly moglobinuria esult of hemoglobin entering urine through glomerulus. cells lls ysed en uring scopic xamination diment ce moglobin ysis f xcretory way itro.

Moderate o lood loudy ed, brown, r ine olor olors ve rans parent pearance emains er entrifugation hemoglobinuria. ute lood isible olor hange ually vident. en blood ccurs hen bviously olored blood ut lood etected hemical ysis. ore or mation about hematuria found section on microscopic examination inary diment hapter

Hematuria, hemoglobinuria, and myoglobinuria can

Hemoglobinuria usually result intravascular hemolysis. Hemoglobin om roken own ravascularly rmally rotein toglobin. hen hemoglobin toglobin, oes ough glomeruli. If intravascular hemolysis overwhelms binding ability f toglobin, moglobinemia globinuria, ecause ee moglobin ers ough lomeruli. Hemoglobinuria ed ositive or moglobin without diment, egree reaction ften reater ccounted or umbers diment. hen moglo bin oncentration ufficiently red oloration, emains ed er entrifugation. If oloration ct clear ove ellet er entrifugation. artial learing er centrifugation moglobinuria maturia. The resence moglobin ither ee moglobin ust onfirmed ick ther evaluation y oscopic xamination.

Hemoglobinuria may be seen with many conditions intravascular hemolysis. Conditions intravascular hemolysis include immune-mediated hemolytic anemia, isoim mune hemolytic of neonates, incompatible blood transfu sions, leptospirosis, babesiosis, certain heavy metals (e.g., copper), and he ngestion ertain oisonous lants. ther onditions moglobinuria lude vere ypophosphatemia, postparturient moglobinemia molysis occurs hen uantities er er eing unable to obtain water (e.g., after long period of low tempera tures ozen er ce).

If ine ute ery moglobinuria originate om ysis ondition must be considered hematuria, because intact were initially present. Often ghost may be found when hemoglobinuria d y elease moglobin om itro.

Because or lood etects moglobin uria, hematuria, myoglobinuria, other considerations include sediment xamination, ory, hysical xamination dditional oratory rocedures etermine of ositive or lood ontamination reagent strips or collection containers with oxidizing agents, such leach, ositive or lood

Myoglobin rotein uscle. evere uscle damage yoglobin om uscle ells blood. yoglobin ough lomeruli xcreted urine. Urine contains myoglobin usually very dark brown to black, but low concentrations, urine may have color to seen patients with hemoglobinuria. Distinguishing myoglobinuria from hemoglobinuria may be istory uggest uscle help o etermine hether ositive moglobin by he resence yoglobin. yoglobinuria requently een rses ith xertional habdomyolysis.

Several thods ve een ry globin om yoglobin, ut thods ompletely reliable. These conditions may sometimes be differentiated on basis f heir ifferent olecular eights nd ifferent olubility nium ulfate.

Presumptive vidence ukocytes obtained ith ukocyte eaction ertain eagent rips. However, many false-negative reactions occur with species, oscopic valuation cessary onfirm ositive result. The leukocyte reagent strip test valid for because roduces ositive esults.

Analyzers or valuation are enerally miautomated ypes nly or eading ecording esults. yzers d inalysis eagent ick hich applied y echnician. ick aded analyzer, results are read recorded appropriate time Fig. Larger reference laboratories generally have fully automated yzers erforming reater number f valuations han he emiautomated nalyzers. any of yzers valuate ross haracteristics ., urbidity).

Automated urinalysis analyzer.

Chapter eview uestions ppendix

- The hemical ysis erformed reagent icks.
- Color ching olor hanges eagent used o etermine hemical alues.
- Tablet ests vailable or hemical ysis urine ually onfirm normality etected with ick
- The ected ient's
- The presence of protein urine usually abnormal, rimarily ributable inary ract.
- Glucosuria ketonuria are associated with diabetes mellitus.
- Bilirubinuria en ariety onditions, luding bile uct bstruction, molytic ver
- Hematuria, moglobinuria, yoglobinuria ccur simultaneously

After studying this chapter, you will be able to:

- Describe rocedure or reparing or oscopic examination.
- Describe rocedure or erforming oscopic examination diment.
- List ells ncountered diment, xplain nificance.
- List ystals ncountered sediment, nificance.
- Describe ormation significance
- List escribe asites ncountered urine diment.
- Discuss nificance cteria diment.

Dry-mount Urine Cytology,
Constituents of Urine Sediment,
Erythrocytes,
Leukocytes,
Epithelial ells,
Casts,
Crystals,
Microorganisms,
Parasite va icrofilaria,
Miscellaneous Components of Urine,
Mucus hreads,
Spermatozoa,
Fat oplets,
Artifacts,
Urolithiasis,
Key Points,

Ammonium biurate
Calcium carbonate
Calcium oxalate
Casts
Cellular casts
Crystalluria
Cystine
Fatty casts
Granular casts
Hyaline casts
Leucine
Renal epithelial cells
Struvite
Transitional epithelial cells
Tyrosine
Uric acid
Uroliths
Waxy casts

The microscopic examination of urine sediment important part of complete urinalysis, especially for recognizing of he rinary ract. any bnormalities rine ample annot be etected eagent rips lets, ften re specific information may be obtained by observation of urine sediment. In addition, urine sediment examination occasionally stemic microscopic analysis of urine sediment usually performed only hen ients mptomatic hen normalities evident physical chemical urine examinations. However, many veterinary practitioners routinely request urine sediment examination very

With xception rse abbit rmal urine f omestic oes ontain sediment. mall umbers pithelial ells, eads, ed blood ells RBCs), hite lood ells WBCs), **hyaline casts** nd crystals f arious ypes rmal animals. The urine of horses and rabbits ually has large amounts **calcium carbonate** ystals. rine ollected leanly, because cteria errant ubstances resent urine een ontaminated uring ollection.

The est or diment xamination rning or collected after several hours of water depriva tion. ecause uch re oncentrated, hances

the results. Report cells and bacteria in numbers per high-power

the average number seen in 10 microscopic fields or a range that represents

of ormed lements eased. ediment examined while urine fresh, because bacteria will multiply the ample s llowed tand oom emperature or eriod of ime. ther hanges ccur Crystals y orm ools, y ve oided ollected, ream referred, ecause ely ontaminated by ells, cteria, ebris om xternal enital urfaces. Urine collected by cystocentesis best for microscopic examination. xamined of ollection, efrigerated reserved.

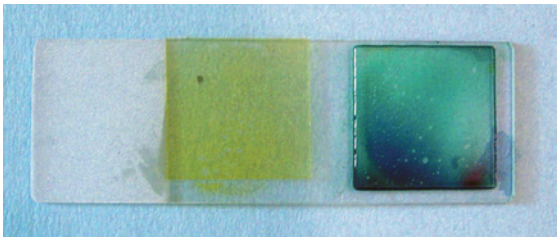
For miquantitative urements ormed lements ine, olume olume diment obtained ecorded. ufficient olume een obtained, ell-mixed ced raduated onical entrifuge ube entrifuged or utes proximately pm, epending adius f entrifuge. xcessive orce ompacts ment ort upt ormed lements. roce dure dized or ticular entrifuge ield uniform esults. fter entrifugation, olume diment ecorded, upernatant ently oured ve approximately ottom ube. sediment esuspended ently ottom centrifuge ube ers ently pipette rocedure

The ova diment stem ycor iomedical Garden rove, rovides thod or dization of ial olume, olume resuspend packed sediment, distribution of elements on e. ch ecimen rocessed ecially ed conical ic ube ed pening or ling. hen supernatant poured off after centrifuging, fixed volume retained along with sediment. The specially designed pipette then used to dispense fixed volume of resuspended sedi ment into special chambered slide for microscopic examination Fig. This unique system provides even distribution of microscopic lements roves isualization.

The sediment may be examined stained or unstained. Exam diment lows or etter valua tion f ecimen. diment, drop f uspended diment ced covered ith overslip, xamined diately. ubdued light partially refracts elements must be used to examine unstained diment. chieved tially hragm djusting ondenser ownward optimal ontrast chieved. oo uch resent, structures djustment microscope ontinuously djusted epth of bject ell ructures.

diment entify erent ell ypes. owever, ften roduce tifacts diment, ticularly precipitate material bacteria. vailable urine sediment include Sternheimer-Malbin (Sedi-Stain, Becton, Dickinson, Franklin thylene lue, which ontains ormalin. op ed ith uspended diment efore op sediment n oscope overslip ced ver drop f diment. lumination critical hen ecimen or unstained ne, educed lumination visualization of substances by providing contrast. The quan tifying f lements he ediment hould ever one ith stained e, ecause utes nificantly. thod inalysis rocedure prepare o ops diment microscope op dded e entify ells, hereas be d o uantify lements

The specimen must be initially scanned under low power objective ns) valuate verall uality reparation



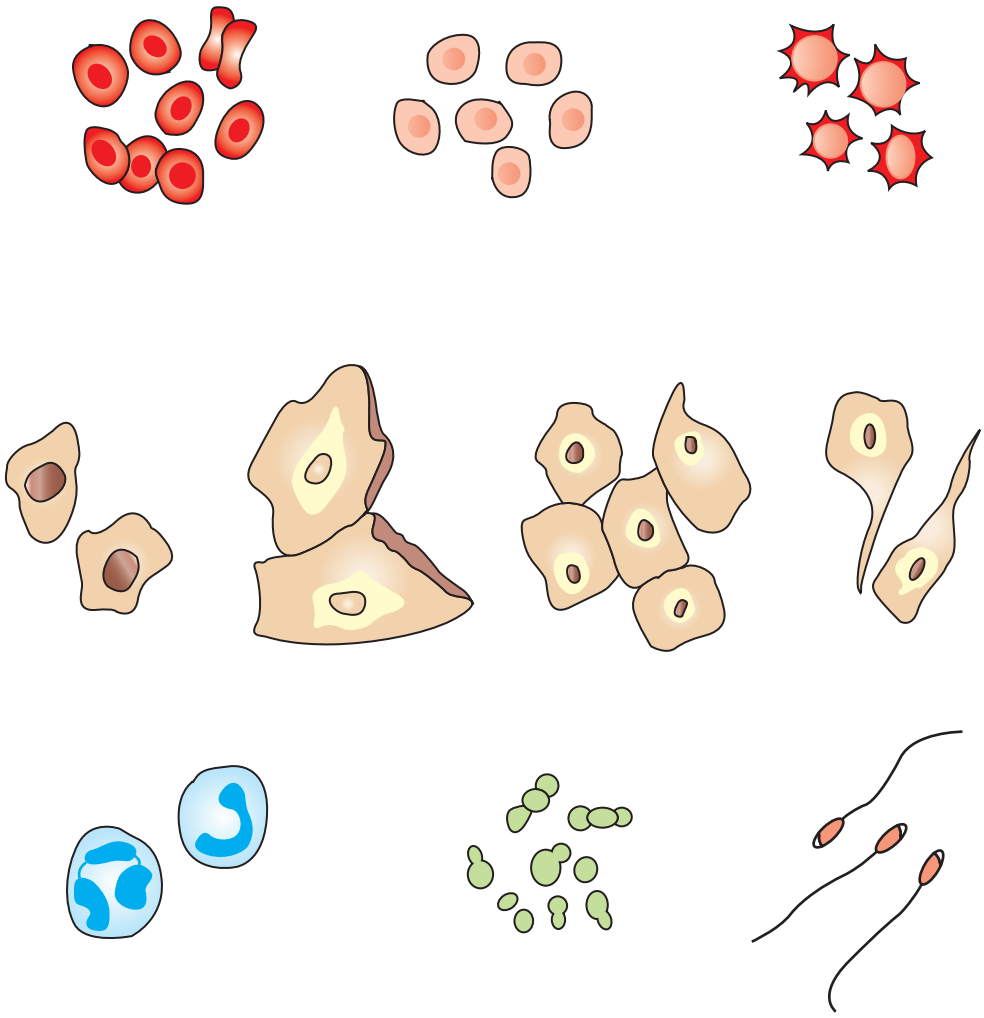
to identify larger elements, such or aggregates of cells. The entire area under coverslip be examined, because casts tend o migrate oward the edge the coverslip. Casts and crystals e entified eported umber bserved er low-power h-power bjective necessary o entify bjects ccurately, etect cteria, o erentiate ell ypes. oscopic fields be observed with high-power lens. Epithelial cells, eported verage umber bserved per h-power acteria eported ew, derate, many, their morphologic characteristics (e.g., cocci, bacilli) are ed. lternatively, lements eported ange en. or ells er h-power would ly very oscopic xamined had ell our. acteria ystals miquantified

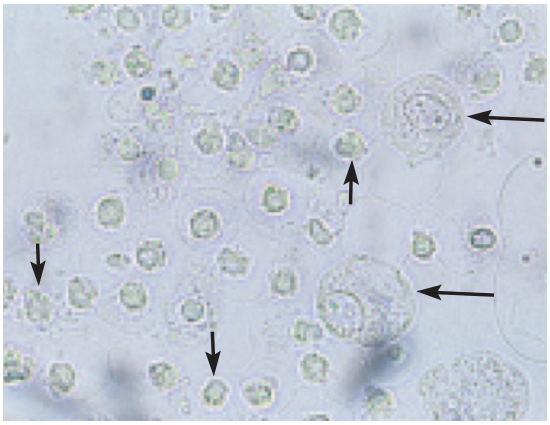
When cells appear abnormal with standard wet-mount prepara tions or when bacterial characteristics be readily evaluated,

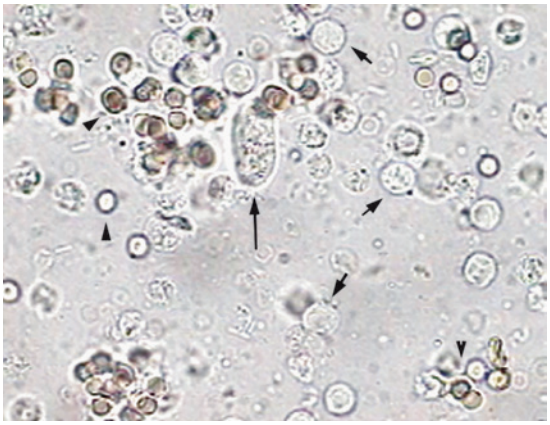
y-mount reparation de om ment. he rocedure escribed or (see Chapter Samples must be thoroughly air-dried so hat he ample dheres lide. ry-mount amples be xamined propriate tology added. f eels uring reparation e de rmal rum to efore dditional rotein added o om rum dherence uring

Normal ine diment ontain ew crystals, epithelial cells, mucus threads, r ecently red emales, ermatozoa. oplets, artifacts, ontaminants en. re few erythrocytes, leukocytes, hyperplastic or neoplastic epithelial cells, ystals, asite va, cteria, enti fied ine diment, onsidered normal, ther diagnostic ests erformed

Erythrocytes ve veral erent pearances, depending oncentration, lapsed between ollection xamination. are small, round, usually smooth edged, somewhat refractile, yellow r range, ut olorless moglobin d uring smaller ve iconcave e. oncentrated enate. Crenated ve uffled dges, htly ker, even pear ranular esult mbrane regularities. In ute r ell yse. wollen ve dges ellow range. ysed may appear colorless rings shadow cells" or ghost cells") ary owever, ysed ecially hen they esult om ked y—often ve be ound ith oscopic xamination. ormally ment ontains ess han wo hree BCs er igh-power eld. Because ontain ucleus, confused with globules or yeast. However, their light yellow or orange color usually allows them to be differentiated from other lements. urthermore, ariation whereas lobules ary ythrocytes ually indicate bleeding somewhere urogenital tract or occasionally genital system. voided from female proestrus or rus r er turition ontaminated Both emales ory onditions genital stem ve ollected ee ch or xpression ladder. rine ollected terization from emales ory enital ract usually ot contaminated, ut rine rom ales ith enital ract inflammation may be contaminated. Even slight trauma occurs esult terization, stocentesis, expression ladder htly umber diment. nerally stocentesis oes



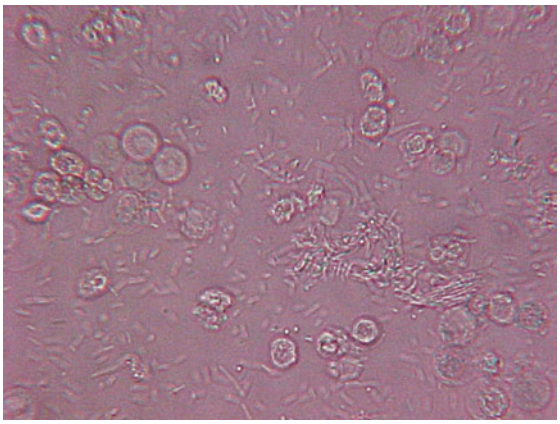




Atlas

and several
. (From

editors: *Clinical textbook for veterinary technicians,* ed 7, St Louis, 2009,



White blood cells and bacteria in unstained canine urine.

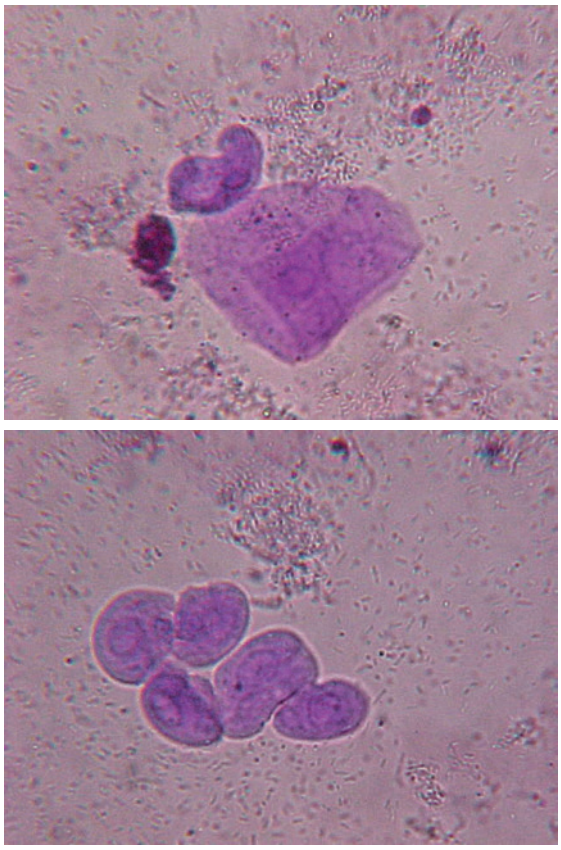
much increase numbers. The veterinary technician note thod ollection oratory eport to lp etermine nificance

Leukocytes (WBCs) are larger erythrocytes smaller

renal epithelial cells eukocytes herical ve dull ray r reenish-yellow olor. hey entified sediment by their characteristic granules or by lobulation of ucleus heir pearance ributable fact utrophils, hich ontain large number of granules. Few leukocytes are found in the urine of without urinary or genital tract shrink oncentrated ell ute eukocytes usually low numbers urine to per high-power field). re ee er h-power ory rocess mewhere inary enital tract. The term for excessive urine *pyuria* Pyuria ive ory ectious rocess uch nephritis, yelonephritis, stitis, ethritis, eteritis. rine with eased umbers ukocytes ured or bacteria, ven rganisms bserved oscopic examination.

few epithelial cells urine are considered normal occur esult f rmal ells. ked indicates inflammation. The three types of epithelial cells found inary diment ransitional, enal. differentiation ransitional om enal pithelial ells ften n eporting ells nsquamous thelial ells cceptable.

Squamous epithelial cells derived from urethra, vagina, vulva, or prepuce are occasionally found voided Their presence ually onsidered cells ith omogeneous ppearance re he argest ells ound ine diment. hey ften ve raight dges inct corners, which sometimes curl or fold Fig. They may have small, round nucleus. Squamous epithelial cells are normally found btained stocentesis terization.

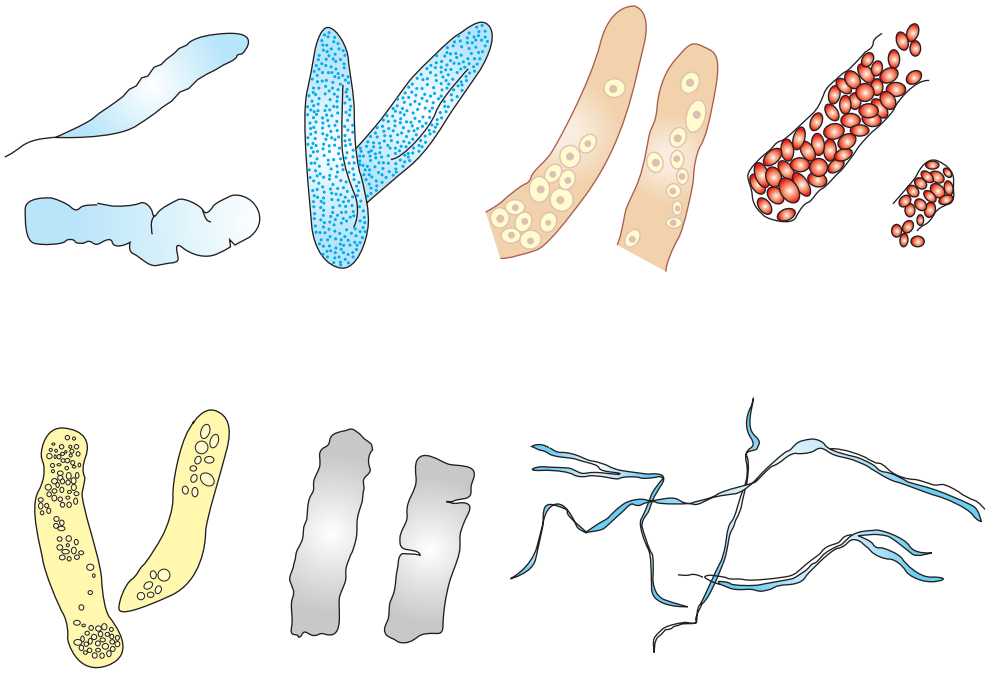


sitional epithelial cells in stained canine urine.

Transitional epithelial cells come from bladder, ureters, renal pelvis, proximal urethra. They are usually round, but y ear-shaped hey ranular, have l uclei, er ow numbers f ransitional ells er h-power may e ound inary diment esult of ells, ut eased umber uggests stitis yelo nephritis. Increased numbers may be seen catheterization d o btain

Renal pithelial ells pithelial ells bserved ine. hey riginate enal ubules, nly htly larger ften onfused enal epithelial ells enerally ontain ucleus ngranular ly ranular toplasm. hey arely found er h-power ncreased umbers ells ccur enchyma.

Casts are formed lumen of collecting tubules of y, here oncentration cidity greatest. n enal ubules, creted rotein recipitates acidic conditions forms are shaped like tubules hich orm. hey omposed rix rotein from mucoprotein secreted by tubules. They are

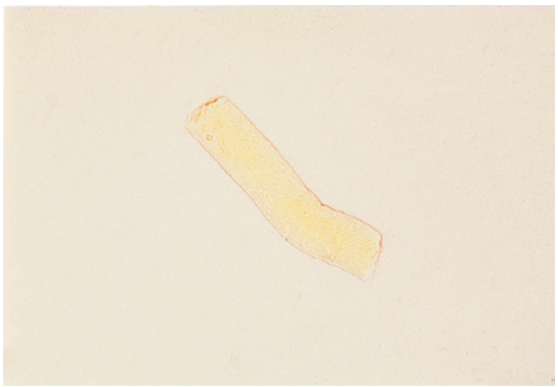


Various types of casts that may be found in urine.

commonly classified on the basis their pearance as yaline, epithelial, **cellular** pithelial ells, ranular, y, ed hich ype resent epends uickly rate ving ough ubules uch ubular resent. aster-moving rate ubule usually vident yaline lower-moving rate lows time or ells orporated trate ving ery wly, ells ill egenerate continues ough ubules; ill pear granular ranular esult hen ellular ebris from ed ubule ells orporated

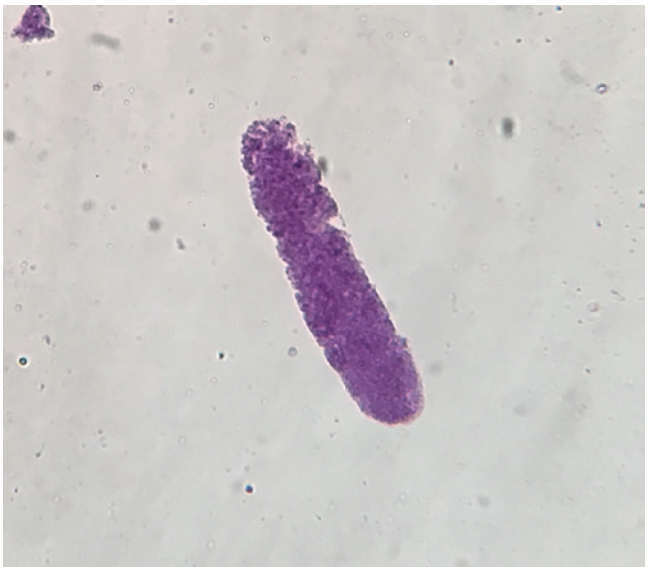
All e lindrical ructures allel their idth etermined umen hich they are formed. Their ends may be tapered, irregular, or round. Any cells or structures area may be incorporated into reby ting rphologic eatures low them o e ecifically entified ve urine, identification be performed fresh ve ecome ecause ve uickly arely en the ediment erbivores, hich haracteristically ave lkaline urine. asts upted h-speed entrifugation ough ling. ew yaline **granular** o er h-power en rmal urine, ut er umbers enal tubules. The number of observed reliable indicator of verity inary

Hyaline lear, olorless, mewhat ransparent structures omposed nly rotein. hey

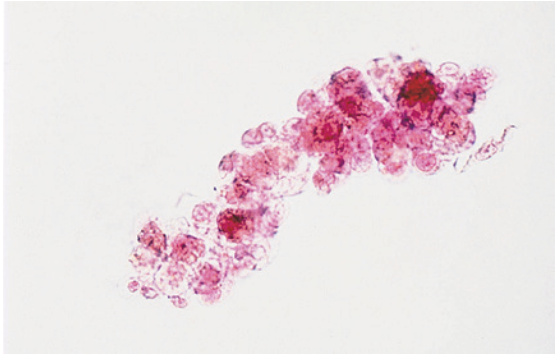


to e, ually entified nly yaline e lindrical, allel ually ounded ends Fig. They are easier to identify stained sediment unstained sediment. Increased numbers of hyaline indicate orm enal ritation. heir umbers increased with fever, poor renal perfusion, strenuous exercise, or eneral

Granular which are hyaline contain granules, are common type of seen Fig. The granules come from tubular epithelial cells, or became incorporated the cast and then egenerated. Cellular degeneration ccur ubules roduce ranular hich oarse pearance. ther rials eleased om ells inary ract ecome



Granular cast in stained canine urine.



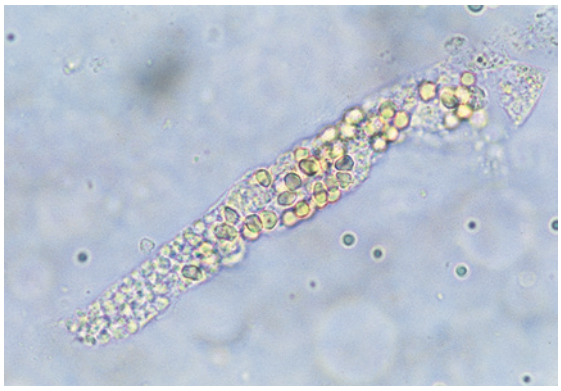
nostic cytology and hematology of the dog and cat,

embedded ranular en umbers with cute phritis re vere ompared yaline

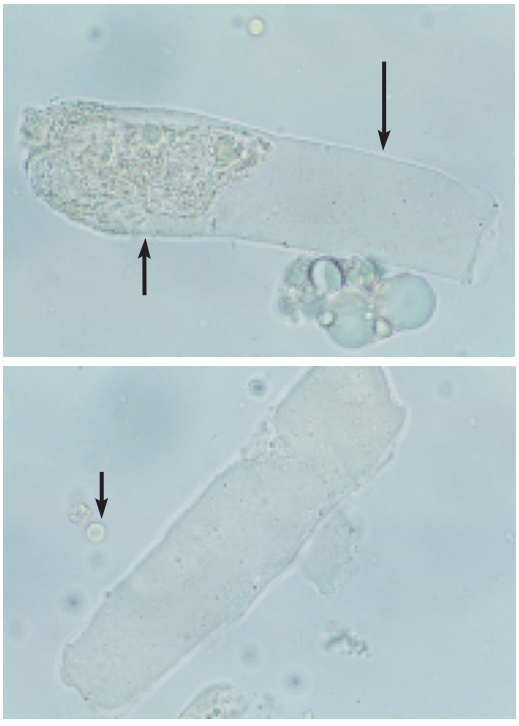
Epithelial onsist pithelial ells om enal ubules that ecome mbedded yaline atrix Fig. 9.12). pithelial cells ways enal pithelial ype, ecause nly pithelial ell resent ormation. These ormed pithelium ubules. They e en cute phritis onditions egeneration enal ubular pithelium.

Leukocyte contain predominantly neutrophils. These e eadily entified ellular egeneration occurred. he resence ukocyte inflammation enal ubules.

Erythrocyte eep ellow range olor. membranes may or may be visible. Erythrocyte contain



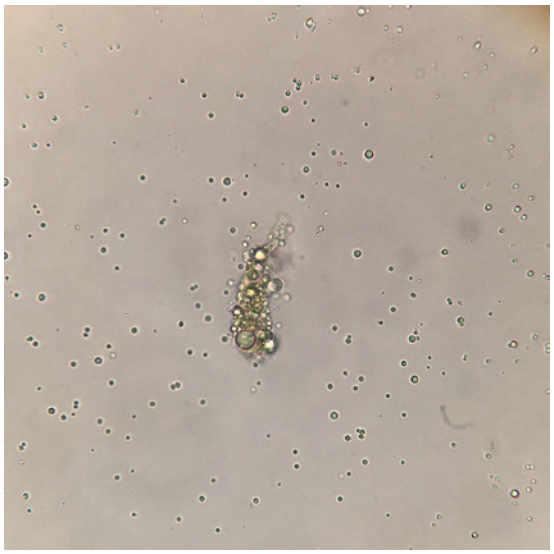
Atlas of canine and feline cytology,



Unstained granular casts develop into waxy casts as

orm hen gregate umen tubule . ythrocyte enal leeding. Bleeding rictly om morrhage esults om trauma r leeding rders, ccur inflammatory

Waxy casts resemble hyaline but they are usually wider, with square nds ather ull, mogeneous, pearance hey olorless ray highly refractile. They indicate chronic severe degeneration of enal ubules.



Fatty cast in unstained urine sample.

Fatty casts ontain oplets pear refractile odies. hey equently en with enal ecause ve enal en chyma. hey ccasionally en ogs etes litus. e umbers uggest egeneration enal ubules.

The presence of crystals urine called **crystalluria** Crystal luria may or may be of clinical significance. Certain crystals form consequence of their elements being secreted into urine y rmal enal ctivity ome ystals orm onsequence tabolic onditions to ystal ormation ormation inary he ypes ystals ormed epend concentration, emperature ubility ments Table lowed cool efore xamination, umber ystals increases, ecause erials ystals soluble wer emperatures. efrigerated ften ve many more crystals warm, fresh Sometimes crystals dissolve when refrigerated warmed to room tempera ture. rystals enerally eported ccasional, derate, or y lthough ystals **uroliths** e often entified rphologic haracteristics, nly definitive methods be used to identify crystals are x-ray diffraction hemical ysis.

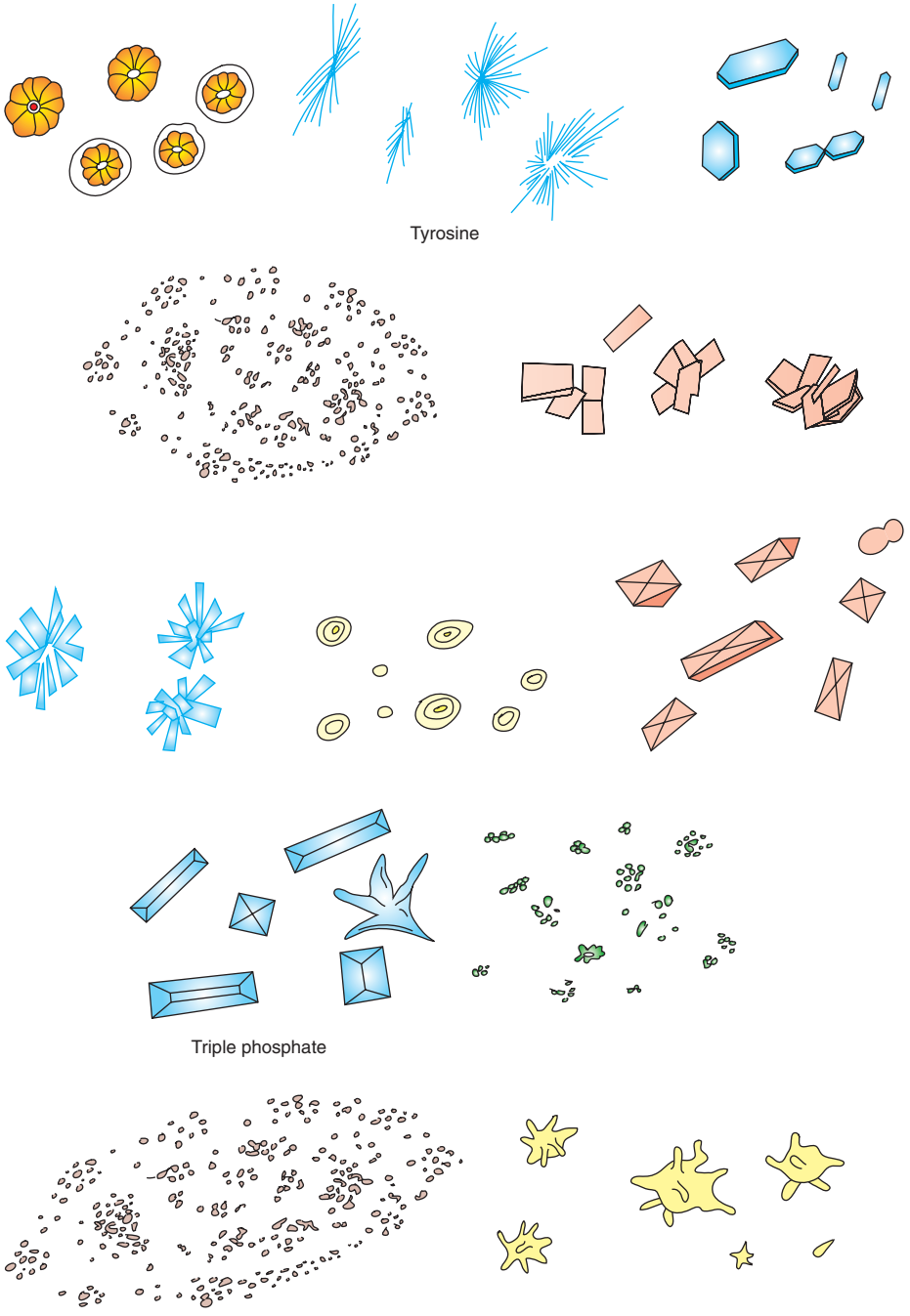
Struvite ystals e metimes eferred riple hosphate crystals or magnesium ammonium phosphate crystals. They are found to slightly acidic urine. Generally, struvite crystals are o ight-sided risms ering ruvite ystals ypically escribed esembling coffin ids, lthough hey ay ake ther hapes. ccasionally they y ern-leaf ecially hen contains oncentration

Calcium oxalate ydrate ystals enerally pear squares, and hey contain an X" across the ystal that resembles ck f nvelope alcium xalate nohy drate ystals umbbell-shaped, be longated ointed ch esembling from ket ence) alcium ydrate ystals are ound utral ommonly seen small numbers dogs horses. The urine of ve een oisoned thylene lycol ifreeze) ften contains umbers xalate ystals, ecially calcium monohydrate crystals. with oxalate urolithiasis may have large numbers of calcium oxalate crystals their urine, large numbers of oxalate crystals may indicate predisposi tion o xalate

Uric acid ystals e ariety ut ually diamond r homboid hey pear ellow yellow-brown, ommonly ogs xcept ogs).

Amorphous phosphate crystals are common urine appear granular precipitate Fig. morphous urates appear granular precipitate to amorphous phosphates (see . morphous ates en whereas rphous hosphates

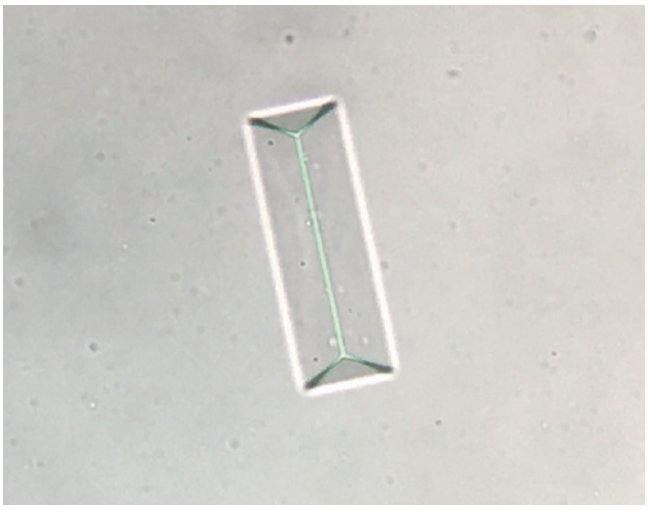
Calcium bonate ystals ommonly en horses abbits. hey adiating from ir enters, pear ranular Fig. hey ve umbbell hey linical nificance.



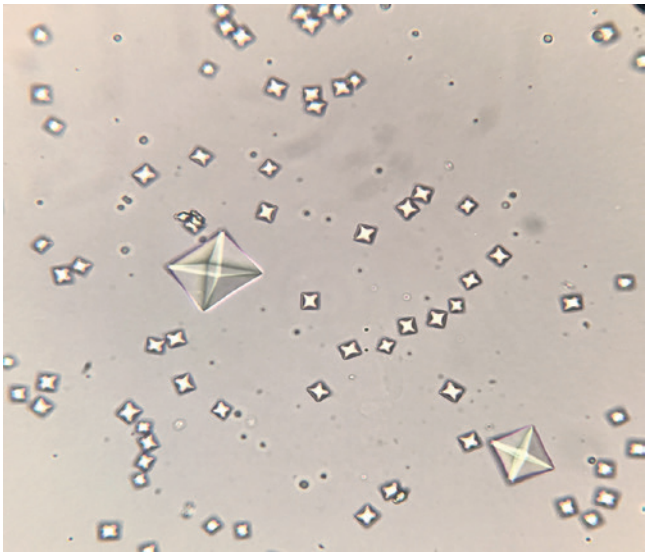
Crystals that may be found in urine.

Ammonium biurate ystals e en htly utral, or ystals rown olor, are round, with long, irregular they have thorn-apple" e) en acture remaining ystal rown, adiating hey ommon vere ver orta caval unts).

Sulfonamide crystals may be seen are being treated with ulfonamides. ulfonamide ystals ually dark, ith vidual ystals adiating om enter. hey are ely bserved ecause more uble revention recipitation of ystals enal ubules ed ine ncouraging

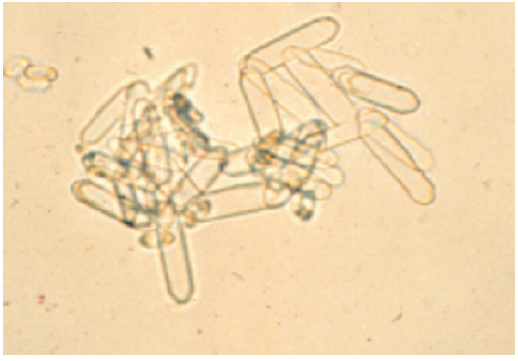


Struvite crystal that resembles a coffin lid shown in unstained

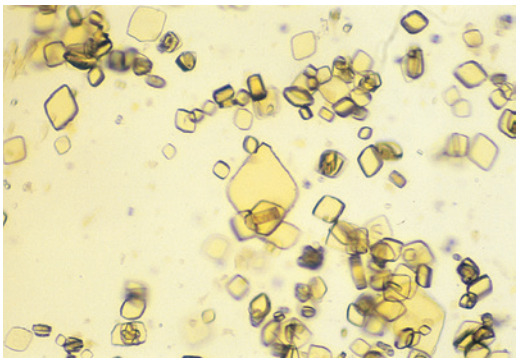


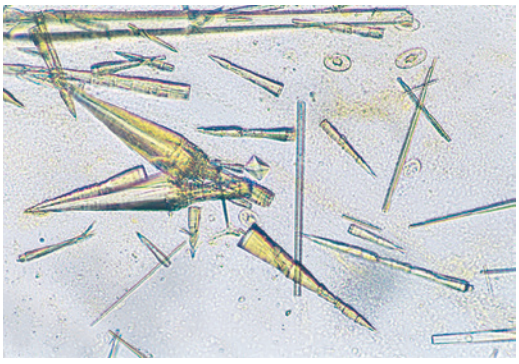
Calcium oxalate (dihydrate form) crystals in unstained canine

Bassert JM, editors: *Clinical textbook for veterinary technicians,*

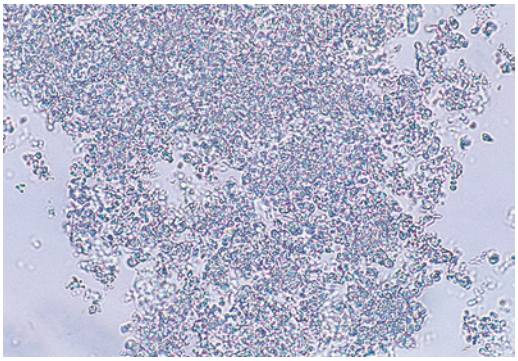


Calcium oxalate (monohydrate form) crystals in unstained canine urine. (From VanSteenhouse JL: Clinical pathology. In McCurnin DM, Bassert JM, editors: *Clinical textbook for veterinary technicians,*

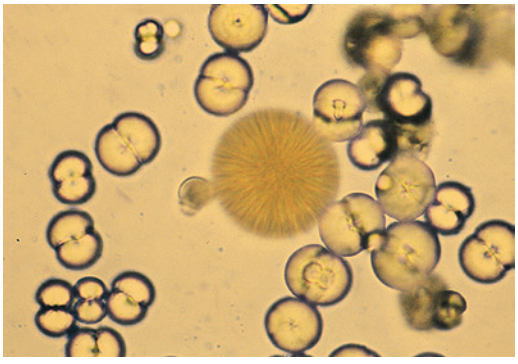


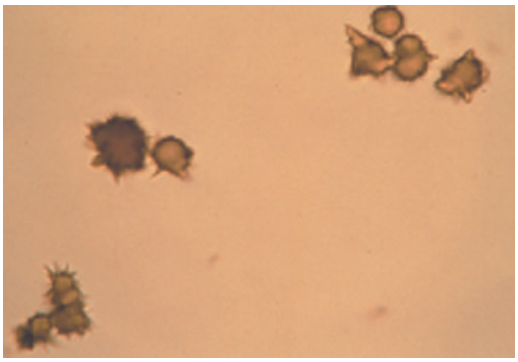


nium biurate uroliths. A calcium oxalate dihydrate crystal is also present

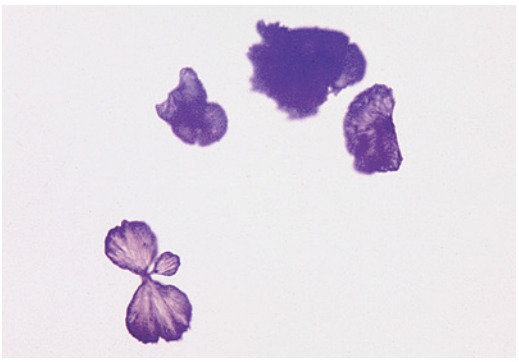


Amorphous phosphate crystals, unstained. (From Raskin RE,



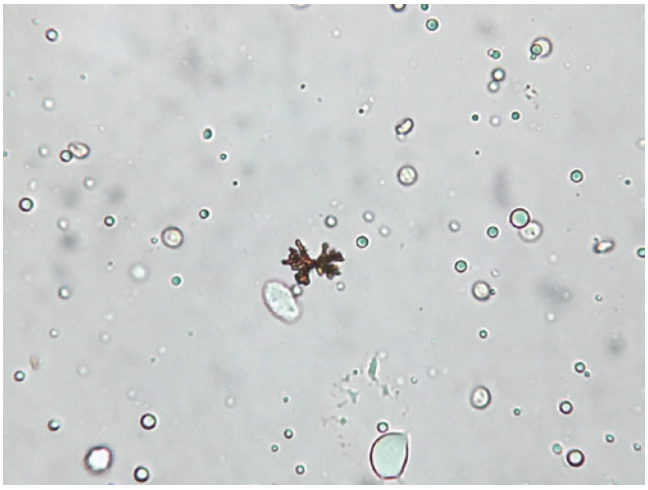


Unstained urine sediment with ammonium biurate crystals.

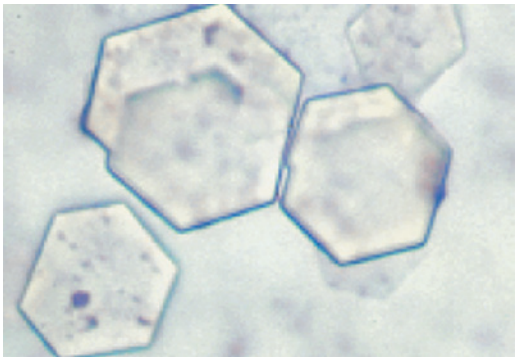


Diagnostic

JM, editors: *Clinical textbook for veterinary technicians,* ed 7, St Louis,



Bilirubin crystals in unstained urine.



Unstained cystine crystals. (From VanSteenhouse JL: Clinical

Golden-brown round to oval crystals with radial striations may be present have been fed diets contaminated with melamine r anuric

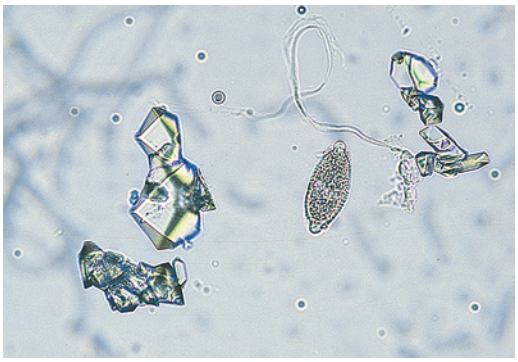
Bilirubin crystals may be seen normal acidic urine Fig. n ecies, resence ilirubin ystals abnormal vestigated for erlying rocess.

Leucine ystals e heel pincushion" ed ellow or rown olor ver may ve ucine ystals

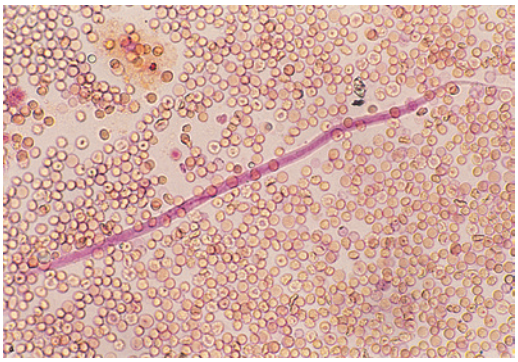
Tyrosine crystals are dark, with needle-like projections, they are hly efractile hey ften clusters. nimals ver ve yrosine ystals their urine. They are common dogs

Cystine crystals appear to be They are six-sided (hexagonal), colorless, Fig. They be associated with renal tubular ysfunction stine

ariety f oorganisms diment, including cteria, rotozoa. ormal ee bacteria, but may be contaminated by bacteria residing on epithelium agina, ulva, repuce uring ination. Normal ine ollected stocentesis terization oes contain bacteria therefore considered sterile. Because bacteria ften roliferate rine hat een eft tanding or some ime, ecially oom emperature, immediately xamined efrigerated xamined. Bacteria entified nly er nification. hey be ound occi) od-shaped cilli), ually efract pear uivering esult rownian vement. They e eported ew, derate, y, oo umerous count TNTC). umber cteria ccompanied large umber uggests ection ion of inary ract stitis, yelonephritis) enital tract ., rostatitis, tritis, aginitis). acteria are significant when they are identified within cytoplasm of These be submitted for cterial ure



Ova of Pearsonema plica in unstained urine sediment. (From



Yeasts e ften onfused oplets, ut they ually haracteristic udding, ve double efractile ually ontaminants because yeast infections of urinary tract are rare domestic ection xternal enitalia east o resent oided ungi found ine. ungi ntous ually ranching. Fungal ections inary ract ommon ut uite serious hen ccur.

Parasite va en diment urinary asites, esult om ecal ontamination ime ollection ome asites inary ract lude *Pearsonema plica* formerly *Capillaria plica*), hich s ladder orm ogs nd ats Fig. 9.27), nd *Dioctophyma renale*, which kidney worm of dogs. Microfilaria (e.g., *Dirofilaria immitis* y e en diment dogs ith dult tworms, culating ofilaria be en morrhage ccurs ither om or esult rauma uring ollection

Mucus eads ften onfused ut have well-delineated edges of They resemble twisted ribbon re rmally present quine ecause rses ve enal elvis eter. urethral ritation ontamination enital secretions.

Spermatozoa are occasionally seen urine sediment of intact hey ecognized ve significance. Sperm may be present recently bred females. Large of sperm urine may produce false-positive results or rotein.

In ine diment, oplets htly reen-tinged, hly refractile, herical odies arying ecause ary e, y inguished om hich tend o e orm diment or ew moments before being examined, droplets rise to just beneath overslip, hereas ormed lements op f herefore, oplets ften of focus of other formed elements. Small round structures found er overslip ually lobules. niformly sized round structures found lower are usually In sediment been stained with Sudan drop lets pear range ed olor. requently oplets om catheter ubricants om ily urfaces ollecting pipettes may contaminate urine. Fat urine, which called lipuria, en egree en with obesity, diabetes mellitus, hypothyroidism, rarely, after h-fat

Many rtifacts ay nter he rine ample uring ollection, rans portation, r xamination. ecognition ructures relevant rmal diment valuation. However, contaminants may be source of great confusion. Air bubbles, oil droplets (usually result of lubricated eters), starch granules (from surgical gloves), hair, fecal material, ores, ollen, otton ers, ticles bacteria, fungi may contaminate urine. The ova of intestinal parasites y bserved esult ecal ontamination ine

Uroliths are (stones) composed of various minerals are found anywhere urinary tract; their occurrence termed urolithiasis. They may blockage of urine outflow from ladder ethra; dge ethra severe cute ility inate; emain ladder ion leeding. etermining om position f itical, ecause revention animal's rognosis epend entification om position. fter omposition etermined, roper rapy may e iated emove revent eoc currence. rolithiasis ticular roblem rated

uminants. dging ethra bstructs tflow hich roblem steers, ticularly ed h-concentrate ations. The ommon ecies omposed calcium, nesium, nium bonate magnesium, nium hosphate.

The ysis ral omposition determined ubmitting ct eference oratory or quantitative ysis. casionally easonable resumption about omposition de ross and adiographic ppearance nd he rystal ypes ound he urine sediment. Uroliths of dogs are usually struvite, cystine oxalate uroliths may be observed. Urate uroliths are seen mostly Dalmatians, because breed excretes large f

Chapter eview uestions ppendix

- Subdued light must be used when examining sediment under oscope.
- The adjustment knob on microscope be con tinuously djusted arious ructures en.
- To entify erent ell ypes cteria ccurately, high-power bjective d.
- RBCs diment ve veral erent pearances, depending on concentration, time elapsed between collection xamination.
- WBCs e er entified characteristic ranules bulation uclei.
- Casts e ormed umen ollecting tubules f y.
- Casts e lindrical ructures allel present cidic
- Crystal ormation epends oncentration, emperature ubility lements.

Clinical Chemistry

Unit Outline

Chapter 30: Sample Collection and Handling,
Chapter 31: Automated Analyzers,
Chapter 32: Protein Assays and Hepatobiliary Function Tests,
Chapter 33: Kidney Function Tests,
Chapter 34: Pancreatic Function Tests,
Chapter 35: Electrolytes and Acid–Base Status,
Chapter 36: Miscellaneous Tests,

The objectives for this unit are:

List and describe the types of clinical chemistry analyzers available for the veterinary practice laboratory.
Describe proper sample collection and handling for clinical chemistry assays.
List the commonly performed clinical chemistry evaluations and the significance of abnormal test results.
Describe electrolyte and acid-base analyses and the significance of abnormal test results.

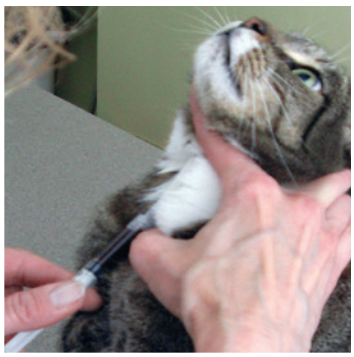
In both human and veterinary medical practice, current trends indicate a move toward greater point-of-care capabilities. This translates into better customer service, and it enhances the practice of veterinary medicine. Determinations of levels of the various chemical constituents in blood can be an important aid in the formu lation of an accurate diagnosis, the prescription of proper therapy, and the documentation of the response to treatment. The chemicals being assayed are generally associated with particular organ functions. They may be enzymes associated with particular organ functions or metabolites and metabolic by-products that are processed by certain organs. Analysis of these components usually requires a carefully collected blood serum sample. Plasma may be used in some cases.

Many veterinary practices own or lease chemistry analyzers to perform routine chemical assays. This focus on in-house laboratory work makes veterinary technicians' laboratory skills perhaps their biggest asset to the practice. As the person most likely to be in charge of the laboratory, the veterinary technician must become familiar with the types of analytic instruments available, the variety of testing procedures used, and the ratio nales underlying the analyses. The most important contribution that the technician can make to the practice laboratory is providing accurate and reliable test results. In vitro results must reflect, as closely as possible, the actual in vivo levels of blood constituents.

There are literally hundreds of biochemical tests that can be performed on serum samples. The average veterinary practice laboratory probably performs a few dozen of these. The more common ones are included in this unit. Additional tests that are commonly performed at veterinary reference laboratories are also discussed. Although the tests are categorized primarily by the organs involved, it should be noted that some tests may be affected by the function of more than one organ or system. For example, amylase has multiple organ sources, and protein levels can be affected by many factors, including liver and kidney damage, metabolic status, and dehydration.

For additional sources for this unit see the Resources Appendix at the end of this textbook.

Sample Collection and Handling



After studying this chapter, you will be able to:

- Describe roper rocessing or rum
- Describe roper rocessing or
- Discuss ffects uality esults.
- List ommon ompromise mechanisms

Plasma,
Serum,
Factors That Influence Results,
Hemolysis,
Chemical ontamination,
Improper eling,
Improper ample andling,
Patient nfluences,
Reference Ranges,
Key Points,

Hemolysis
Icterus
Lipemia
Reference range
Serum

Most hemical yses equire ollection reparation serum hole lood lood y e d for me est thods ecific ypes quipment. instructions ccompany hemistry yzers consulted or ype equired. ollection high-quality hich erform ect effect n uality esults. dverse nces uality voided eful onsideration ollection ling.

Chemical measurements be completed within after lood ollection. esting ill elayed, eezing ill reserve egrity onstituents. Freezing y erfere thods, wever. thawed, e-frozen. ertain icoagu may interfere with particular chemical analyses. Many factors other influence results of chemistry tests. These ctors reanalytical, ytical, ostanalytical (see nit hapter

Samples should be analyzed within 1 hour after

Specific lood ollection rotocols ary epending patient ecies, olume lood eded, thod restraint, ypes eded. lood or chemical esting ways ollected efore reatment iated. dministration ertain dications treatments ect esults iochemical esting. re prandial om en for referred. ostprandial collected er en) roduce rroneous results. amples en er ient en roduce alues or umber lood omponents, luding glucose, ea, egardless thod lood collection, eled diately er been ollected. ube eled time f ollection, wner's ient's ient's entification umber. ill submitted o oratory, lude equest orm on l cessary entification ion of hich ests equested. dditional ecific ormation regarding eneral lood ollection rotocols rder which o aw arious ypes resented Chapter

ortion lood hich ells are uspended. omposed proximately er dissolved constituents, such proteins, carbohydrates, vita hormones, enzymes, waste materials, antibod rocedure escribes method or btaining be ontaminated ells om ottom ube after entrifugation. entrifuged , ust efrigerated. parinized een stored overnight after separation or been frozen, e entrifuged emove rin rands may ve ormed. reezing ect ertain esults; test instructions be consulted for all of tests must be un efore ozen.

Always check the information provided by the analyzer

Serum om hich rinogen rotein) been emoved. uring lotting rocess, uble rino gen onverted uble rin rix. When lood ueezed cellular lot rum. eps or btaining rum escribed rocedure entrifuging eeds re

or refrigerate or freeze it, as appropriate.

o pm or rolonged esult **hemolysis** erum eparator ubes SSTs) ontain el hat orms physical barrier between serum or blood cells during centrifugation. ube ontain particles ctivation. lood ollected be mixed by inverting tube several times then allowing to clot for minutes before centrifugation. Serum separator transport tubes are available. These contain approximately ouble el SST. he dditional el rier erac tion between serum cells after centrifugation test esults ely ected elayed. ny prolonged elays esting equire rum emoved from ced erile ube. ube be efrigerated ozen. reezing ect esults; therefore, ructions onsulted or ust e efore rum ozen.

Many factors other influence results of chemis try ests. emolysis, **lipemia** **icterus** ertain dications, inappropriate handling all lead to inaccurate results. Effects f ompromise ummarized able

Hemolysis may be produced artifact when blood drawn into moist syringe, mixed too vigorously after collection, orced ough edle hen eing ransferred ube, r ozen lood ringe completely dry before used, because water syringe may molysis. edle om ringe emoved before lood ransferred ube. orcing lood ough small edle pening upture ells. hen ransferring blood ube, eterinary echnician xpel lood wly om ringe ithout ubbles form. emolysis esult hen xcess

*Variable effect, depending on the analyte and test method used.

clean he kin llowed ry efore eginning he lood collection rocedure.

Hemolysis, egardless reatly er eup of serum or For example, from ruptured blood cells dilute thereby resulting falsely lower concentrations onstituents ctually resent

Certain constituents are normally found high concentrations serum or escape from ruptured blood cells, thus falsely elevated concentrations

Hemolysis may elevate levels of potassium, organic phosphorus, and ertain nzymes lood. emolysis lso nterferes ith ctivity ilirubin eterminations. rum are frequently preferred types over whole blood, serum equently referred ver

Sterile tubes are necessary for collection of blood for routine chemical says. However, the ubes must chemically e. etergents ompletely insed om eusable tubes etergents erfere esults.

Serious rrors esult ube ontains eled diately er ollected. ube e eled ollection, patient's identifying number or The veterinary technician ouble heck entification equest form, one used, prepared test run.

Ideally, l hemical urements ompleted f ollection, ut ways easible. roperly led ored vels f hemical onstituents proximate ient's ody ollection. be lowed o ecome oo etrimental estroy hemicals ctivate rs (e.g., nzymes). rum een ozen, ust e roughly ed entle version er wing to void oncentration radients.

If ractical, btained om The blood glucose level be elevated, inorganic phosphorus vel ecreased, diately er ddition, postprandial lipemia results turbid or cloudy or serum. Kidney assays are affected result of transient increase lomerular ration ate er ing. ater ed e estricted efore btaining lood

Reference ranges e wn rmal alues. efer ence range for particular blood constituent range of values een erived hen oratory epeatedly yed from significant number of clinically normal of iven ecies ecific thods. herefore, efer ence anges er epending ecific thod yzer umerous gy books reference ranges of blood constituents for domestic species. lternatively, reference ranges may be formulated by local diagnostic oratories vidual ractice oratories. Appendix ontains eference anges or ommon iochemi onstituents.

Establishing reference range values for any laboratory time consuming xpensive. eference alues for laboratory, veterinary technician would have to assay from significant number of clinically normal Some investigators recommend analysis of rs ecommend ysis re with haracteristics. ther onsiderations lude variety f reeds ecies ften en eterinary practice; he ender nd eproductive tatus e.g., ntact, eutered) of tested environment, including husbandry utrition, limate onsideration, because astic hanges ect esults.

Chapter eview uestions ppendix

- Clinical hemistry esting ually equires ither rum
- Consult ufacturer ractice for roper ype.
- Samples must be nalyzed within hour to avoid hanges to ect esults.
- The ideal collected from preprandial patient before iation rapy.
- Common erferences lude molysis, emia, terus.
- Samples molyzed, teric, emic rovide inaccurate esults.
- Reference anges ary erent thods analyzers.

After studying this chapter, you will be able to:

- Describe rinciple efractometry.
- Describe rinciple hotometry.
- Differentiate etween tic oint ys.
- List eatures enefits ommon yzers.

Photometry,
End Point Versus Kinetic Assays,
Units of Measurement,
Ion-Selective Electrode and Electrochemical Methods,
Features and Benefits of Common Chemistry Analyzer Types,
Instrument Care and Maintenance,
Key Points,

Beer's law
End point assay
Ion-selective electrode
Kinetic assay
Optical density
Reflectometer
Spectrophotometer

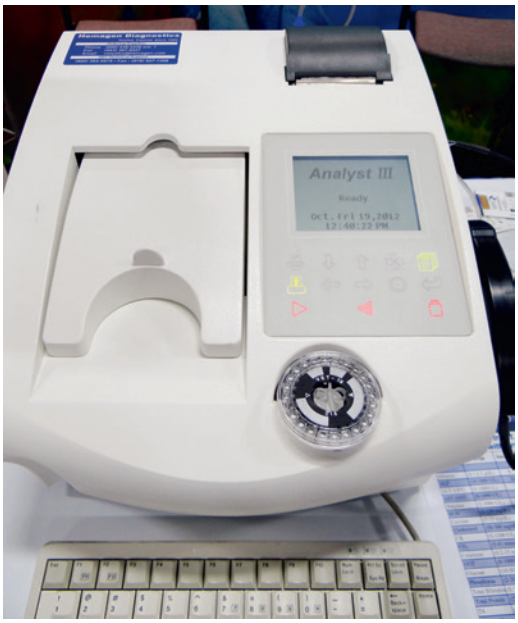
variety of different chemistry analyzers are available for eterinary ractice oratory. eterinarians etter to nose nitor ient rapy hen esults are available immediately. Most chemistry analyzers used veterinary practice principles of photometry to quantify onstituents lood. nalyzers lectro chemical thods vailable or

Several ypes hotometers nostic equipment **Spectrophotometers** e esigned measure of light transmitted through solution. The basic components spectrophotometers are same regardless of ecific ufacturer quipment. ll ectropho tometers ontain ight ource, rism, avelength elector, photodetector, readout device Fig. The light source ypically ungsten gen rism tions fragment light into component wavelength segments. The majority of spectrophotometric tests wavelengths are isible ortion lectromagnetic ectrum. ew are vailable velengths -infrared ultraviolet portions of spectrum. The wavelength selector usually nly lows ecific velength to o hotodetector eceives hatever light rbed hotodetector n ransmitted eadout evice. epending model f rument, eadout ercent transmittance, percent absorbance, **optical density** or concentra tion Some automated analyzers variations of photometric procedure. The type of photometer filter to lect velength eferred olorimeter. nother type etects eflected ubstance ather transmitted light. This type referred to **reflectometer** Fig. eferred eflectance ys.

The velength or urement lood onstituent hosen rbance properties f onstituent eing ured. velength of ht hosen or iven urement esults reatest rbance transmission) ough or ution appears blue-green, will transmit greatest of blue-green light, whereas light red portion of spectrum will be rbed. herefore, velength ed ortion spectrum rovides rbance or urement omponent. or ution sured ith ectrophotometry, ution dhere principle f **Beer's law** hich wn eer–Lambert's

law. This principle states direct relationship exists between concentration of analyte light absorption when monochromatic light light of single wavelength) passed through The law states transmis sion of monochromatic light through concen tration of analyte have inverse exponential relationship. The degree of color change proportional to solution's concentration.

Most photometric analysis procedures are **end point assays** In other words, reaction occurs between reagent reaches stable end. The analyzer then either one-point calibration or internal standard curve to calculate patient results. Either method requires of standard. standard nonbiologic solution of analyte, usually distilled water, with known concentration. For one-point calibration, standard analyzed concurrently manner patient reaction characteristics are

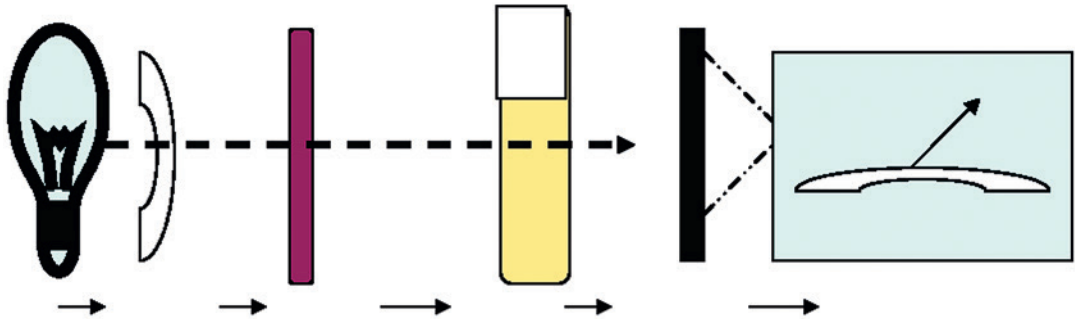


Diagnostics, Columbia, MD.)

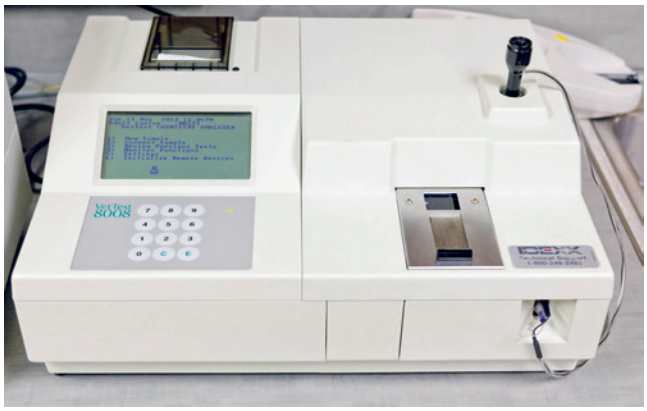
mathematically compared with patient The specific type of calculation varies, depending on the analyzer. In general, however, ratio of optical density of reacted standard is compared with the OD of the patient sample. OD is logarithmic function describes degree to which light transmitted through medium. An example of type of calculation follows:

The internal standard curve created when analyzer calibrated. To perform standard curve, serial dilutions of standard solution are created, each analyzed to determine absorbance or transmittance of light. The results from each dilution are plotted on graph straight The concentra tions of subsequent patient are determined by locat intersection of absorbance of reacted patient with on graph. Analyzers standard curve methods must be recalibrated each time new reagent purchased.

Some analyzers kinetic methods rather end point methods. These are primarily used for enzyme assays or when reagent enzyme based. Enzymes induce chemical changes other substances (called substrates), but they are inher ently changed. An enzyme may increase rate of biochemical reaction by acting catalyst to reaction. Most enzymes are formed function intracellularly, they are found their highest concentrations within cells. For reason, blood level of enzymes low healthy The blood level of enzyme may be elevated enzyme leaked out of damaged cells or cells have increased production of enzyme excess leaked out of cells into blood. Each specific enzyme catalyzes reaction of one specific substrate. Each enzymatic reaction produces specific product from interaction of substrate enzyme. The reaction forms product, but there change enzyme. Because blood levels of enzymes are low, directly measuring enzyme concentrations The tests performed to deter enzyme concentrations blood indirectly measure enzyme concentration present by directly measuring rate of formation of product of enzymatic reaction



Principles of spectrophotometry.



tion of chemical components in the sample.

or ate f eduction ubstrate. **Kinetic assays** o reach le oint. eaction esults ecorded specific time after initiation of reaction even though reaction ontinues eyond hat ime. easurements hat re ot made orrect ually ccurate. oint calibrations enerally erformed or tic ure ments. However, several points be evaluated, change absorbance for both standard patient be d o ient esults. hen ves created or tic thods, raph eated hoosing reaction time during which graphed rates of absorbance form lose raight ossible. ecific after iation eaction hen eaction to erent or ch yte.

Enzymes e ctive hen ubstrate oncentration h roduct oncentration ero. nzyme concentration patient exceeds substrate avail able est eagents, nzyme ctivity nger proportional roduct ormed, valid. (The ubstrate oncentration ecome ctor or nzymatic eaction.)

Substrate oncentrations ept nough do validate urement. matic/kinetic are manufactured large of substrate initially present to avoid problem. If of enzyme present ient oubled, ate eaction oubled roduct ormed oubled, long onstant. nzyme resent ient ut oubled, roduct oubles. herefore, enzyme oncentration ept onstant, ate reaction y etermined.

The ortant ameters nce roper measurement of any enzyme are time temperature. Enzyme activity ontinues ubstrate vailable. measurement be recorded appropriate time reac tion. Enzyme activity may be inhibited by low temperatures accelerated by high temperatures. Other factors interfere with enzyme activity include ultraviolet light presence of of vy tals opper, rcury). roteins, they may be denatured by temperature extremes or by rganic vents. ven hange nzyme's oly peptide hain ructure ctivity, for nzyme ys led

Each nzyme ptimal emperature hich orks efficiently. This temperature typically listed instruc tions ccompany yzer. ys performed temperatures of between to For every above optimal temperature, enzyme activity doubles. Close monitoring of incubator or er emperature nzyme ys ortant.

Enzyme Units Into International Units

Enzyme oncentrations ured ctivity. f urement onfusing. or nzyme activity proportional to enzyme concentration only under certain conditions. Each investigator who developed enzymatic analytic thod ned wn urement to esults, ften eflected eveloper's Examples lude odansky, omogyi, igma-Frankel Because each of assays performed under different conditions (e.g., temperature), correlation of results reported in one unit ith those of another unit ecame difficult. To void his onfusion, he nternational nion iochemistry established of enzyme activity known International Unit r ccording stem, nzyme oncentra tion xpressed liunits er liliter er liter or per milliliter International Unit efined nzyme er iven conditions ticularly emperature ill yze conversion omole ubstrate er ute. ox shows w onvert arious nternational Some oratories ve eplaced nternational stem with ne etter elated ystème nternational

t f hich tric stem. stem, nzyme ctivity, reflects of activity converts of substrate per cond.

Enzymes e ually or ubstrate hich act r iochemical eaction hich ticipate. enzyme or nzyme yzes iochemical eactions esult ydrolysis ctate ehy drogenase ticipates eaction volves xidation or ehydrogenation ctate yruvate.

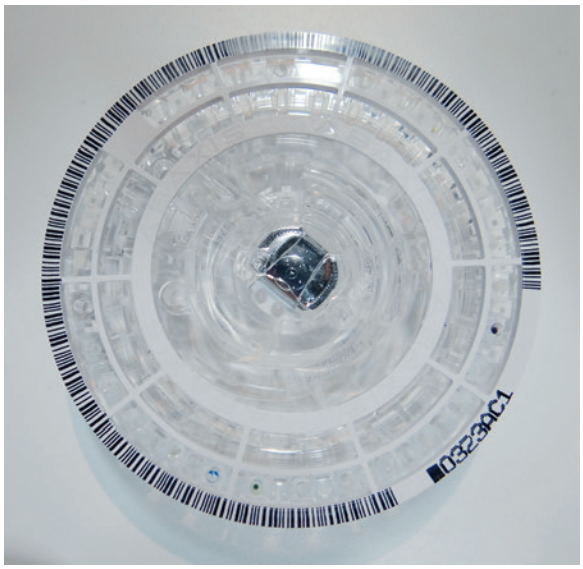
Some nzymes erent issues ccur isoenzymes. enzyme roup nzymes ytic ctivities ut erent hysical roperties. serum concentration of enzyme occurs isoenzymes otal f oncentrations enzymes resent from arious issues. entification hich enzyme present lows or entification source of particular isoenzyme. For example, serum phosphatase issues, ticularly eoblasts patocytes. rum hosphatase vel levated, ertaining hether om ed bone ells r ed ver ells ossible. owever, respective levels of various isoenzymes of phosphatase are assayed, levels of isoenzyme from damaged tissue are elevated, reby entifying ed issue. phosphatase assay performed practice laboratory for total serum phosphatase, because individual isoenzyme assay methods ve et een eveloped or ractice ora tory. nother linically enzyme eatine he enzymes idely ributed issues, with hest ctivity rain, diac, eletal uscle. Fractionation of into three primary isoenzymes, (brain type), (hybrid type), (muscle type) more sensitive indicator of specific pathological conditions.

to measure blood gases and electrolytes. (Courtesy International Tech

Some analyzers related principles of electrochemistry or **ion-selective electrode** echnology etermine yte concentrations. ew yzers ombine lectrochemical photometric ethods ithin elf-contained artridges. lectro chemical nd ethods re ost ften sed or valuation of electrolytes other ionic components. These types of tests vary considerably configuration, but they function manner Fig. analyzers, which are sometimes referred to otentiometers, esigned ecific lectrodes are onfigured low or eraction or each ion being evaluated, analyzer contains electrode ecific or wn oncentration This referred to reference electrode. The interacts with cond n-specific lectrode eates lectrical potential or voltage difference. The difference electrical potential between two electrodes proportional to concentration of ions Electrochemical analyzers incorporate elec trodes within biosensor reagent strips or cartridges. The interacts ith eagents regnated iosensor device creates measurable current corresponds to ion oncentration

Most tomated yzers eagents, eagents, slides ontain eagents. eagents chased ulk ized osable vettes. eagents are vailable ized orm. eagents expensive, but they require additional handling storage space. Some reagents are flammable toxic. The purchase of unitized reagents eliminates hazards are associated with handling eagents. eagents storage oncerns ut end re xpensive.

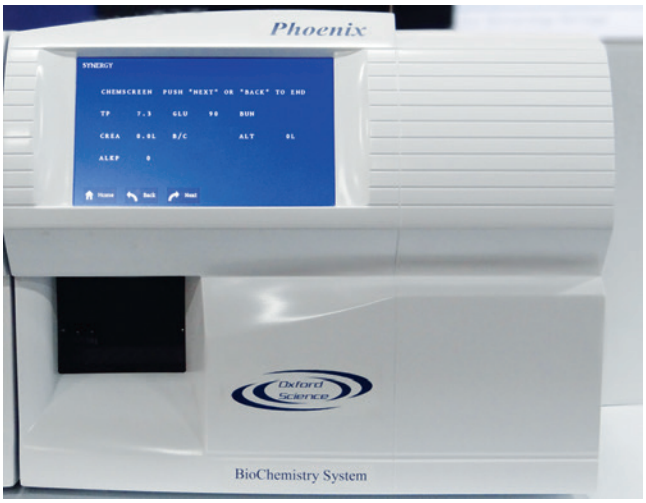
can be loaded into the analyzer at one time.



Reagent rotor for use in some liquid chemistry systems.

Analyzers stems lude eagent-impregnated slides, pads, or cartridges. Most of reflec tance assays. Dry systems tend to have comparatively higher costs associated yzer ypes. any configured for veterinary species have fairly high incidences of ejection, ticularly emic or molyzed ome om owever, do ve enefit equiring eagent ling, performance of single tests relatively Running profiles large numbers of tests on single patient on some of types of systems tends to be bit more time-consuming ompared yzer ypes. ew type yzers low or ading umbers nce, reby equired repare analyzer o valuate rofile ome stems reagent rips or hemical testing.

Liquid stems lude yophilized reagent or already prepared liquid reagent. The common type of lyophilized reagent system for veterinary clinical practice rotor technology. The rotors consist of individual cuvettes to which iluted amples re dded Fig. 1.7). uvettes re ptical-quality reservoirs used photometer, they may be plastic or glass. Rotor-based systems tend to be quite accurate, although some are configured for veterinary species. They are usually cost-effective for profiles, but they not configured to run ngle tests. ther stems ommon lude unitized eagent vettes eagent. ized stems have dvantage equiring eagent ling, ut tend to be expensive of all of liquid reagent systems. In ddition, rofiles stems mewhat time-consuming, but single testing Bulk reagent systems may supply reagent either concentrated form, which must be diluted, or working strength. orking-strength reagent systems do usually require any special reagent handling. These analyzers are ersatile erform ither rofiling or single testing with relative Fig. Most require preparation owever, ew ve xtensive enance



time, ticularly ration ameters. ome systems eagent ve ell ead cuvette. ample nd eagent an spirated irectly hrough he analyzer ithout ed or ransfer eactants cuvettes. Regardless of test methods or tests available for ecific yzer, ly ufacturers rovide ability to integrate all analyzer types hematology, chemistry, blood oagulation, lectrolytes) ftware stem enables results to be recorded automatically integrated with patient ecords

Dedicated-use yzers vailable or ertain any of tilize lectrochemical echnology or nly one ubstance, uch lood lucose edicated analyzers nly equested emergency uations.

Fig. 31.9 Most analyzer manufacturers allow for the integration of multiple analyzers with programs to record results in patient records.

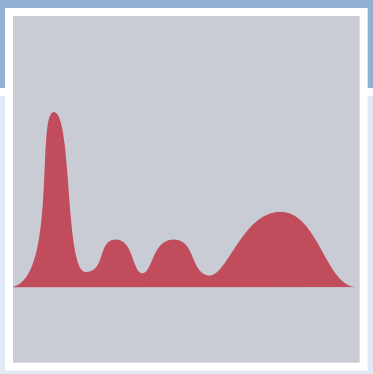
over the counter in many pharmacies.

Chemistry yzers nsitive ruments carefully eterinary echnicians ollow manufacturer's operating instructions. Instruments generally have m-up eriod low ce, hotodetector, incubator, resent, each quilibrium efore Ideally, oratory ersonnel urn rument rning ve y. rument re fore eady o uring y, ecially uring emergency uations.

Following manufacturer's maintenance schedule prolongs e f hemistry yzer. hedule et established or ch rument oratory low or uick eview enance ory instrument. Most manufacturers have toll-free number to call roblems

Chapter eview uestions ppendix

- Most linical hemistry yzers ectrophotometric methods rinciples eer's w.
- Analyzers reflectance methods are widely available.
- Chemistry ys oint tic thods.
- Electrochemical yzers rimarily or lectrolyte assays.
- Chemistry yzers ve erent dvantages, enefits, limitations.



Protein Assays and Hepatobiliary

After studying this chapter, you will be able to:

- List otential erations rum roteins.
- Describe ommonly erformed ure protein
- Describe ommon thod or etermining lobulin concentration.
- List ommonly erformed or valuation patobiliary stem.
- Describe tabolism ilirubin.
- Differentiate etween onjugated onjugated bilirubin.
- List nzymes, nificance altered nzyme ctivity.
- Describe culation nificance of ered ile ctivity.

Protein Assays,
Total rotein,
Albumin,
Globulins,
Albumin/Globulin atio,
Fibrinogen,
Acute-Phase roteins,

Hepatobiliary Assays,
Hepatocyte unction
Enzymes eleased rom ed epatocytes,
Enzymes ssociated
Other ests ver unction,

Key Points,

Acute-phase proteins
Alanine transaminase
Albumin
Alkaline phosphatase
Aspartate transaminase
Bile acids
Bilirubin
Cholesterol
Conjugated bilirubin
Gamma glutamyltransferase
Globulins
Glutamate dehydrogenase
Hepatoencephalopathy
Hyperlipoproteinemia
Hyperproteinemia
Hypoalbuminemia
Hypoglycemia
Hypoproteinemia
Iditol dehydrogenase
Jaundice
Protein

Although rotein ssays re ot pecifically onsidered iver unc tion ests, rity **proteins** e roduced liver. dditional roteins roduced system eticuloendothelial issues, ymphoid issues, ells). roteins ve tions ody, alterations rotein oncentrations ccur ariety of conditions, especially of liver kidneys. More proteins exist. Some protein con centrations change markedly during certain be used nostic ther rotein oncentrations hange during ge-related changes protein con centrations en. ajor rotein tions summarized ox rotein hemical ys e ommonly erformed eterinary lude total rotein, **albumin** rinogen.

Total rotein urements lude rinogen alues, whereas otal rum rotein eterminations ure protein actions xcept rinogen, hich emoved uring lotting rocess. rotein oncentration affected y ered patic ered rotein ribu tion, ered rotein reakdown xcretion, ell dehydration verhydration.

Total protein concentrations are especially valuable for deter mining n nimal's tate ydration. ehydrated nimal sually elatively levated rotein oncentration **hyperpro teinemia** whereas overhydrated usually relatively decreased total protein concentration **hypoproteinemia** Total protein oncentrations eening for ients dema, rhea, eight patic enal lood lotting roblems.

Two thods ommonly or etermination total rotein vels: efractometric thod iuret photometric thod. efractometric thod ures refractive ex rum efractometer Procedure efractive ex tion of oncentration ticles primary are proteins. This method good screen est, ecause xpensive, ccurate. iuret method ures umber ontain re ee eptide onds rum thod commonly ytic ruments oratory. thod ields ccurate esults. ther hemi ests o ure rotein lude ye-binding thods precipitation methods. These tests are commonly performed eterinary ractice. hey ually ure f rotein erebrospinal ecialized tests to separate various protein populations are performed some reference laboratories research facilities. These methods include fractionation, chromatography, gel electropho resis Fig. These tests be performed on other rum erebrospinal ther tests e ariety ody lude ulfosalicylic andy ulfosalicylic acid est escribed hapter

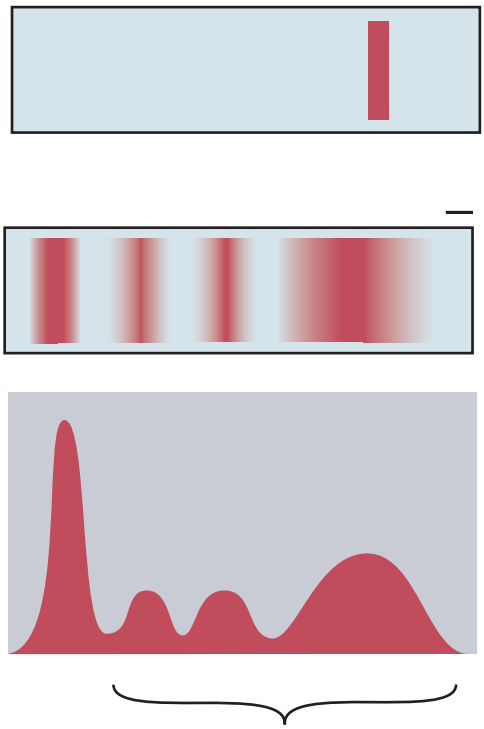
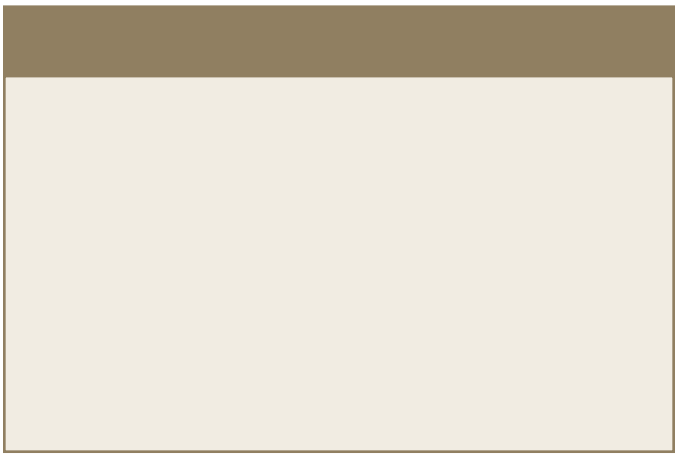


Fig. 32.1 Schematic diagram showing the results of the electrophoresis

Albumin ortant roteins serum. t rotein ypoproteinemia ely epatocytes albumin, ver esult ecreased albumin enal tary estinal protein absorption may influence albumin level. Albumin major binding transport protein blood, responsible for osmotic pressure of The primary photometric test for albumin veterinary patients romocresol reen ye-binding thod. Box summarizes conditions are associ ated ith erations rum roteins.

The **globulins** are complex group of proteins. lpha globulins are ed ver rimarily ransport proteins. Two important proteins fraction are high-density lipoproteins ery-low-density oproteins. eta lobulins include complement transferrin, ferritin. They are responsible for iron transport, heme binding, fibrin formation lysis. globulins (immunoglobulins) are synthesized by cells, they are responsible for antibody production (immunity). The immunoglobulins (Ig) have been identified gD,



Direct hemical urements lobulin arely er formed. lobulin oncentration ormally stimated eter erence etween rotein concentrations.

An eration rmal atio lobulin equently ion rotein normality. ratio yzed onjunction rotein rofile. be used to detect increased or decreased albumin globu oncentrations. any gic onditions er However, albumin globulin concentrations are reduced qual roportions, uch morrhage, eration ill resent.

The determined by dividing albumin concentration by globulin concentration. In dogs, horses, sheep, goats, umin oncentration ually reater lobulin concentration re oncentration ually qual lobulin oncentration

Fibrinogen ed patocytes. recursor fibrin, hich uble rotein orms rix blood clots, one of factors necessary for clot forma tion. f rinogen vels ecreased, lood oes orm stable clot or does clot all. Fibrinogen makes to of otal rotein ontent. ecause emoved om by clotting process, fibrinogen found serum. Fibrinogen assays are performed part of coagulation profile but y e ool hemistry rofile. cute inflammation issue levate rinogen levels een etect ubclinical ion horses. ne ethod brinogen valuation he eat recipi tation test described Unit Chapter The fibrinogen value calculated by subtracting total protein value of heated tubes from of unheated tubes. This protein surement of heated tubes be lower because fibrinogen een emoved om ome tomated yzers may rovide rinogen alues.

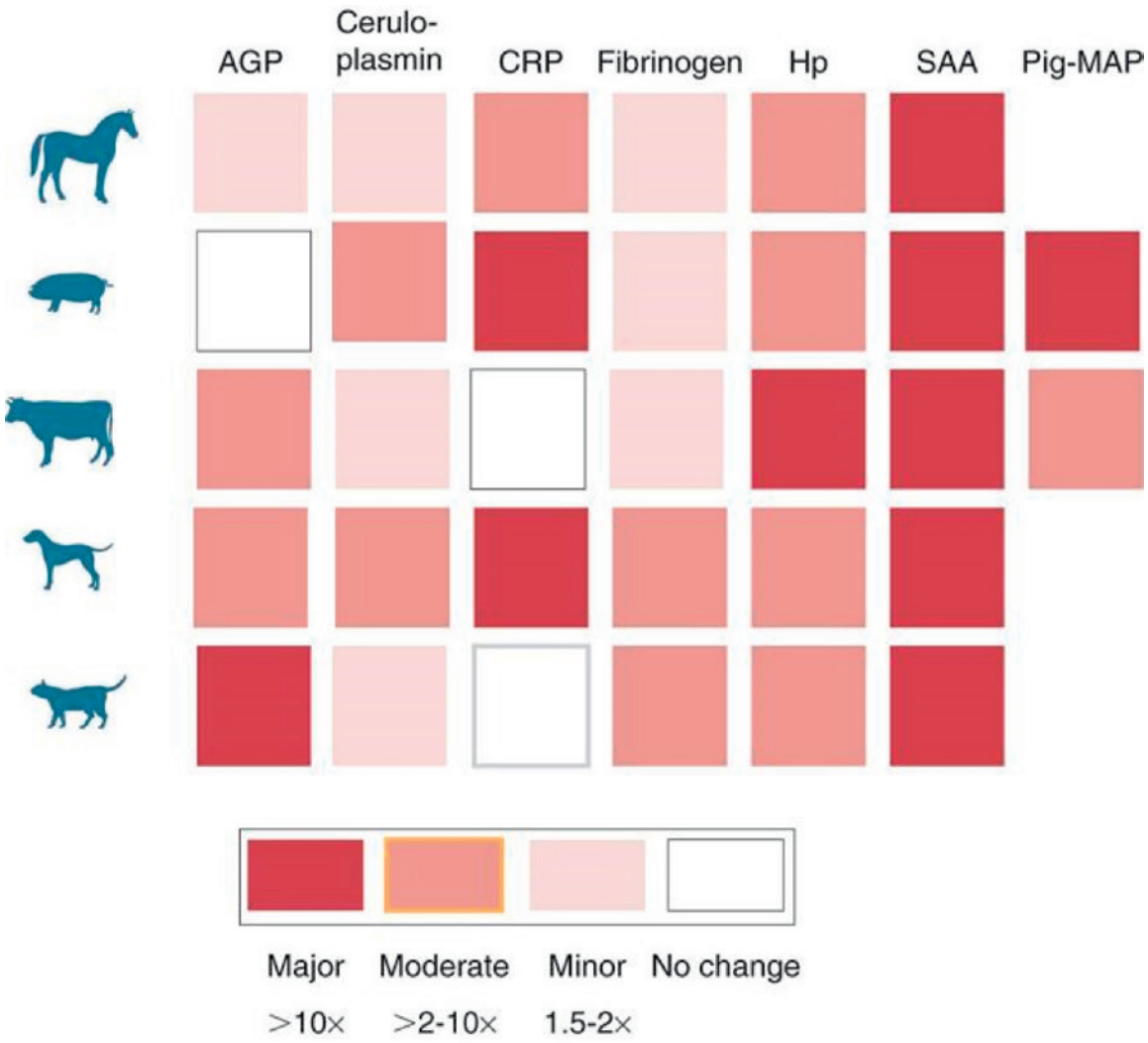
Acute-phase proteins e rimarily roduced patocytes immediately ollowing njury nflammation. here re bout recognized acute-phase proteins, different species produce different nes erent vels. ddition rum yloid cute-phase roteins omestic include C-reactive protein fibrinogen, haptoglobin ceruloplasmin, -acid lycoprotein cute-phase protein Fig. lbumin transferrin are referred to negative acute-phase proteins because their concentra tion decreases following injury or inflammation. lthough serum electrophoresis entifying eased vels cute-phase roteins, urements ecific cute-phase roteins re ful. cute-phase roteins nificance domestic are measured with immunoassays, although hemical vailable or urement toglobin. ld ortable yzer vailable or urement rses.

Serum yloid cute-phase rotein species of of particular importance rses. erum yloid vels ollowing injury or inflammation. The magnitude of increase determining presence of specific states. For example, healthy generally below Increases are orrelated ecific Table values are used to differentiate infectious from noninfec tious rses rack esponse rapy.

C-reactive rotein iomarker or ion produced liver. Levels of rise dramatically variety of states, including cardiac sepsis, neoplasia. The response occurs within hours after inflammatory event or rauma vels op rapidly riggering vent reated.

The liver largest internal organ. It complex structure, function, gic haracteristics. tions, including tabolism bohydrates, **cholesterol** roteins, lotting ctors; estion rption utrients related o ile ormation; cretion **bilirubin** ile; limination, uch etoxification oxins catabolism ertain ugs. tions ontrolled enzymatic reactions. The gallbladder closely associated with liver, both anatomically functionally. Its primary function storage site for bile. Malfunctions liver or gallbladder result predictable clinical signs of **jaundice** **hypoalbuminemia** problems with hemostasis, **hypoglycemia** **hyperlipoproteinemia** **hepatoencephalopathy**

Hepatic ells xhibit xtreme versity tion capable f egeneration ed. esult, re



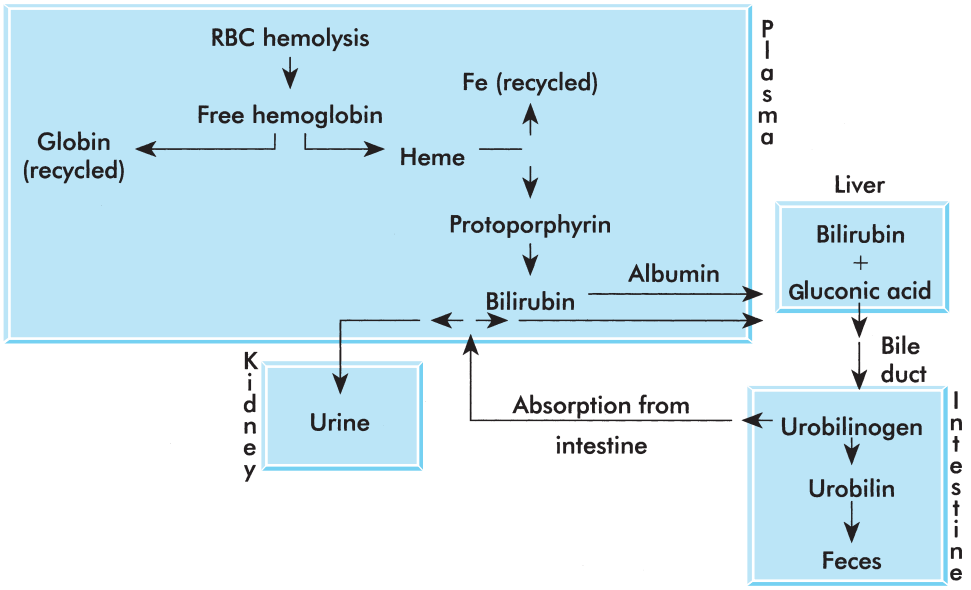
Species differences in the major acute-phase proteins produced by domestic animals. (From Tizard

Expected SAA Value (mg/L)

different ypes vailable valuate ver tion. Usually ver rogressed nificantly efore signs pear. ome ver tion esigned ure substances roduced ver, dified ver, or released when hepatocytes are damaged. Other liver function tests measure enzymes have altered serum concentra tions result of cholestasis. Liver cells compartmentalize

ork one ver ect all ver tions. ver tion ually erformed with rial eterminations veral erent ypes ver tests ompleted erifying tional rgan. uperior or etecting hepatobiliary ew eing eveloped low for etection patic efore ver verely damaged. The primary tests used veterinary medicine for evaluation of liver gallbladder are summarized Box

Many substances are taken up, modified, produced, secreted by ver. lteration ility erform ecific functions rovides verview ver tion. tocyte function are performed veterinary practice include bilirubin nd ther ubstances roduced epatocytes are less-sensitive indicators of liver function, because test results may show abnormalities until two thirds to three fourths of liver tissue damaged. These less-sensitive tests include albumin holesterol.



Bilirubin metabolism.

Enzymes Released From Damaged Hepatocytes

Bilirubin uble erived om reakdown of moglobin crophages en. ound o ransported ver. patic cells tabolize onjugate ilirubin bilirubin lucuronide. creted om hepatocytes, becomes component of bile. Bacteria within gastrointestinal system act on bilirubin glucuronide produce group of compounds collectively referred to urobilinogen. Urobilinogen broken down into urobilin before being excreted eces. ilirubin lucuronide obilinogen may e rbed ectly lood xcreted ys ilirubin roken own exposure to light. It important be protected from light o nsure ccurate esults.

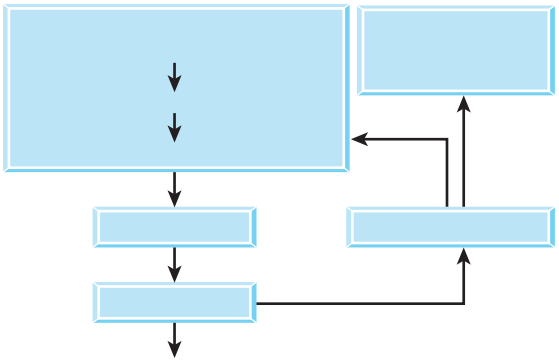
Measurements of circulating levels of various popu lations f ilirubin oint aundice. Differences elative ubility ch allow m vidually uantified. prehepatic (bound to albumin) bilirubin comprises approximately two thirds of total bilirubin serum. Increases popula tion indicate problems with uptake (hepatic damage). Increases **conjugated bilirubin** e ile uct bstruction.

Increases in the unconjugated bilirubin population

Assays ectly ure ilirubin onjugated rubin us onjugated ilirubin) onjugated ilirubin. Conjugated ilirubin ometimes eferred irect ilirubin, because est ethods irectly easure he mount onjugated bilirubin nconjugated ilirubin metimes referred o ect ilirubin, ecause oncentration indirectly ed ubtracting onjugated ilirubin concentration rom otal ilirubin oncentration ample.

Bilirubin yed etermine aundice, evaluate ver tion, heck ency ucts. Blood vels onjugated ect) ilirubin levated hepatocellular uct ury bstruction. Blood vels onjugated ect) ilirubin levated with xcessive rythrocyte estruction efects rans port chanism low ilirubin nter patocytes or conjugation.

Bile cids rve tions. hey rption enabling he ormation icelles he astrointestinal ystem modulate cholesterol levels via bile acid Bile acids are ed patocytes om holesterol, conjugated lycine onjugated secreted cross mbrane, each duodenum iliary stem. allbladder ores bile acids (except horse) until contraction associated with feeding. hen each ransported



Circulation of bile acids.

to ortal culation ravel ck ver. pproxi mately ctively esorbed um, emaining xcreted feces. he eabsorbed ried ver, here they e econjugated xcreted nterohepatic circulation

Spillover ile om nterohepatic culation y etected rmal rum oncentra tions f ile orrelate ortal oncentrations. result, ostprandial rum oncentrations higher han asting oncentrations. ny rocess hat mpairs he hepatocellular, biliary, or portal enterohepatic circulation of bile acids results elevated SBA levels. The great advantages of SBA determinations ver tion valuate major omic omponents patobiliary stem y e itro.

The A vel rmally levated er ecause allbladder ontracted eleased eased of ile o uodenum. aired rum erformed after ostprandial eded to perform test. The difference bile acid concentration of eported. rses, ested. Inadequate ontaneous allbladder ontraction increase fasting bile acid levels. Exposing patient to even aroma f food result in spontaneous gallbladder ontraction. Prolonged rhea ecrease

Elevated A vels ually ver uch congenital ortosystemic hronic patitis, patic rhosis, holestasis, oplasms. vels ecific with regard to type of liver problem exists are there fore used screening test for liver Bile acid levels may detect ver roblems efore ecomes teric. hey may e ollow rogress ver uring treatment. ncreased ile cid oncentrations an lso esult rom extrahepatic secondarily affect liver. decreased bile cid oncentration en estinal rp tive rses, eased oncentrations be esult patobiliary ecreased eed The eference anges or ows idely ariable. Bile cid esting nsitive or ows.

Bile cids etermined veral thods, ommonly nzymatic thod. ydroxy bile cids eact ydroxisteroid ehydrogenase with ormazan. olor eneration ured oint spectrophotometry. Lipemic postprandial must be cleared by entrifugation void nterference ith pectrophotometry. ile cid est unologic thods nzyme-linked unosorbent vailable or veterinary linic.

Cholesterol oprotein roduced rimarily ver, ested ood. increase rum holesterol ecies. owever, differences xist oprotein rofiles erent ecies, learance oproteins ell haracterized eterinary ecies. umber tomated yzers available rovide holesterol oprotein alues. Hyperlipidemia ften condary onditions ox rimary yperlipidemia are, ciated inherited onditions reeds.

holesterol metimes eening for ypothyroidism. hyroid rmone ontrols estruction holesterol ody. nsufficient yroid hormone ypothyroidism) esults ypercholesterolemia, because ate holesterol estruction elatively wer rate of Other associated with hyper cholesterolemia include hyperadrenocorticism, diabetes mellitus, nephrotic syndrome. Dietary causes of hypercholesterolemia are rare but may include high-fat diets postprandial lipemia.

Cholesterol by itself does grossly lipemic seen after eating; triglycerides are usually present. The administration orticosteroids ay lso ause levated lood cholesterol oncentration. uoride xalate icoagulants may levate nzymatic thod esults.

When hepatocytes are damaged enzymes into blood, etectable lood vels nzymes ciated liver ells ccurs. hese omponents, hich re ommonly eferred to leakage nzymes," lude ransferase nzymes **alanine transaminase** ransferase) T) **aspartate transaminase** (aspartate aminotransferase) (AST), well dehydrogenase enzymes **iditol dehydrogenase** (sorbitol dehydrogenase) **glutamate dehydrogenase** Transaminases atalyze the reactions that ransfer mine roups from eto uring roduction

acids. The enzymes are therefore found tissues have high rates of protein catabolism. Although other trans are present hepatocytes, only readily available tests are for ALT AST. Dehydrogenases catalyze transfer of hydrogen groups, primarily during glycolysis. Transaminases dehydrogenases are found either free cytoplasm of hepatocytes or bound to cell membrane. The serum levels of enzymes vary different species, have nonhepatic sources.

The common enzyme tests of liver function that are

In dogs, primates, major source of ALT hepa tocyte, where enzyme found free cytoplasm. ALT considered liver-specific enzyme species. Horses, rumi pigs, birds do have enough ALT hepatocytes for enzyme to be considered liver specific. Other sources of ALT are renal cells, cardiac muscle, skeletal muscle, pancreas. Damage to tissues may result increased serum ALT levels. The administration of corticosteroids or anti convulsant medications may also lead to increases in serum ALT. ALT used screening test for liver because precise enough to identify specific liver No correlation exists between blood levels of enzyme severity of hepatic damage. Increases ALT are usually seen within hours of hepatocyte damage, peak levels are seen to hours. The serum levels will return to reference ranges within few weeks chronic liver insult present.

present hepatocytes, which are found free cyto well bound to mitochondrial membrane. More severe liver damage required to release membrane-bound AST. levels tend to rise more slowly do ALT levels, they return to normal levels within day provided chronic liver insult present. found significant many other tissues, including erythrocytes, cardiac muscle, skeletal muscle, kidneys, pancreas. An increased blood level of may indicate nonspecific liver damage, or may be caused by strenuous exercise or intramuscular injection. The common of increased blood levels of are hepatic muscle inflammation or necrosis, spontaneous or artifactual hemolysis. If level elevated, serum or be examined for hemolysis. Creatine activity be assessed to rule out muscle damage before attributing increase to liver damage.

The primary source of hepatocyte. Smaller of enzyme are found kidney, small intestine, skeletal muscle, erythrocytes. present hepatocytes of all common domestic species, but it is especially useful for evaluating liver damage large such sheep, goats, swine, horses,

Large hepatocytes do contain diagnostic levels of ALT, offers liver-specific diagnostic test. The level of rises quickly with hepatocellular damage or necrosis. assay be used all species to detect hepatocel lular damage or necrosis, thereby eliminating need for other tests (e.g., ALT assay). The disadvantage of analysis unstable serum, activity declines within few hours. If testing delayed, be frozen. tests are readily available to average veterinary laboratory. Samples to be sent to outside laboratories be packed ice for transport.

mitochondrial-bound enzyme found high con centrations hepatocytes of sheep, goats. An increase enzyme indicative of hepatocyte damage or necrosis sheep. could be enzyme of choice for evaluating ruminant avian liver function, but standardized test method been developed for veterinary practice laboratory.

Blood levels of certain enzymes become elevated with cholesta (bile duct obstruction), metabolic defects liver cells, administration of certain medications, result of action of certain hormones, especially of thyroid. These enzymes are primarily membrane bound. The exact mechanism induces increased levels of enzymes when cholestasis present well documented.

Alkaline phosphatase present isoenzymes many tissues, particularly osteoblasts bone, chondroblasts tilage, intestine, placenta, cells of hepatobiliary system liver. The isoenzymes of tend to remain circulation for approximately to days, with exception of intestinal isoenzyme, which circulates for just few hours. corticosteroid isoenzyme of been identified dogs with exposure to increased endogenous or exogenous glucocorticoids. Because occurs isoenzymes various tissues, source of isoenzyme or location of damaged tissue may be determined by electrophoresis other tests are performed commercial or research laboratories.

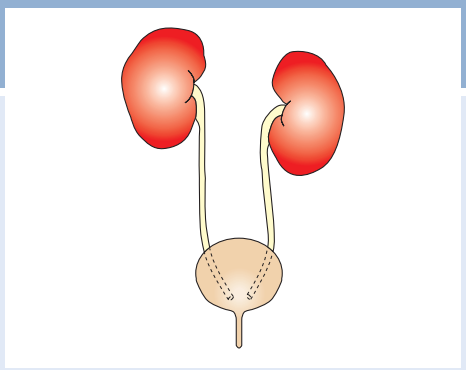
In young comes from osteoblasts chondroblasts result of active bone development. In older nearly all circulating comes from liver bone development stabilizes. The assays are used for practice laboratory determine total blood concentration. concentrations are often used to detect cholestasis adult dogs Because of wide fluctuations normal blood levels sheep, test useful for detecting cholestasis species.

Gamma glutamyltransferase r metimes eferred to amma-glutamyl ranspeptidase. tissues, luding enal pithelium, pithelium ticularly during lactation), biliary epithelium, but primary source ver. rses, ep, oats, irds ve higher lood ctivity ogs ther ces of include kidneys, pancreas, intestine, muscle ells. lood vel levated ients liver ecially bstructive ver

e umber dditional eterinarian to evelop rognosis. any biochemical ciated rgans ell, us ecific or ver or blood lucose enerally ciated eatic tion. However, liver modulation of glucose values, liver ften esults yperglycemia ypoglycemia. Additional ver tion ye xcretion, caffeine learance) rimarily erformed nly esearch settings.

Chapter eview uestions ppendix

- Total protein measurements include fibrinogen values, whereas otal rum rotein eterminations ure rotein actions xcept rinogen.
- Total protein concentrations are especially valuable for deter ydration.
- Albumin omprises rotein
- Serum yloid cute-phase rotein orrelated to ecific ecies.
- Some liver function tests are designed to measure substances that e roduced the liver, dified the liver, or eleased when patocytes ed. ther ver tion measure nzymes ve ered rum oncentra tions esult
- In unconjugated bilirubin comprises approxi mately o ilirubin rum.
- Bile acids absorption modulate cholesterol levels via ile cid
- Elevated vels ually ver uch congenital ortosystemic hronic patitis, patic cirrhosis, holestasis, oplasms.
- The leakage nzymes" lude ransaminase, tate ransaminase, ehydrogenase, lutamate dehydrogenase.
- ALT ommonly erformed ver tion ests or
- Alkaline hosphatases erived om rgans.



After studying this chapter, you will be able to:

- Discuss ys meostasis.
- Describe ommonly erformed function.
- Describe elationship etween rogen creatinine.
- Define *azotemia.*
- Discuss or ffective enal glomerular ration ate.
- Discuss reakdown rogenous roducts role f ys.

Allantoin
Azotemia
Blood urea nitrogen
Creatinine
Effective renal plasma flow
Enzymuria
Fractional excretion of electrolytes
Glomerular filtration rate
Uric acid

The ys meostasis Their primary functions are to conserve water elec trolytes imes gative er lectrolyte limination ositive excrete or conserve hydrogen ions to blood within normal to conserve nutrients (e.g., glucose, proteins), to emove roducts rogen tabolism **creatinine allantoin** blood levels of end products remain low. dditional functions include production of renin enzyme involved control of blood pressure), erythro poietin rmone cessary or rythrocyte roduction), prostaglandins (fatty acids used to stimulate contractility of uterine other smooth muscle). The kidneys function to lower lood ressure, egulate cretion omach, to regulate body temperature and platelet aggregation, to control inflammation, ctivation.

The kidneys receive blood from renal arteries. The blood enters lomerulus phrons, here ly er small dissolved solutes into collecting tubules. Each nephron ontains ctions tion eabsorb crete specific utes. The resorption of glucose ccurs in the proximal convoluted ubule. cretion eabsorption ral ccur ending op enle onvoluted ubule. phron ecific esorptive capability for each substance, which called renal threshold. Most er eabsorbed ell. As esult er eabsorp tion, olume xcreted olume originally ntered y. lood eturns om ys to est f ody ough enal eins, hich onnect to ena va. rine lood yzed evaluate tion. etails inalysis rocedures. The primary serum chemistry tests for kidney function are urea

nitrogen eatinine. ther lude arious ys have een esigned valuate ate fficiency merular ration.

Some references term serum urea nitrogen rather **blood urea nitrogen** UN). rea rincipal product f reakdown vels are d o valuate tion ility of y emove rogenous om lood. Under normal conditions, all urea through glomerulus nters enal ubules. pproximately reabsorbed ubules, emainder xcreted urine. f tioning roperly, ufficient emoved om reby eased BUN vels.

Contamination lood ease-producing bacteria (e.g., *Staphylococcus aureus, Proteus* spp., *Klebsiella* spp.) may esult ecomposition ubsequently decreased UN vels. revent ysis om pleted ithin veral ollection, be efrigerated. ariety hotometric vailable or urement rogen. ll ve ccept able vel f ccuracy recision. hromatographic ick tests e vailable rovide miquantitative rum urea rogen esult thods end accurate nly uick eening

Urea uble xcreted h olume er. ehydration esults eased retention of urea blood. High-protein diets strenuous exercise may elevated BUN level result of increased acid breakdown rather decreased glomerular filtration. Differences rate of protein catabolism versus female well young versus older will affect UN vels.

A dipstick test for blood urea nitrogen.

Creatinine ormed om eatine, hich eletal muscle, part of muscle metabolism. Creatinine out of uscle ell ody luding lood. If hysical ctivity emains onstant, eatine metabolized eatinine emains onstant, lood level f eatinine emains onstant.

atinine tion uscle nder normal onditions, rum eatinine ered ough glomeruli eliminated urine. ny condition alters **glomerular filtration rate** GFR) ill lter he erum reatinine levels. Creatinine may be found sweat, feces, vomitus, y ecomposed cteria.

Blood eatinine vels valuate enal tion on ility lomeruli er eatinine from lood liminate eatinine ccurate or tion, ecause ly f issue nfunctional efore lood creatinine vels ommonly thods or rum creatinine lude affe thod ell veral nzymatic methods. Postprandial decreases creatinine occur response to ransient er

Because BUN creatinine both have wide range of reference intervals, their indicators of renal function limited. The y e ecreased uch elow rmal before hanges en rum eatinine vels. In addition, healthy often have values are below reference anges. ients enal yperplasia enal issue ly enal ure. atio of UN o eatinine or f enal lthough et ell lished for veterinary species, be used to patient's status during reatment.

BUN creatinine have inverse logarithmic relationship. The eciprocal eatinine racked ver track rogress ffectiveness reatment. roportionate ehydration, dietary reatment ure, wner ncompliance reatment egimens.

The quantitative assessment of renal proteinuria of diagnostic significance or enal nce ory cells roteinuria lomerular or ccurate etermination roteinuria, inary protein alue etermined. edious errors e ommon. matical thod ompares urine rotein vel eatinine vels urine re ccurate omprehensive. protein-to-creatinine atio oncept ubular oncentration inary protein eatinine oncentrations qually.

This method been validated for species. Usually to of urine are collected between ref erably by cystocentesis. The urine be kept or ored entrifuged, uperna used. The protein creatinine concentrations for each be determined by variety of photometric methods. The ine atio or ogs urine atio etween ve rerenal rigin (e.g., yperglobulinemia, moglobinemia, yoglobinemia) tional rigin xercise, ever, ypertension), hereas ine atio reater enal

Uric acid metabolic by-product of nitrogen catabolism, ound ver. ric ually ransported to ys compound asses hrough he lomerulus, nd argely eab sorbed y ubule ells. onverted lantoin excreted ogs, efect uptake o patocytes esults ecreased onversion allantoin. herefore, reed xcretes ather allantoin)

Uric cid roduct rogen tabolism avian species. It constitutes approximately to of total rogen xcreted vian ctively creted by renal tubules. The measurement of or serum uric acid used index of renal function birds. Uric acid e eased tifactually om oenail lippings because f ecal ate ontamination. ric oncentrations will increase after carnivorous birds. renal uric acid concentrations increase when kidney more f tional city.

TECHNICIAN NOTE

In ients otemia mptomatic or renal ithout otemia, veral dditional performed o valuate tion. learance require ollection imed, uantified with concurrent Two primary types of clearance studies e erformed: **effective renal plasma flow** he ubstances liminated both glomerular filtration renal secretion (typically amide p-aminohippuric volves stances are eliminated only by glomerular filtration (typically creatinine, ubstance dministered, ine ollected. are n ed ollows:

/ /

epresents ubstance resent ligrams per liliter), epresents ollected ver efined eriod ligrams er gram er ute), epresents oncentration ubstance.

The ERPF uses test substances that are eliminated

Because eatinine pears lomerular rate ligible ubular cretion, ural racer lomerular filtration. ortunately, rt-term lood oncentrations stable nough learance ormula or eady infusion udies puric elatively ox ure lood eatinine accurate timed urine collection are required for test. Precision tmost ortance. loppy ladder eterization esults, ecially briefer thods. ladder inse efore er the est, ith he fter-rinses aved ith he rine or reatinine analysis. learance ed viding inary eatinine excretion rine eatinine oncentration rine olume) plasma reatinine oncentration. he stimate, mprecise, ro vides ractical ormation.

To avoid errors, creatinine be determined with of combination creatinine PAP test instead of Jaffe method. he ombination eatinine nzymatic chromogenic thod etermine eatinine oncentration.

voided urine is collected over a specified time frame (most commonly

Uv Uc/

urinary bladder is catheterized and rinsed with saline after a specified

The affe thod etermines ncreatinine hromogens hich pear xcess rum etones, glucose, roteins ly levate imates chromatic erference oss-reactivity.

Exogenous eatinine learance ccurate thod or suring eatinine oncentration increases, reby oncentration ncre atinine hromogens gligible. lows or plication of Jaffe method to determine creatinine concentrations Box 33.2 voiding dehydration the animal itical or the per formance of test; free access to water must be ensured before any lomerular ration erformed.

Iohexol clearance be used to estimate dogs Iohexol adiographic ent iven rave dose after during which patient free access o er. erum en after administration sent to reference laboratory for evaluation f ransit ohexol alculation FR.

Inulin excreted entirely by glomerular filtration, without tubular secretion, eabsorption, atabolism. esult, nulin learance tests onstant ate uantita tive ine onsidered thod or evaluation f FR. ingle-injection nulin learance impler method alternatively may be used. fter (free access o er ermitted uring cted intravenously dosage of or (body surface calculation gives more accurate results); serum are then obtained minutes. Total inulin clearance calculated om ecrease rum oncentration by o-compartment del. ormal ogs ve of o f ody urface

Polyuria r olydipsia uspicions out y, which y rroneous. iuresis ubsequent olydipsia may phrons tion upted hyperadrenocorticism ushing's etes llitus, nephrogenic diabetes insipidus. The kidneys may be normal, but they may receive signal to concentrate urine, which occurs with neurogenic diabetes insipidus. In addition, diuresis may be totally appropriate renal compensation for pathologic water intake chogenic olydipsia).

Vasopressin idiuretic rmone om rohypophysis ys etain er ollecting uct's ermeability er. ater t f ollecting uct ypertonic enal medulla, reby oncentrating emains ehind ollecting uct. stem propriate diuresis), either neuroendocrine pathway releases response to hypovolemia or hyperosmolarity been interrupted phrons espond.

This est volves bserving ient's esponse ndog enous r xogenous ehydrate patient safely until definite stimulus exists for endogenous elease ually proximately ody eight That end point may vary. hen denied water, patients dehydrate different rates must be monitored for weight clinical signs of dehydration, increased urine osmolarity or specific gravity. t oint, er rictest endocrine rders oncentrate ontinued uresis dilute urine indicate lack of endogenous or unresponsive nephrons. n ogs ure, esponsiveness precedes otemia.

Contraindications lude ehydration temia. ehydrated ients ypovolemia ck. hey already have release; they could concen trate urine, they would. Under conditions, test useless dangerous, especially for with diabetes insipidus or neurogenic etes zotemia eady emonstrates kidney dysfunction. gain, test reveals new adds rerenal omponent otemia.

When ients emonstrate reviously ntioned when rior er-deprivation asopressin response test indicated. The vasopressin response test simply challenge with exogenous focuses on ability of kidneys o espond. rine ity ecific ravity index f tion. ormal ys oncentrate with echnique, espite ient's ee ccess er. Vasopressin must be handled carefully, because labile drug ttles uspensions. ures esult om of or poorly mixed solutions. In addition, intramus asopressin ction esult asopres sin's asomotor ctivity, oretically ontraindicated during regnancy.

In both tests, even normal kidneys may be unable to concen trate ine o rmal xtremes. iuresis uickly utes from enal dulla, eakening radient draws water from collecting ducts. Gradual water deprivation over o day period efore the ater eprivation est recommended to renew renal solutes allow evaluation of ct ehydration

Deprivation/Vasopressin Response

and hydration and central nervous system (CNS) status are evaluated at

test is ended when the animal is clinically dehydrated, appears ill, or

The water deprivation vasopressin response tests may be ombined rotocol erentiate veral of polyuria polydipsia Box The modified water-deprivation ecifically ontraindicated or ients known enal emia esults om rerenal primary renal disorder, or with suspected or obvious dehydration.

The actional learance hich eferred **fractional excretion (FE)—of electrolytes** matical manipulation describes excretion of specific electrolytes (particularly dium, otassium, hosphorus) elative he ommonly dium. Bicarbonate hloride esting arely erformed. tests differentiate prerenal from postrenal azotemia. Random, concurrent lood equired. calculated ollows:

/ /

lectrolyte urement hich our dium, otassium, hosphorus, hloride); e ine oncentrations, espectively, of ecific lectrolyte; e ine concentrations of creatinine, respectively. Normal results are ollows:

- Dogs: dium, otassium, hloride, hosphorus,
- Cats: sodium, potassium, chloride, phosphorus,

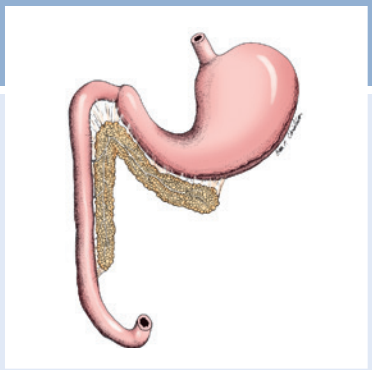
Serum rganic hosphorus ually eciprocal serum um. ormally, rum eabsorbed tubules. his chanism er rmonal ontrol parathyroid rmone, ected rum nitially, renal e ers ecreased inary eased rum ubsequent erations Pi ead o ncreases erum alcium nd ecreases erum i. See electrolyte information later chapter for additional information out esting or

Enzymuria refers to presence of enzymes urine. Many of hemical erformed rum be erformed resent ine ients enal lude inary urinary N-acetyl- -d-glucosaminidase (NAG). Urinary AG e nzymes eleased om ed ubule cells. omparison AG er ligram of creatinine indicate extent of renal damage. Both and AG ncrease apidly ith ephrotoxicity, nd ncreases ccur sooner hanges rum eatinine, eatinine learance, or actional xcretion lectrolytes.

Chapter eview uestions ppendix

- The kidneys play major role maintenance of homeosta by modulating water electrolyte concentra tions, blood conserving nutrients, removing waste roducts, roducing enin, rythropoietin, prostaglandins.
- The rimary rum hemistry or tion urea rogen eatinine.
- Urea rincipal roduct reakdown
- Dehydration esults eased etention blood, ecause xcreted olume water.
- Any ondition ers ill er rum atinine vels.

- In Dalmatian dogs, defect uric acid uptake into hepa tocytes results excretion of uric acid urine.
- Uric acid major end product of nitrogen metabolism avian species.
- Clearance studies may be performed azotemic patients include
- The test substances eliminated by both glomerular filtration renal secretion.
- The fractional excretion of electrolytes mathematical manipulation describes excretion of specific elec trolytes relative to
- Serum usually reciprocal of serum calcium.



Pancreatic Function Tests

After studying this chapter, you will be able to:

- Differentiate etween ndocrine tions of eas.
- List escribe ommon or valuation xocrine
- Explain elationship etween lucagon, blood lucose.
- Describe ommon valuate patients or yperglycemia.
- Discuss eneral oncepts volved erformance of lucose olerance

Acinar
Amylase
Amyloclastic
Endocrine
Fructosamine
Glucagon
Glucose
Glucose tolerance
Glycosylated hemoglobin
Hyperglycemia
Insulin
Pancreatic lipase immunoreactivity
Trypsin
Trypsinogen

The pancreas actually two organs—one exocrine other **endocrine** e ogether roma. xocrine portion, hich eferred eas, om prises reatest ortion rgan. ortion cretes nzyme-rich uice ontains nzymes cessary or digestion estine. ee rimary eatic enzymes are **trypsin** **amylase** These digestive enzymes are released into lumen of other organs through duct system. Trauma o eatic issue ften ciated eatic duct inflammation results backup of digestive enzymes into eripheral culation.

Photo om hristenson *Veterinary medical terminology* d Louis, aunders.

Interspersed within exocrine pancreatic tissue are arrange ments of cells histologic section, take on appear ance of "islands" of lighter-staining tissue. These are called islets of Langerhans. Four types of islet cells are present, but they be distinguished on of their morphologic characteristics. The four cell types are designated pancreatic polypeptide cells. The cells comprise of islet cells, they secrete somatostatin pancreatic polypeptide, respectively. -Cells comprise approxi mately of islet, they secrete **insulin** The remaining nearly of islet consists of -cells secrete **glucagon** somatostatin. The pancreas regenerative ability. When pancreatic islets are damaged or destroyed, pancreatic tissue becomes firm and nodular, with areas of hemorrhage and necrosis. These islets are longer able to function. Diseases of pancreas may result inflammation cellular damage leakage of digestive enzymes or insufficient production or secretion of enzymes.

The tests are commonly performed to evaluate acinar functions of pancreas include amylase Trypsin like immunoreactivity serum **pancreatic lipase immuno reactivity** are available tests for pancreatic function. In serum amylase activities have been shown to have limited clinical significance for diagnosis of creatitis. In experimentally induced pancreatitis serum amylase actually decreases. The serum activities of both enzymes are frequently normal with pancreatitis. Several immunoassays are available provide either quanti tative or semi-quantitative evaluation of specific of dogs to rapidly differentiate pancreatitis from other states.

The primary source of amylase pancreas, but amylase produced in the salivary glands and the small intestine. Increases serum amylase are nearly always caused by pancreatic especially when accompanied by increased levels. The rise blood amylase level always directly proportional to severity of pancreatitis. Serial determinations provide information.

Amylase functions to break down starches glycogen sugars, such maltose residual **glucose** Increased levels of amylase appear blood during acute pancreatitis, flare-ups of chronic pancreatitis, or obstruction of pancreatic ducts. Enteritis, intestinal obstruction, or intestinal perforation may result increased serum amylase from increased absorption of intestinal amylase into bloodstream. In addition, because amylase excreted by kidneys, decrease glomerular filtration rate for any reason lead to increased serum amylase. Serum amylase activity greater three times refer ence range usually suggests pancreatitis.

Two amylase test methods are available: saccharogenic method **amyloclastic** method. The saccharogenic method measures production of reducing sugars amylase catalyzes breakdown of starch. The amyloclastic method measures disappearance of starch broken down to reduce sugars through amylase activity. Calcium-binding anticoagulants (e.g., EDTA) be used, because amylase requires pres ence of calcium for activity. The presence of lipemia may reduce amylase activity. The saccharogenic method ideal for because may artificially elevate assay results. Normal feline amylase values be to times higher found human beings. Therefore, may have to be diluted tests designed for human are used.

The majority of serum derived from pancreas. The function of to break down long-chain fatty acids of Excess normally filtered through kidneys, levels tend to remain normal during early stages of pancreatic Gradual increases are seen progresses. With chronic progressive pancreatic disease, damaged pancreatic cells are replaced with connective tissue produce enzyme. As occurs, gradual decrease both amylase levels are seen.

Chemical test methods for determination of levels are usually based on the hydrolysis of an olive oil emulsion into fatty acids by present patient serum. The quantity of sodium hydroxide required to neutralize fatty acids directly proportional to activity Newer tests for are capable of detecting feline pancreatic by immunologic methods are available. These tests have been demonstrated to have high degree of sensitivity for diagnosis of pancreatitis dogs assay may be more sensitive for detection of creatitis compared with amylase assay. The degree of activity, like amylase activity, directly proportional to severity of pancreatitis. Determinations of blood amylase activities are usually requested time to evaluate pancreas.

Increased activity seen with renal hepatic dysfunction, although exact mechanisms for are unclear. Steroid administration is correlated with increased lipase activity, with concurrent change amylase activity.

The comparison of amylase activity peritoneal with serum may provide additional diagnostic information. of higher amylase activity peritoneal serum strongly suggests pancreatitis, provided intes tinal perforation first been ruled out.

Trypsin proteolytic enzyme digestion by catalyzing reaction breaks down proteins of ingested food. Trypsin activity more readily detectable feces blood. For reason, trypsin analyses are performed on fecal Trypsin normally found feces, absence abnormal. variety of fecal testing methods are available reference laboratory.

and Chronic Pancreatitis in Dogs and Cats

Exocrine pancreatic insufficiency; pancreatic lipase immunoreactivity; trypsinlike immunoreactivity.
From Nelson R, Couto C:

Serum rypsinlike unoreactivity adioimmuno assay ibodies rypsin. etect **trypsinogen** rypsin. ibodies ecies ecific. Trypsin trypsinogen are both produced only pancreas. With eatic ury, rypsinogen eleased xtra cellular ce onverted rypsin, hich bloodstream. vailable nly or og rovides nsitive ecific or exocrine eatic ufficiency ogs. ogs have rum ormal ogs ve range of to Dogs with other of malassimilation may ve rmal rum vels. ogs hronic atitis y ve rmal alues alues etween ormal ve ates hereas ith I ve ates ssays or olate cobalamin (vitamin $_{12}$ are usually performed conjunc tion with to evaluate extent of gastrointestinal disorders.

Serum ecreases allel tional eatic he ion ciated cute robably chronic eatitis nhance rypsinogen rypsin om ddition, decreased lomerular ration ate rypsinogen l lomerular er). Serum ortant or tional eatic t ormative oupled enzoyl-l-tyrosyl-p-aminobenzoic acid fecal results to characterize nose ion.

Serum er ecially roteins), ut values emain eference ervals. ddition, atic nzyme xogenous) upplementation oes er Therefore, ood ithheld or preferably efore lood en. lood oagulated oom emperature rum ored il ce.

Serum eline eatic unoreactivity eatic unoreactivity ecific for eatitis, ecommended ead reviously alidated rum rum patients with symptoms of pancreatitis. More information regarding unoassays Table escribes dvantages dvantages various ests or cute hronic creatitis ogs

ariety f vailable valuate ndocrine tions f ddition raditional lood

glucose tests, other tests are available include **fructosamine** -hydroxybutyrate, **glycosylated hemoglobin** Urinalysis, serum cholesterol, triglyceride tests provide informa tion about function of pancreas.

The regulation of blood glucose levels complex. Glucagon, thyroxine, growth hormone, epinephrine, glucocorticoids are all agents favor **hyperglycemia** They boost blood glucose levels by encouraging glycogenolysis, gluconeogenesis, lipolysis while discouraging glucose entry into cells. Insulin hypo glycemic hormone. By promoting glucose into target cells, triggers anabolism, which process converts glucose into other substances. This regulatory effect prevents blood glucose concentration from exceeding renal threshold spilling of glucose into urine.

The pancreatic islets respond directly to blood glucose concentrations, they release insulin (from cells) or glucagon (from cells) needed. Glucagon release directly stimulates insulin release. Epinephrine under direct sympathetic neural control; hyperglycemia one aspect of classic "fight or flight" state. The other hormones mentioned respond to hypothalamic/pituitary command. At any point time, of agents are acting to blood glucose concentration or down.

Because only insulin lowers blood glucose levels, aberrations of insulin action have obvious clinical effects. Hypo function (diabetes mellitus) or hyperfunction (hyperinsulinism) occur.

The blood glucose level used indicator of carbohydrate metabolism body, may be used measure of endocrine function of pancreas. The blood glucose level reflects net balance between glucose production (e.g., dietary intake, conversion from other carbohydrates) glucose utiliza tion, which involves expended energy conversion into other products. It may reflect balance between blood insulin glucagon levels. Glucose levels fluctuate significantly due to variety of factors, including nutritional status stress. An individual blood glucose measurement reflects level present time collected.

Glucose utilization depends on of insulin glucagon produced by pancreas. As insulin level increases, does rate of glucose thereby resulting decreased blood glucose levels. Glucagon acts stabilizer to prevent blood glucose levels from becoming too low. As insulin level decreases (e.g., with diabetes mellitus), does glucose thereby resulting increased blood glucose concentration.

Many tests are available for blood glucose. Some of react only with glucose, whereas others may quantitate all sugars blood. point kinetic assays are available. The kinetic enzymatic assays tend to be accurate precise. Samples must be taken from properly fasted Serum for glucose testing must be separated from erythrocytes immediately after blood collection. Glucose levels may drop per of left contact with erythrocytes room temperature. Even of serum separator tube may be adequate to prevent Mature erythrocytes glucose for energy, blood they may decrease glucose level enough to give false-normal results original had elevated glucose level. If originally had normal glucose level, erythrocytes may enough glucose to decrease level to below normal or to zero. If be removed immediately, anticoagulant of choice sodium fluoride to of blood. Sodium fluoride may be used glucose preservative with EDTA at 2.5 mg/mL of blood. Refrigeration slows glucose use by erythrocytes.

Glucose levels may drop 10% per hour if the sample

Glucose bind variety of structures, including proteins. Fruc tosamine represents the irreversible reaction of glucose bound to protein, particularly albumin. When glucose concentrations are persistently elevated blood, occurs patients with diabetes mellitus, increased binding of glucose to serum proteins occurs. The of increased fructosamine indicates per sistent hyperglycemia. Because half-life of albumin dogs to weeks, fructosamine provides indication of average serum glucose over period. Fructosamine levels respond more rapidly to alterations serum glucose does glycosylated hemoglobin. However, serum fructosamine may be artifactually reduced patients with hypoproteinemia.

Glycosylated hemoglobin referred to hemoglobin represents irreversible reaction of hemoglo bin bound to glucose. When hyperglycemia present, there increased binding of hemoglobin glucose thus increased glycosylated hemoglobin. The of increased glycosylated hemoglobin indicates persistent hyperglycemia. The test result reflection of average glucose concentration over life of erythrocyte, which to months dogs to months Therefore, indicates blood glucose concentration over longer period of time either fructos or single blood glucose measurement. more specific diagnostic indicator of diabetes mellitus more sensi tive monitoring control of diabetes. With older test methods, patients were anemic often had artifactually reduced levels of glycosylated hemoglobin. Newer test methods are immunoassays are subject to errors from reduced hemoglobin.

Ketone bodies be detected The ketone produced greatest abundance ketoacidotic patients -hydroxybutyrate. However, many tests for serum ketones only detect acetone. Tests for -hydroxybutyrate enzymatic,

colorimetric thods ecoming vailable or eterinary

Glucose tolerance ests ectly hallenge glucose load measure insulin's effect by evaluating blood or urine glucose concentrations. If adequate insulin released target cells have healthy receptors, then artificially elevated blood lucose vel utes er estion egins to op, eaching rmal alue lucose appears rmal lucose lood vel postprandial etes llitus. rolonged yper glycemia lucosuria onsistent etes llitus. Profound ypoglycemia er hallenge lucose-responsive, yperactive -cell umor may e etermining ostprandial glucose.

Oral lucose olerance ected normal estinal function nteritis, ypermotility) xcitement from astric ubation); ravenous lucose olerance preferred. The intravenous test only practical option for ruminants. ravenous lucose olerance ro cedure hallenge lucose ad cted er (except ruminants). Blood glucose subsequently checked, progress mapped tolerance curve. Results are standardized disappearance half-lives or glucose turnover rates xpressed ercent er ute:

Turnover 693/ 100

dL in patients with suspected hyperinsulinism (do not fast ruminants or dogs

infusion with the use of sodium fluoride as an anticoagulant, and submit all

the glucose values on semilogarithmic graph paper, and determine

in 30 to 60 minutes and return to baseline values after 120 to

Decreased glucose tolerance increased half-life, decreased turnover rate) occurs with diabetes mellitus consistently with hyperthyroidism, hyperadrenocorticism, hyperpituitarism, and severe ver disease. Increased glucose tolerance (i.e., decreased half-life, increased turnover rate) observed with hypothyroid ypoadrenocorticism, ypopituitarism, yperinsulin owever, esults rroneous. ormal are onsuming w-carbohydrate diabetic curves." he ffect ed roviding animal ith ays igh-carbohydrate eals efore esting. The ravenous lucose olerance esults ariable rmal rses, epending ing, ful.

Glucose olerance ually cessary btain diagnosis f etes llitus. ersistent yperglycemia glucosuria—frequently ory olyuria, olydipsia, polyphagia, eight ufficient or diabetes llitus. alue or etecting yper insulinism, ecause -cell umors rapidly esponsive lucose. hey ven etic glucose olerance ves, ecause ulin-antagonist rmones are eleased esult ypoglycemia. atient stress hemical estraint ect lucose olerance results. Serum glucose measurements themselves may be errone ously w lood ubjected icoagulants e lowed oom emperature. owever, ill d.

The est or lucose olerance borderline hyperglycemia without persistent glucosuria. However, test cost-effective for owner, may result significant therapeutic change. This dilemma often seen which high renal thresholds for glucose stress-induced hyperglycemia ommon ding. xtra ormation may e btained om ravenous lucose olerance immunoreactive insulin concentrations are followed simultane ously. This protocol may differentiate diabetes mellitus results from ute ck ype om target-cell nsitivity ype propriate release ype

The ulin olerance robes etes mellitus. ecifically, hecks esponsiveness et cells o hallenge egular ystalline rt-acting) ubcutaneously ramuscularly. erum lucose levels e ured lood ve een btained before insulin injection fasting blood glucose) every minutes after injection for hours. If serum glucose level to op o oncentration utes of ulin ction esistance), receptors e esponsive ction eing verely antagonized. The latter may occur with hyperadrenocorticism acromegaly. Insulin resistance profoundly influences prognostic rapeutic ecisions. ulin-induced ypoglycemia persists for hours hypoglycemia unresponsiveness), hyper insulinism, hypopituitarism, or hypoadrenocorticism be suspected. ypoglycemia, ient

may uffer om onvulsions. lucose ution always be on for rapid intravenous administration.

The indications for glucagon tolerance test are repeated normal r orderline esults nded ulin/glucose ratio est discussed later this chapter) the ck of insulin assay. he lucagon olerance rovides of yperinsulinism. ucagon imulates eatic ells both ectly ectly lood vel. In normal glucagon injection intravenously o otal ogs ransiently elevates lood lucose vel reater In rmal oncentration vel reater rmal oncentration eturns fasting oncentrations. rmal vel occurs utes eclines oncentration utes. ype etic resent esponse. If eatic -cell umor, rum lucose level eak wer rmal. ollowed hypoglycemia serum glucose level because excessive insulin secreted by stimulated neoplasm.

To erform ient ed rum glucose vel elow ually Glucagon cted, dium ride icoagulated blood btained efore lucagon ction utes er ction nitor lucose esponse. nfortunately, nsitive, y ypoglycemia onvulsions er. Patients must be fed immediately after test then observed for s.

The f yperinsulinism taneous urements rum lucose vels fasting Hypoglycemia normally inhibits insulin secretion. Pancreatic -cell umors yperactive esponsive to lucose crete undance propriate for prevailing blood glucose concentration. lthough fasting serum ulin oncentrations ften rmal ients hyperinsulinism, ratios of insulin-to-glucose concentrations are usually errant.

The ute atio lucose nded increase agnostic ccuracy. The ended insulin/glucose ratio subtracts om rum lucose oncentration. rum glucose vel rmally etect able, ero lucose insulin hysiologic ce. ecause normally high ulin oncentrations re bvious rum glucose oncentrations, nded ulin/glucose atio aluable onfirmed ypoglycemia nsulin lucose ve ured from rum hich oint rial etermina tions y e erformed lect oncentration hypoglycemia. However, test totally dependable. If results are unconvincing, then procedure be repeated or other tests tried. Specifically, diagnostic imaging insulin like rowth ctor ried onfirm paraneoplastic ypoglycemia.

When esults lucagon esponse nded insulin/glucose atio re quivocal, lucose, pinephrine, eucine, tolbutamide, or calcium challenges may be attempted. These sub stances, like glucagon, may provoke hyperinsulinemic response from eatic ell umors, reby esulting ecreased serum glucose levels. However, tumors vary with regard to their sensitivity o ents, gative esults response) occur. These tests are dangerous, because they recipitate vere rolonged ypoglycemia.

Chapter eview uestions ppendix

- The ndocrine tions.
- The tests are commonly performed to evaluate acinar functions f lude ylase
- Immunologic vailable or etection eatitis.
- Tests f ndocrine tions lude glucose, uctosamine, lycosylated moglobin.
- Glucose depends on of insulin glucagon produced y
- Glucose tolerance tests challenge pancreas with glucose load ure ulin's ffect valuation lood or ine lucose oncentrations.
- Serum for glucose testing must be separated from rythrocytes diately er lood ollection.
- Increased uctosamine ersistent yperglycemia of o eeks' uration ogs
- Increased lycosylated moglobin ersistent hyperglycemia nths' uration ogs nths' uration
- The etone roduced reatest undance etoacidotic patients -hydroxybutyrate.
- Prolonged yperglycemia lucosuria er lucose tolerance est onsistent etes llitus.

Electrolytes and Acid–Base Status

After studying this chapter, you will be able to:

- Describe lood uffer stems maintenance
- Explain ffect espiratory ate balance.
- Define *respiratory acidosis, respiratory alkalosis, metabolic acidosis, metabolic alkalosis.*
- List ions escribe their oles.
- List ommon onditions elated ered rum electrolyte vels.
- Describe valuation, calculated.

Acid–Base Balance,
Bicarbonate uffer,
Potassium uffer,
Protein uffer,
Acidosis and Alkalosis,
Respiratory cidosis
Metabolic cidosis
Base xcess,
Electrolyte Assays,
Sodium,
Potassium,
Chloride,
Bicarbonate,
Magnesium,
Calcium,
Inorganic horus,
Anion ,
Key Points,

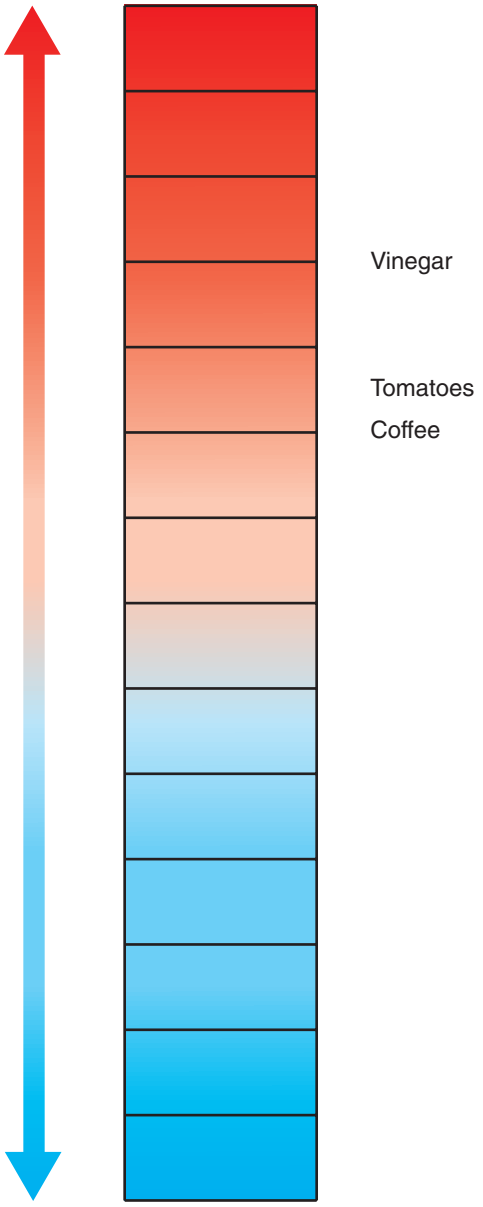
Acid–base balance
Acidosis
Alkalosis
Anion
Anion gap
Base excess
Bicarbonate
Buffers
Calcium
Cation
Chloride
Electrolytes
Hypercalcemia
Hypercapnia
Hyperkalemia
Hypernatremia
Hyperphosphatemia
Hypocalcemia
Hypocapnia
Hypokalemia
Hyponatremia
Hypophosphatemia
Inorganic phosphorus
Magnesium
Potassium
Sodium

Electrolytes e gative **anions** ositive **cations** f lements ody organisms. ome tions lectrolytes lude maintenance of water balance, osmotic pressure, normal muscular nervous functions. Electrolytes function maintenance ctivation veral nzyme stems acid–base egulation. epends lectrolytes, o erpreted ogether.

Acid–base balance efers o eady body. used to describe hydrogen ion concentration Fig. very hange umber ower-of-10 erence ydrogen oncentration. Normal ly row ange proximately alues egin tside ange, tioning ody's roteins or estroyed. hen alue ody ondition led **acidosis** hich haracterized excess ydrogen hen alue re condition led haracterized hydrogen n oncentration.

Normal tabolic rocesses ontinually enerate other processes work to counteract effect of acids. Buffer systems e esponsible or ounteracting ffects. **Buffers** are ubstances er ydrogen oncentration. Buffers e cated racellularly xtracellularly, multiple uffering stems resent ody. ome components uffer stems ve eely etween intracellular extracellular compartments, they are capable of either binding or releasing hydrogen ions response to blood

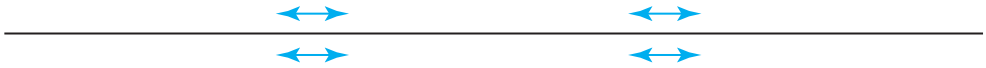
Both respiratory renal systems work to regulate resence espiratory stem works matter of minutes whereas renal system continues to function for days to restore level within normal



increases, the solution becomes more
concentration increases,
the solution becomes more basic or alkaline, and the pH value increases.
Clinical anatomy and physiology for veterinary

blood become acidic, **bicarbonate** binds to xcess ee ydrogen orm bonic). Carbonic acid then broken down to water carbon dioxide eaction diated bonic ydrase. arbon dioxide removed from body through normal respirations. The ys egulating oncentration of icarbonate ctively creting esorbing om filtrate esponse lood nder rmal onditions, icarbonate–carbonic uffer lood quilibrium wn hemical quation Note eactions eversible.

Changes oncentration **potassium** xtracellular ect oncentration hydrogen ions. Potassium hydrogen ions are both positively charged ns eely ve etween racellular ecreases oncentration potassium ause otassium ove rom ells hydrogen ns ve om ells. onversely, increased otassium vels esult otassium ving into ells ydrogen ving here fore, potassium affects acid–base balance, acid–base balance ects otassium oncentration.



Numerous roteins eleasing hydrogen ions. The hemoglobin molecule serves blood buffer esult f ility bon xide ydrogen. The bon xide ransported ungs, eleased, liminated ough espiration.

Acidosis are categorized by of condi tion. espiratory cidosis espiratory esults om abnormalities espiratory stem. etabolic cidosis metabolic esults om normal respiratory tions. ortant ondi tions e errelated ccur ously. s acidosis or develops, respiratory renal systems will both attempt to work to correct imbalance. For example, s etabolic cidosis lkalosis evelops, espira tory system will react by increasing or decreasing respiratory rate propriate.

If respiratory rate decreases, rate which carbon dioxide liminated ecreases. xcess bon xide eacts water to form carbonic acid. Carbonic acid then dissociates into water ydrogen ill videnced increased partial pressure of carbon dioxide blood **hypercapnia** bnormalities espiratory stem espiratory ate yperventilation) esult decrease concentration of carbon dioxide blood ubsequent ecrease lood **hypocapnia**

Any tabolic ondition esults uildup body creates condition of metabolic acidosis. For example, excess etones roduced hen lucose tabolism abnormal etes llitus) verwhelm uffer systems. The result generally decrease blood bicarbon ate vels. isorders er lectrolyte vels omiting) tabolic ubsequent blood icarbonate vels.

Base excess of strong acid or required to itrate lood held onstant g. his lue enerally alculated rom matocrit urements. xcess used to evaluate degree of metabolic acid–base disturbances.

gative alue tabolic cidosis, ositive value tabolic

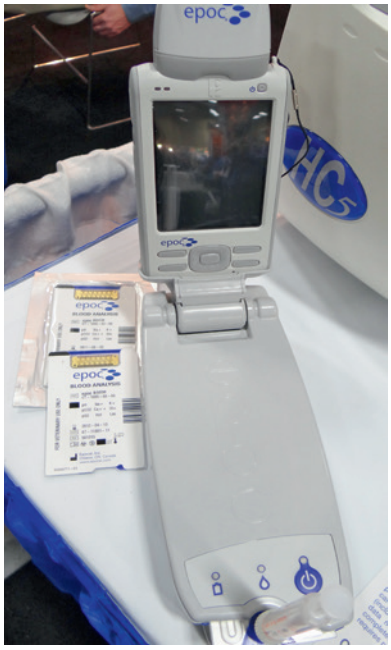
The r lectrolytes **calcium inorganic phos phorus magnesium sodium** otassium, **chloride** icar bonate Table hanges lectrolyte oncentration result om eased ecreased lectrolytes between eased enal etention electrolytes eased lectrolytes ys, gastrointestinal ract, espiratory stem.

Automated yzers or valuation lectrolytes readily available reasonably priced, many veterinary prac tices ve ility erform lectrolyte esting. any analyzers are capable of performing blood gas analysis Fig.

Volume cement ypically ects lectrolyte measurement, although method dependent. increased concentration esults olume ecreased water content. Electrolytes are distributed aqueous portion of ortion. herefore, ro cedures ure lectrolytes olume er f uch ect otentiometry, ill esult artifactually ecreased lectrolyte lues. his ill ccur nly very emic riglyceride oncentrations re rocedures ure lectrolytes aqueous hase nly er er), uch ect potentiometry, will result accurate electrolyte concentrations.

Arterial or ysis lectrolytes blood gases. enous have significantly different normal reference anges. yzed diately er collection. xposure oom esults lterations on centration f dissolved gases the and fects sample

Sodium major cation of ECF. It plays impor ole er ribution ody ressure



maintenance. In kidney, sodium filtered through glom eruli esorbed ck ody eded ough tubules xchange or ydrogen r, dium plays ital egulation balance. **Hypernatremia** efers o levated lood vel sodium. **Hyponatremia** ecreased lood vel dium. The odium alt eparin hould ot sed nticoagu ecause ly levate esults. emolysis oes nificantly er esults, ut ute erythrocyte uid nd ause alsely ower esults. ome onditions associated ith erations dium vels ummarized Table

Potassium racellular ion. ortant or normal uscular tion, espiration, diac tion, rve transmission, carbohydrate metabolism. In acidotic otassium ve eplaced hydrogen ns, reby esulting levated otassium levels, r **hyperkalemia** he otassium vel be elevated in resence of cellular damage or necrosis, which elease otassium lood. ecreased potassium levels, or **hypokalemia** may be associated with inadequate otassium esult of omiting rhea. ome onditions ciated with erations dium vels ummarized able preferred because platelets may release potassium uring lotting rocess tificially vated otassium vels. emolysis voided, ecause oncentration otassium rythrocytes her concentration Hemolysis releases potassium into esults tificially levated otassium levels. he efrigerated een parated om ells, ecause ooler emperatures promote otassium om ells ithout vidence of hemolysis. Samples be frozen without first separat lood ells, ecause esulting molysis uitable or esting.

Chloride redominant xtracellular ys important role maintenance of water distribution, osmotic pressure, normal anion/cation ratio. Chloride usually included lectrolyte rofiles ecause elationship to sodium and icarbonate vels. Hyperchloremia an elevated blood hloride vel, ypochloremia ecreased lood chloride vel. emolysis ect esults uting ith rythrocyte rolonged orage ithout separating lood ells htly esults.

Potassium is the major intracellular cation. Chloride

Bicarbonate cond ommon
kidneys help to regulate bicarbonate levels body by excret excesses after resorbing all needed. Bicarbonate levels are equently imated om lood bon xide vels. bicarbonate level approximately 95% the otal carbon dioxide measured. rterial lood ample hoice or icarbonate determinations. parin coagulant f hoice. hilled er to revent lycolysis om ering omposition. Freezing esults molysis. thods require ion

Magnesium fourth common cation body cond ommon racellular ion. agnesium found l ody issues. ore nesium ody ones, losely elated hosphorus. agnesium ctivates nzyme stems involved production decomposition of acetylcholine. Imbalance of magnesium/calcium ratio result muscular tetany result of release of acetylcholine. Cattle sheep are he nly omestic nimals hat how linical igns elated magnesium deficiencies. Hypermagnesemia refers to elevated blood nesium vel. ypomagnesemia ecreased lood magnesium vel. nticoagulants parin ti ficially ecrease esults. emolysis levate esults through eration nesium om rythrocytes.

More ody bones. The remaining or major functions body, which lude enance uromuscular xcitability tone (decreased calcium result muscular tetany), maintenance ctivity nzymes, cilitation blood coagulation, maintenance of inorganic ion transfer across cell membranes. Calcium whole blood found entirely or serum. Erythrocytes contain calcium.

Calcium oncentrations re sually nversely elated nor ganic phosphorus concentrations. general rule, calcium concentration rganic hosphorus oncentration falls. **Hypercalcemia** levated lood oncentra tion. **Hypocalcemia** s ecreased lood alcium oncentration.

Samples or lcium esting ollected DTA or xalate r rate icoagulants, ecause ubstances bind ith vailable or y. emolysis results ecrease oncentration om uptured rythrocytes utes

More hosphorus ody bones. he emaining tions, uch energy orage, elease, ransfer; volvement bohy drate metabolism; composition of many physiologically important ubstances, uch ucleic hospholipids.

Most f hosphorus lood erythrocytes organic phosphorus. The phosphorus rum rganic hosphorus, hosphorus assayed laboratory. Inorganic phosphorus levels rum rovide ood ion phorus or serum phosphorus calcium concentrations versely elated. hosphorus oncentra tions ecrease, oncentrations

TECHNICIAN NOTE

Hyperphosphatemia increased serum or phos phorus concentration. **Hypophosphatemia** decreased serum or lasma hosphorous oncentration. emolyzed amples hould e d. rganic hosphorus erated om ruptured rythrocytes ydrolyzed rganic phorus, which results falsely elevated inorganic phosphorus concentration. rum parated om lood ells on ossible er lood ollection before ored.

Under normal circumstances, total number of positive charges (cations) quals umber gative harges This electrical neutrality maintained through buffer systems. Any erence etween ositive harges negative harges **anion gap** his lue s alculated rom he measured lectrolyte alues, rimarily entify metabolic cidosis. eneral, nly ommonly ured electrolytes ion.

The n ap ed ollows:

The rmal urement proximately q/L dogs to Increases are usually seen with lactic acidosis, renal failure, diabetic ketoacidosis. Hypoalbuminemia common of decreased anion gap.

- Electrolyte assays performed veterinary practice laboratory include sodium, potassium, chloride.
- Some electrolyte analyzers evaluate calcium, phosphorus, magnesium, bicarbonate, blood gases.
- The major electrolytes are calcium, inorganic phosphorus, magnesium, sodium, potassium, chloride, bicarbonate.
- Changes electrolyte concentration result from increased or decreased intake, of electrolytes between ICF, increased renal retention of electrolytes or increased of electrolytes via kidneys, gastrointestinal tract, or respiratory system.
- Arterial venous blood have different normal values (reference ranges) for electrolytes blood gases.
- Sodium major cation of ECF, whereas chloride predominant extracellular anion.
- Potassium major intracellular cation.
- Calcium concentrations are usually inversely related to inorganic phosphorus concentrations.
- The phosphorus serum inorganic phosphorus. Phosphorus within erythrocytes organic phosphorus.

Miscellaneous Tests

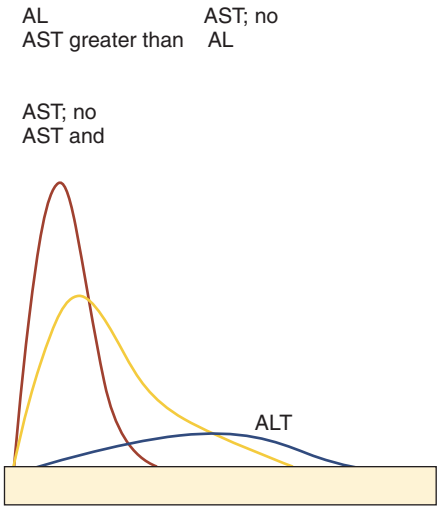
After studying this chapter, you will be able to:

- Describe elevance eatine esting or diagnosis f ver eletal uscle
- Describe ctate valuation or itical ients.
- Discuss ions or drenocorticotropic rmone stimulation examethasone uppression
- Discuss roduction ction yroxine.
- Describe hemical astrointestinal tion.
- Describe or oxicology esting.
- Describe ommon oxicology esting.

ACTH stimulation test
Addison's disease
Adrenocorticotropic hormone
Cortisol
Creatine kinase
Cushing's disease
Dexamethasone suppression test
Ethylene glycol
Hematochezia
Hyperadrenocorticism
Hyperthyroidism
Hypoadrenocorticism
Lactate
Melena
Mucin clot test
Plumbism
Thyroid-stimulating hormone
Thyroxine

iety f other says performed in veterinary ractice. These lude ys esigned etect normalities endocrine systems well other biochemical tests are specific o rgan stem ut ill rovide nostic and rognostic nformation e.g., lood actate easurements). Other lood ctate urements, hich performed eterinary ractice oratory, tests e erformed ut ather ef erence r eferral ractice oratories. ome vailable immunoassays erformed

Creatine kinase found wide variety of tissues. Small are present bladder, gastrointestinal tract, thyroid, kidney, ung, en, rity ctivity eletal and cardiac muscle brain. hen skeletal muscle, luding diac uscle, ed estroyed, t f ells roduces levated lood vel. equently yed levated lood aspartate ransferase vel ut ws



atinine kinase, aspartate transaminase, and alanine transaminase levels

differentiate between predominantly liver or skeletal muscle injury. (From *Veterinary laboratory medicine: interpretation and*

signs of liver Fig. evaluated cere brospinal because measurement been suggested ancillary diagnostic test for nonspecific damage to ural issue ural ypoxia, rauma, ion, compression pace-occupying esion uch umor). he alue refore or rognosis neurologic premature foals. Increased values may e bserved er izures.

Although ything uscle ell mbrane cause n ncreased lood evel, ractionation he soenzymes of ce exists three primary isoenzymes: (brain type), (cardiac ype), eletal uscle ype). ncreases diac uscle uscle rauma.

Muscle eased em om intramuscular injections, persistent recumbency, surgery, vigor xercise, lectric ck, ceration, ruising, ypother yositis yopathies levated lood vels. vels tificially eased oxidizing agents such bleach, ethylenediaminetetraacetic acid (EDTA), rate, ride, xposure unlight, elay performance y.

Cardiac muscle damage be evaluated with troponin assay. Cardiac troponins are proteins involved regulating iation eletal uscle ontraction. ncreases levels diac uscle vel etermining egree lapsed since damage occurred. Brain natriuretic peptide hormone secreted by myocytes function maintenance of blood ressure. vated vels ccur eased entricular filling ressure ure. Both unoassays. esults erpreted onjunction nostic

Lactate ctic cid) roduced erobic ellular tabo Its presence does indicate any specific However, increased lactate levels indicate hypoxia or hypoperfusion. Lactate levels y ured eritoneal Hypoxia f ction owel esults eased ctate production, uch hich eritoneal vity before nters culation emoved ver.

f ed lood eritoneal ctate urements been advocated diagnostic equine colic The blood ctate oncentration rmal rses ways reater of peritoneal Horses with gastrointestinal disor ders enerally ve eritoneal ctate oncentrations are reater orresponding lood alues. vere gastrointestinal disorders (e.g., impactions) tend to smaller difference etween eritoneal uid nd lood actate oncentra tions compared with more serious conditions (e.g., intestinal torsion isted ction owel]). eritonitis peritoneal ctate alues.

Increased lactate levels generally indicate hypoxia

The used for lactate measurement blood or peri toneal be collected fluoride oxalate or lithium heparin nticoagulant ube. he uoride tops ellular etabo f lucose onsequent roduction ctate, xalate revents lotting. andheld ctate ters have been validated for veterinary species are available Fig.

Lactate ehydrogenase rum nzyme yzes onversion ctate yruvate. isoenzymes. ifferent enzymes resent different issues. issues ve ver, muscle, rythrocytes ces eased blood vels. ompared nitude rise dramatic after muscle injury. values are frequently included biochemistry profiles. This enzyme considered organ-specific, ecause ces ecause concentrations ch issue nough esult significant levations.

In ddition o ancreas, riety rgans issues elease hormones tion ndocrine stem. rimary

Principles and practice

organs of endocrine system are adrenal glands, thyroid athyroid uitary produce nd ecrete ormones irectly nto apillaries, nd hey have ariety et rgans ffects.

Adrenocortical function tests are commonly performed. drenal dysfunction increasingly common often result of misuse of corticosteroids. The adrenal starts with hypothalamus. Stimuli originate brain (e.g., result of stress) hypothalamus to secrete corticotropin-releasing factor. Under influence of corticotropin-releasing factor, adenohypophy cretes **adrenocorticotropic hormone** CTH), hich hormone imulates drenocortical rowth cretion, particularly of glucocorticoid-synthesizing tissue. **Cortisol** major rmone eleased omestic turn, eeds ck orticotropin-releasing ctor CTH elease, reby ompleting ed stem.

True or mimicked hyperfunction of the system common complaint. Brain or pituitary tumors lead to secondary bilat eral adrenal hyperplasia, idiopathic adrenal hyperplasia, or neopla of one or both glands may excessive cortisol release **hyperadrenocorticism** Overenthusiastic glucocorticoid therapy common of cortisol excess. Because exogenous (like endogenous) glucocorticoids inhibit adrenotropic hormones, iatrogenic hyperadrenocorticism accompanied by paradox of rophied drenal udden ithdrawal xog enous lucocorticoids drenal ypofunction. owever, **hypoadrenocorticism (Addison's disease)** y efinition, ncludes mineralocorticoid eficiency, hich oes ccur ro genic caused by rapid withdrawal of glucocorticoids. Addison's esult om veruse (Lysodren; or drenal yperplasia) om

Overenthusiastic glucocorticoid therapy is the most

Screening ests or yperadrenocorticism efully interpreted, ecause ogs nadrenal diabetes llitus), ver enal ve positive esults. hereafter, yperadreno corticism de onjunction with several of various laboratory tests. Conversely, negative laboratory esting ccurs onsistent vidence, etested er persist.

ACTH ortisol oncentrations lpful tic or erentiation rimary drenal-dependent) hypoadrenocorticism om condary uitary-dependent) hypoadrenocorticism. owever, urement limited fulness, ecause vels tuate urnal cycle. More often, measurements are taken baseline compared with obtained from challenge to adrenal gland with or dexamethasone. with functioning adrenocortical umors ve oncentrations esult gative eedback ffect. uitary-dependent hypoadrenocorticism have higher concentrations. Low to undetectable concentrations occur with secondary son's hereas rmal eased) oncentrations expected with primary ddison's labile protein, special handling of needed. protinin (protease inhibitor) within EDTA tube or immediate freezing of equired. or ortisol immunoassays, some are available to veterinary practice laboratory. Some tests are performed on serum, while others be erformed nly ew performed on urine Urine cortisol/creatinine ratios have een eening or drenal tion.

Animals with suspected hypoadrenocorticism (Addison's or yperadrenocorticism **(Cushing's disease)** y e valuated with CTH response test. In addition, test indicated to distinguish mong atrogenic nd pontaneous yperadrenocorti rocedure fficacy ketoconazole, or metyrapone therapy. The **ACTH stimulation test** evaluates degree of adrenal gland response to administra tion f exogenous CTH. The egree of response to stimulation by lucocorticoid roportion lands' development. Hyperplastic adrenal glands have exaggerated responses, whereas hypoplastic adrenal glands show diminished responses. he est an etect hese bnormalities ut ot eveal their imate esponse eening test. drenal yperactive om oplasia be nsitive onetheless, rent est re ccurate or adrenocortical yperfunction og re accurate or

Hormone Stimulation Test

synthetic adrenocorticotropic hormone (ACTH; cosyntropin) via

administration in dogs and cats and 4 hours after ACTH administration in

normal post-ACTH cortisol concentration does not rule out Cushing's

Cortisol Level

the same protocol as described previously, except increase the dexa

Note: Successful suppression is defined as a 50% decrease in the plasma cortisol concentration from the baseline value. In 15% of dogs with pituitary-dependent hyperadrenocorticism, the plasma cortisol level is not suppressed

that remain above these are considered adequate for suppression (i.e., greater

Dexamethasone suppression tests valuate drenal differently by adrenal feedback loops. The low-dosage test onfirms eplaces esponse or yperad renocorticism ushing's h-dosage oes further erentiate uitary yper adrenocorticism om drenal rocedure only h-dose examethasone uppression uitable.

Dexamethasone, which potent glucocorticoid, suppresses ACTH elease om rmal uitary esults drop ortisol oncentration. yperadrenocorti f y tiology ually uppression om small examethasone ecause uitary abnormally insensitive to drug continues to elaborate excessive CTH, although of dogs with pituitary-dependent hyperadrenocorticism have post-dexamethasone cortisol level f ess han g/dL 0% he aseline oncentration. Neoplastic drenal tonomously creting ortisol independent ndogenous CTH ontrol. he xcessive ortisol production uppresses cretion rmal pituitary gland through negative feedback inhibition. Small doses of examethasone ect ortisol urements. However, such doses may complicate test results may differen tiate only normal from with hyperadrenocorticism.

With er examethasone re erences pear. The nsitivity uitary examethasone omplete; examethasone vercome abnormally high cortisol concentrations fall. However, normal drenal ontinue crete ortisol autonomously. hus, ortisol oncentrations unresponsive examethasone robably by primary adrenal gland Suppression by large (but small) oses uggests uitary accuracy for differentiating pituitary from adrenal dogs sensitivity for diagnosing hyperadrenocorticism dual high-dosage dexamethasone test response test e escribed rocedure lthough ombined protocol ep ver, ossibly esults cessitate more ests xpense. esponse gment test particularly prone to error. Because dexamethasone alters adrenal esponsiveness nhances uch responsiveness, epending he uration ctivity), he iming of test crucial. Normal standards must be newly established for y hanges rotocol.

This est ed erentiate etween uitary-dependent hyperadrenocorticism primary hyperadrenocorti ortisol oncentration

Corticotropin Stimulation Test

mmediately administer synthetic adrenocorticotropic hormone (ACTH) intra

for cortisol determination at 30 minutes

administration in dogs and cats and 4 hours after ACTH administration in

elevated er orticotropin-releasing rmone imulation dogs ith drenal-dependent ushing's

The rotocol onsists btaining retest sample o etermine he ortisol CTH evels, dministering of corticotropin-releasing hormone, obtaining blood ain utes er valuate ortisol CTH vels.

Thyroid rmones ve ervasive ffects, nce tabolic ate, rowth, erentiation ody ells. Because yroid tion umerous onfusing, tion aluable. yroid are overned drenal ortices. hyrotropin-releasing factor TRF) om ypothalamus ncourages erior pituitary to release thyrotropin or **thyroid-stimulating hormone** (TSH). SH nhances yroid rowth, tion, **thyroxine** release. hyroxine eally omposed arieties mones, riiodothyronine yroxine hich ary ir xtent dination. onverted re active tissues. Thyroxine completes regulatory cycle by inhibiting elease.

Thyroid ested rimarily ypofunction dogs, horses, ruminants, and ine and as yperfunction cats. The may be dietary iodine deficiency or excess or goitrogens,

Stimulating Hormone Response Test

determination is made

normal dogs should be approximately twice the baseline value, or it should

which e ommon rimary landular (e.g., neoplasia, autoimmune idiopathic atrophy) comprises whereas pituitary (secondary thyroid omprises ypothyroid ogs. ood ortion, stillbirths, pecia, oiter etuses onates), rum concentrations, serum protein-bound iodine concentrations, pasture iodine analyses. Feeds be xamined or oitrogenic *Brassica* spp.) or for excess calcium, which decreases iodine take.

In-house thyroid testing is generally performed with

Baseline thyroxine concentrations are used diagnostically, but normal values vary dramatically. Immunologic tests are available for urement oncentrations. ome ugs insulin, rogens) oncentrations; rs glucocorticoids, iconvulsants, ithyroid ugs, enicillins, trimethoprim ulfamides, epam, ogens, ulfonylureas) may decrease concentrations. In addition, total (TT may be eased ypothyroid ogs esult resence of i-T ibodies. ecific etermination ctive form of thyroxine non–protein-bound or free more accurate proach yroid tion.

The thyroid-stimulating hormone response test used on small xcept **hyperthyroidism** rses, provides reliable diagnostic separation of patients with normal versus normal yroid tion ox xogenous challenge may sort out borderline separate real hypo thyroid patients from with other illness or drug-depressed thyroxine oncentrations, oint ns.

The test usually used to explore hypothyroidism. fter TSH injected, thyroid response (usually serum levels, which provide eliable ex) ollowed. serum level occurs normal Primarily exhausted or insensitive yroids espond xogenous ndeed, endogenous TSH concentrations are already high from inhibition. Therefore, serum level increased ith uitary rain wever, yroid glands remain responsive. Such lesions result too endog enous yrotropin. lthough rum vel is xpected n nimals ith ituitary esions, ays SH

challenge may be necessary before increased serum levels are seen. The extra TSH required to overcome chronic glandular atrophy, method comparable to "priming pump."

Glucocorticoids seem to inhibit both TSH secretion, euthyroidism with low serum levels only often accompa Cushing's or vigorous glucocorticoid therapy. For tunately, TSH ACTH response tests may be performed simultaneously. In such glands remain responsive to TSH, but absolute values of prechallenge postchal lenge serum are low or low resting with normal post-TSH values. Feline hyperthyroidism usually caused by functional thyroid adenomas. Oddly, with exogenous TSH challenge, or increase occurs serum level, primary hypothyroidism. This phenomenon suggests neoplasm either functions independently of trophic hormone or already manufacturing maximum capacity. lack of TSH responsiveness, appropriate clinical manifesta tions, high baseline concentrations all attest to feline hyperthyroidism.

In horses, iodine-deficiency hypothyroidism rare, because iodized usually offered freely or feeds. Overzealous iodine supplementation with kelp or vitamin mineral mixes provoke hypothyroidism goiter. The excessive of iodine inhibits thyroid function. The normal serum values horses are to which lower what found other species. Hypothyroidism be suspected only with serum concentrations of

Rare tumors of pars intermedia of pituitary, which compress anterior pituitary, may secondary hypothy roidism older horses. Because pituitary damage induces plethora of signs, the TSH response test may be especially helpful.

The thyrotropin-releasing hormone (TRH) response test used on small provides reliable diagnostic separation of patients with normal versus abnormal thyroid function. fraction of thyroxine bound to protein. levels are influenced by nonthyroidal or drugs total concentrations. Exogenous challenge may sort out borderline separate real hypothyroid hyperthyroid patients from those with other illness or drug-depressed thyroxine concentrations. The test usually used to explore hypo thyroidism when TSH available. Baseline serum TT concentrations are determined. Four hours after or 0.2 (total dose) of TRH are injected intravenously, thyroid response (serum TT levels) followed. An increase serum TT concentration of concentration of times compared with baseline concentrations occurs normal The evaluation of levels allows for clearer distinction between euthyroid hypothyroid dogs when TT results are equivocal. The response test may be used to diagnose mild to moderate feline hyperthyroidism. Baseline serum concentrations are determined. Approximately hours after of are injected intravenously, serum levels are determined. An increase of serum of compared with baseline concentra tions occurs hyperthyroid Increases of between are borderline, increases of more rule out hyperthyroidism.

TECHNICIAN NOTE is the fraction of thyroxine that is not bound

Hyperthyroidism common middle-age to United States Great Britain. Diagnosis may be based on resting thyroid hormone concentrations. The determination of both TT may help to distinguish nonthyroidal The combination of high value with low TT level indicative of nonthyroidal illness, whereas high concentration high-normal TT concentration indicate hyperthyroidism. However, some may require functional test to confirm or rule out

Thyroid suppression testing based on expected negative feedback regulation of TSH, which induced by high concentra tions of circulating thyroid hormone. Hyperthyroid have normal pituitary–thyroid regulation. As result, administration of exogenous must induce decrease endog enous feedback TSH regulation altered.

To perform the test, basal and determination is required. Seven doses of given orally every hours are admin istered home. Approximately to hours after seventh dose, blood obtained for determination. Cats with hyperthyroidism have serum concentrations of more or whereas nonhyperthyroid have lower values. Low posttest concentrations indicate invalid test result of failure of exogenous administration.

The diagnosis of acromegaly may be based on docu mentation of elevated growth hormone level. Serial determinations from three to five taken 10-minute intervals) are performed because affected dogs have constant levels of rather fluctuating concentra tions. In addition, affected dogs do respond to stimulation with GH-releasing hormone. This test requires intravenous administration of of GH-releasing hormone or of clonidine. In normal dogs, the posttest plasma GH level increases to posttest clonidine level increases to

The principal functions of gastrointestinal tract are assimilation of nutrients (via digestion absorption) excretion of waste products. Most nutrients are ingested form either too complex or insoluble for absorption. Within the GI tract, these substances are solubilized and degraded enzymatically to molecules may be absorbed across mucosal epithelium.

are common veterinary practice. Specific essential, especially when chronic. In of malabsorption, intestinal biopsy tends to be required to obtain

efinitive unction erformed other onfirm ed or re vasive nostic rocedures.

Malassimilation may be classified by pathophysiologic process into maldigestive malabsorptive forms. Maldigestion results from altered gastric secretion lack of or decreased of digestive enzymes, which are usually secreted by pancreas often, by intestinal mucosa. Malabsorption often d cquired estinal or y cterial vergrowth omes. efore maldigestion are seen, approximately of pancreas must be ither nfunctional estroyed. estine dog tion ell ut re esult short owel ome," hich be ompensated or daptive chanisms.

Laboratory tests may evaluate gastric hydrochloric acid secretion, but of them are directed to detect malassimilation origin. Gastric acid secretion may be indirectly estimated by etermining astric uice rmal ogs ve gastric etween ric uice continuously nitored adiotelemetric echnique.

Malassimilation xamination eces for ecal tary utrients ecal nzyme ctivities ell serum or oncentrations rally dministered ubstrates metabolites ecific or ndogenous ubstances.

Blood into gut another of protein-losing gastroenteropathy. Dramatic bleeding evident black feces **(melena)** or frank fecal blood **(hematochezia)** Less-obvious subtle bleed nificant ers, oplasia, asitism. Chronic low-level bleeding may lead to iron-deficiency anemia.

The eagent etect ccult leeding Impregnated rips lets xidized olored roduct by moglobin eroxidase ctivity eces. eagent responds o tary moglobin yoglobin; refore, patient's t ee or ys efore precaution ertinent rbivores, ut echnician must heck een upplemented or one nother ecal ccult lood vailable unochromatographic thods, ut een validated ith eterinary ecies.

These ests re ecifically robe estinal tion. gain, ent iven rally; lood oncentrations ure of rption.

d-Xylose e-carbon ugar rbed vely jejunum xcreted apidly ys. ecause absorption agent metabolized, fate readily traced. Xylose absorption inefficient often affected by me estinal onetheless, elatively insensitive, ecause ontrol alues ariable.

The est rimarily erformed rses, er formed ogs ell, escribed ox nterference

Absorption Test in Dogs and Horses

are obtained from post-administration blood samples that are collected 30,

from umen ra recludes ral sheep; alternative injection of monosaccharides into abomasum nough are.

Abnormal xylose absorption indicates intestinal tion specifically, malabsorption. However, only slight differences parate rmal normal anges; may have normal results. with lymphangiectasia still may have rmal esults, ecause ymphatics ticipate lose rption. ate rption epends only n iven, rptive intestinal blood circulation, gastric emptying. The latter may be elayed y ypertonic utions, prehension, or feeding. Fasting or radiographs to confirm empty stomach are equired. omiting, wever, ly wers lood alues, does ascites (xylose enters pooled Bacteria have ability to tabolize refore, cterial vergrowth monitored by test. If bacterial overgrowth suspected (e.g., of intestinal or pancreatic enzyme deficiency), test epeated er s' ral etracycline. Finally, renal falsely elevates blood xylose concentrations.

The fate of xylose been followed dogs by collecting ine er ral etermining otal lose xcreted. thod re orious, ut requires nly y.

Cats were thought to have concentrations kinetics ere ogs. ther take be variable concentration increase to vels ound ogs. oncentrations normal ranged between when dosage of ody eight

False-negative results may be caused by delayed gastric emptying, normal estinal ility, educed estinal lood w, bacterial overgrowth, sequestration of xylose ascitic

False-positive results may be caused by decreased glomerular

filtration rate; therefore, ensuring patient fully hydrated azotemic time of testing important.

oral dose of xylose, excreted through kidneys within hours. This test been improved by performing d-xylose 3-O-methyl-d-glucose absorption test com pares differential absorption of two sugars to eliminate nonmucosal effects of d-xylose absorption.

Serum concentrations of folate and cobalamin may be assessed by immunoassay. Both concentrations tend to be decreased with absorption. Folate absorbed proximal intestine, whereas cobalamin absorbed ileum. Bacterial overgrowth may also alter these concentrations; folate synthesis is increased with bacterial overgrowth, whereas some bacteria may decrease cobalamin availability. Assays for folate cobalamin (vitamin are usually performed conjunction with to evaluate extent of gastrointestinal disorders.

Synovial mucin forms clot when added to acetic acid. The nature of resultant clot reflects quality concentration of hyaluronic acid. method used to perform test involves adding of non-anticoagulated synovial to glacial acetic acid been diluted The synovial fluid/acetic acid solution gently mixed allowed to room temperature for before evaluated for presence of clot. The mucin clot generally graded good large, compact, ropy clot clear solution), soft clot slightly turbid solution), fair-poor friable clot cloudy solution), or poor actual clot, but some large flecks present turbid solution). Clot assessment enhanced by gently tube. Good clots remain ropy, whereas poor clots fragment. If only few drops of synovial are obtained with arthrocentesis, an abbreviated **mucin clot test** may be performed. If available after preparation of cytologic possibly after total nucleated cell count), drop of non–EDTA-preserved placed on clean microscope slide. Three drops of diluted acetic acid are added mixed. The resultant clot graded after approximately minute. Assess ment may be easier against dark background.

Numerous agents may be involved in common poisonings of dogs, horses, food These agents include herbicides, fungicides, insecticides, rodenticides, heavy metals (especially lead), household products (including phenols), automotive prod ucts (especially ethylene glycol), drugs (including medications), and various poisonous plants and animals. Often presumptive diagnosis may be attained from accurate history (including environmental factors) thorough clinical examination followed by response to therapy or by necropsy. However, establishment of specific etiologic diagnosis may be some few tests may be performed veterinary practice laboratory. In such situations, personnel must be competent with test procedure, reagents must be outdated, special equipment may be required. These requirements, together with sporadic demand for such tests, frequently dictate practitioners send all toxicologic specimens to specially equipped laboratory for analysis.

Suggestions for appropriate specimens preferred methods of handling, packaging, transport be obtained by consulta tion with toxicology laboratory. Such contact ensures laboratory offers procedures requested. Submitted specimens be free from contamination by extraneous environmental compounds or debris. Specimens be washed, because may remove toxic residues. Samples of different tissues, feeds must be submitted sepa rate, clean, leak-proof (airtight) plastic or glass containers. All containers be individually identified by owner's veterinarian's animal's or identification number, nature of specimen before packaged into large container for submission to laboratory.

Samples of whole blood usually heparin ized), serum (at least mL), vomitus, gastric lavage fluid, feces, urine (approximately may be submitted from live Samples of feed (portions of water, suspected may be helpful some In of poisoning, collected during thorough necropsy include whole blood or serum; urine; gut (especially stomach) contents g, noting site of collection); organ or tissue especially liver kidney but some times brain, bone, spleen, or (generally, where practi of each tissue). Sending too large always better sending enough, because excess be discarded.

In general, serum or blood are best submitted refrigerated, whereas gut contents tissues are best frozen. Preservatives are usually required. An exception would be tissue are submitted for histopathologic examina tion, which require fixation formalin must be frozen. If preservative used on specimen submitted for chemical analysis, probably worthwhile to submit aliquot of preservative for reference analysis. Frozen be insulated from other specimens arrive laboratory while they are still frozen. Dispatch to labora tory by courier recommended.

Because litigation may result from poisoning accurate detailed records be kept from outset of The establishment of good working relationship with toxi cology laboratory, including provision of good history necropsy findings of poisoning) when are submitted, helps to ensure best results.

The advantage of following tests they be performed reasonably quickly practice laboratory. Results are therefore available more rapidly were sent to toxicology laboratory. However, they are best viewed screening procedures be used to suggest appropriate avenues of investigation treatment. The verification of findings (especially positive ones) by reputable toxicology laboratory

Fig. 36.3 Lead poisoning causing recumbency, blindness, and bellowing. There also were uncontrolled jerking actions in this 16-month-old

dvisable, ecially ubsequent gal ction lient ossibility.

Lead ly ommon nvironmental ollutant found ell d-based lead unition), eries, er, oofing materials, petroleum products. Lead poisoning **(plumbism)** occur all species. Clinical signs vary with species, they e hiefly elated ract rvous stem Fig. ematologic xamination lood om with d oisoning eveal philic ippling erythrocytes increased numbers of circulating nucleated red blood ells tarubricytosis). uch is ot nemic nd hat as linical igns hat re onsistent ith lead oisoning rongly uggest umbism.

No simple, reliable in-house ests exist to detect ad blood, feces, urine, or tissues. Blood lead levels be determined readily oxicology oratory om lood been ollected parin, rate lood ubes. issue samples especially ver idney) eces ay lso ested. Histopathologic examination of liver, kidney, or bone stained with iehl-Neelsen echnique eveal haracteristic philic, cid-fast ranuclear lusion odies patocytes, renal ubular ells, eoclasts, espectively.

Nitrate r rite oisoning ccur horses eeds oncentrations om pounds. uch ay he ase ereals, rasses, nd oot rops ve een vily ertilized rogenous ompounds. Water, ecially om eep ells led epage from vily ertilized round, ontain uantities of rate. itrates onverted rites eed estinal ract. itrites rbed om ecrease oxygen-carrying capacity of blood by degrading hemoglobin to themoglobin rythrocytes. onsequently, blood becomes dark-red to brown. The severity of clinical signs related to quantity ingested. Death be acute, many ected.

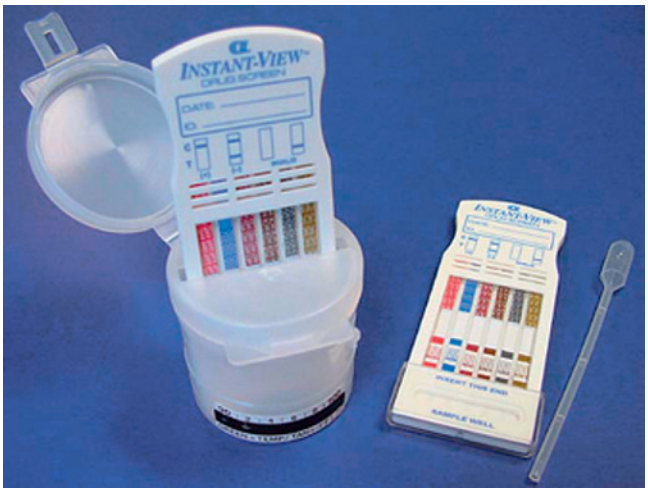
apid ly ecific miqualitative nylamine, hich onverted uinoidal ompounds ensely lue olor rates rites. iphenylamine ved illed er ution made o oncentrated ulfuric ock solution may be used undiluted or diluted with sulfuric acid. he ution plied ortion 's stem. n ense lue olor conds plication of uted ution uggests re rate present thus feed potentially toxic). False-positive results may occur with numerous substances, significant of hich on. uch on enerally tside refore, eful plication cumvents roblem. more dilute diphenylamine solution previous stock solution uted oncentrated ulfuric used to test for nitrates nitrites serum, other body hree ops uted henylamine added o ne op ver hite background. itrate rite roduce ense lue olor immediately. emolysis olor hange.

Anticoagulant rodenticides (e.g., warfarin, diphacinone, pindone) act y iting tabolism ody. latter equired or roduction ctors ver. nticoagulant odenticide oisoning ially ro longs rothrombin ecause ctor depleted. Subsequently, partial thromboplastin time activated oagulation rolonged ctors become depleted. hen bleeding result of such poisoning, rothrombin tial om boplastin ime ctivated oagulation ually prolonged. icoagulant odenticide oisoning often based on screening tests patient's response to reatment

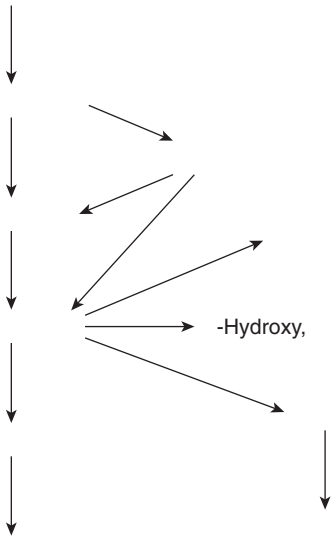
variety of compounds, when ingested, may result damage to oxidative denaturation of) hemoglobin erythrocytes with ormation einz odies. uch ubstances lude paracetamol methylene blue onions (dogs), red leaves (horses), onions brassicas (ruminants). The demonstration of Heinz bodies on blood diagnostic of such poisoning.

Selenium-deficient are more prone to such oxidative injury esult eficiency lutathione eroxidase enzyme erythrocytes helps protect against such damage).

Ethylene glycol major constituent of antifreeze solutions. ccidental estion rious oxicosis, usually ogs thylene lycol tabolites Fig. etected lood rum by oxicology oratory. resence thylene lycol strongly uggested hen diments om oisoned ogs



ed 11, St Louis, 2011, Saunders. Courtesy Alfa Scientific,



Pathways of ethylene glycol metabolism. The pathway by which the toxicologically significant metabolites are generated is shown vertically.

or ontain xalate nohydrate ystals (see nit opathologic xamination ys of ly ected eveals enal ubular phrosis numerous xalate ystals.

Animals ecially ogs xposed ariety of legal rescription dications. any ients are resented or reatment er nset signs. The diagnosis of toxic exposures to human drugs of abuse may e omplicated ck ecific ormation egard otential xposure. lients ware exposure ccurred, rosecution ting o ossessing ugs ithout gal rescription. Clinical signs vary considerably depending on drug otal ested.

Few studies exist regarding mechanisms of action of many of medications In addition, mode of expo sure often differs om that seen humans. Drugs ould normally e cted ually esult xposure estion et dditional om plication f xposure elates otential resence substances ed ugs entifiable or eadily parent. ve emonstrated of licit ugs ontain posed o e, ontain ugs imulants.

Routine biochemical analysis of blood urine rarely dem onstrates any abnormality otherwise healthy patients after acute xposure. ariety eening vailable rity tilize competitive unoassay echnique ontained eral w hly ccurate with rict dherence ollection, rocessing, performance requirements. The tests are to type first marketed or regnancy orrelation indicate egree reement etween oratory field est esults. ommon ypes dipstick ys. vailable umerous onfigurations est or yte or ultiple ytes. ew ests ody ell. ome manufacturers allow for purchase of very small quantities of such ests.

The ccuracy epends mewhat consumed lapsed xposure.

f dized oncentration vels lished by international regulatory authorities, specifically National Institute n buse, orld anization, Substance buse Mental Health Services dministration of epartment ervices. etailed published studies of validity of tests for veterinary species e vailable.

- Creatine be used to differentiate liver from skeletal muscle damage.
- Increased lactate levels generally indicate hypoxia or hypoperfusion.
- ACTH cortisol concentrations may be helpful diagnos tic for differentiation of primary from secondary hypoadrenocorticism.
- Thyroxine composed of two varieties of hormones: triio dothyronine (T thyroxine (T
- Free fraction of thyroxine bound to protein.
- In-house thyroid testing generally performed with immu nologic methods.
- Ethylene glycol ingestion serious or toxicosis.
- Immunochromatographic tests are available to evaluate patients for potential poisoning with human drugs of abuse.

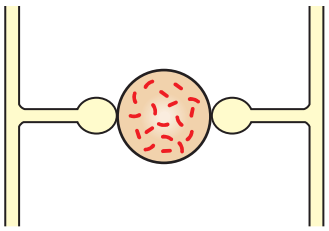
Unit Outline
Chapter 37: Introduction to Microbiology,
Chapter 38: Equipment and Supplies,
Chapter 39: Sample Collection and Handling,
Chapter 40: Staining Specimens,
Chapter 41: Culture Techniques,
Chapter 42: Antimicrobial Sensitivity Testing,
Chapter 43: Additional Testing,
Chapter 44: Mycology,

The Objectives for This Unit Are:
List and Describe the Supplies and Equipment Needed for Microbiology Testing.
Describe the General Characteristics of Bacteria and Fungi.
Discuss Sample Collection Procedures for Bacterial and Fungal Samples.
Describe Commonly Used Staining Procedures for Bacterial and Fungal Samples.
Describe Proper Techniques for Culturing Bacterial and Fungal Samples.
Describe the Proper Procedure for Antimicrobial Sensitivity Testing.
Describe the Procedure for Performing the California Mastitis Test.
List and Describe Common Biochemical Tests Performed on Bacterial Samples.

Microbiology refers to the study of microbes. Microbes are organisms that are too small to be seen with the unaided eye. Bacteria, fungi, and viruses are all microbes. Some parasites are also considered microbes. The fields of study of bacteria, fungi, and viruses are referred to as bacteriology, mycology, and virology, respectively. Virology evaluations in the veterinary clinical laboratory are usually performed with immunologic methods. Bacteria and fungi can be evaluated with a number of routine microbiology procedures. Although some prac tices send all microbiology work to a reference laboratory, most practices do some testing in-house. Bacterial and fungal samples can be collected quickly, easily, and inexpensively, and tests do not require much in the way of specialized equipment. Careful attention to quality control is vital to ensure the diagnostic value of results.

Most microbes found on and in the body are nonpathogenic (i.e., they are normal flora). The intestinal and respiratory tracts, the skin, and parts of the urinary and reproductive tracts all have known normal flora. Samples collected from some locations, such as the spinal column, the blood, and the urinary bladder, should be free of normal flora. Microbes that are considered normal flora and nonpathogenic when found in one location can produce significant disease if they are found in a site where they should not reside.

For additional sources for this unit see the Resources Appendix at the end of this textbook.



Introduction to Microbiology

After studying this chapter, you will be able to:

- Describe eneral haracteristics cteria, viruses.
- Discuss cteria rowth haracteristics equirements for rowth.
- Describe haracteristic rangements bacteria.
- Discuss nificance ore ormation cteria fungi.
- Describe eproduction rganisms.
- Differentiate roups genic
- Discuss eneral thods or iral ecimen ollection handling.
- List thods or valuation suspected iral gens.

Bacterial Cell Morphology,
Spores,
Bacterial Growth,
Fungal haracteristics,
Virology,
Cell ulture,
Immunologic olecular iagnostics xamination,
Key Points,

Ascospores
Bacilli
Basidiospores
Capnophilic
Cocci
Conidia
Endospores
Facultative anaerobes
Fastidious microbes
Flagella
Hyphae
Mesophiles
Microaerophilic
Mycelium
Obligate aerobes
Obligate anaerobes
Prokaryotic
Psychrophiles
Spirochetes
Sporangiospores
Thermophiles
Yeast
Zygospores

erstanding haracteristics cteria, iruses ill eterinary echnician ollec tion, reparation, valuation or ysis. identification cterial gens rimary pose microbiology examinations. Fungal organisms are encoun tered with relative frequency veterinary practice. iral pathogens are umerous, ut echniques or valuation or viral gens enerally erformed ecialty eference laboratories overnment-sponsored esting cations.

Bacteria e **prokaryotic** ells ange om to he cteria equently udied laboratory range from to width from to ngth. ellular rganelles xcept ell membranes, ribosomes. Some contain capsules **flagella** evelop **endospores** acteria ve ecific requirements for temperature, oxygen tension, nutrition.

These equirements onsidered hen ollecting preparing obiology ddition, entification of some bacteria be aided by characteristics. The majority of clinically significant bacterial species require ange f acteria equire xygen urvive are referred to **obligate aerobes** Bacteria are killed presence f xygen hose ith rowth hat nhibited he presence of oxygen are **obligate anaerobes** **Facultative anaerobes** are rganisms urvive nce xygen, ut their growth limited. **Microaerophilic** bacteria prefer reduced oxygen ension, **capnophilic** cteria equire vels carbon xide.

Nutritional equirements ary cteria, ure media types are chosen on of requirements. Some bacteria ve rict equirements eferred **fastidi ous microbes**

Temperature requirements vary among different bacteria. However, nearly all bacteria are pathogenic to grow best to are referred to **mesophiles** Bacteria with lower higher temperature requirements are referred to **psychrophiles** **thermophiles** espectively.

Methods f entification ected oward haracteriz cteria ariety iteria. iteria include size, shape, arrangement, chemical reactivity. These characteristics ften or erentiation ecific bacterial gens.

Bacteria y rganized ollowing ee roups according o

1. Coccus **cocci** herical ells, uch *Staphylococcus aureus,* hich ive ent itis
2. Bacillus **bacilli** ed ods linders, uch *Bacillus anthracis,* hich ive ent eings
3. Spiral **(spirochetes)** ually ccur ly ubdivided into loose spirals, such *Borrelia anserina,* which avian borreliosis; ight uch *Leptospira pomona,* hich red water comma-shaped spirals, such *Campylobacter fetus,* hich ortion
4. Coccobacillus coccobacilli): Some small rod-shaped bac teria, uch rains *Escherichia coli,* y olar pearance cocci
5. Pleomorphic: ange om occi ods

Bacterial cell shapes.

Bacteria e ariety rangements. ome row le ells rs emain ched er viding form hains lusters. any xhibit erns rangement, such following, are important for their identification Fig.

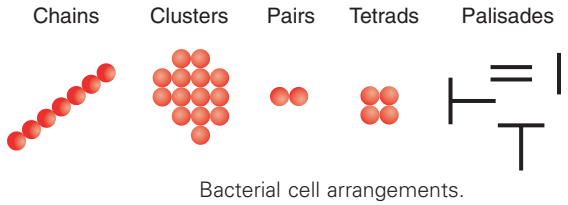
1. Single: Some bacteria occur singly, such spirilla (sing., rillum) cilli cillus).
2. Pairs: ome cteria ccur uch *Streptococcus pneumoniae* coccus).
3. Clusters unches: ome acteria ccur lusters, unches, or groups. For example, *Staphylococcus aureus* forms grapelike clusters.
4. Chains: ome rganisms row rt uch *Streptococcus* ecies.
5. Palisades: ome rganisms ranged de "Chinese tter" ern, uch *Corynebacterium* ecies.

With pleomorphic organism (e.g., member of *Coryne bacterium* species), judging hether the rganism coccus or bacillus may be If Gram-stained made from e ure ells resent efinitely rod-shaped, rganism egarded cillus for poses entification.

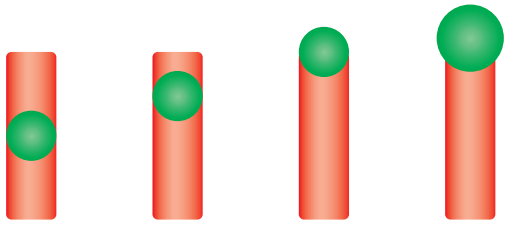
When cultured, few genera of bacteria form intracellular refrac tile bodies called endospores or, more commonly, spores. Organ f enera *Bacillus* *Clostridium* e ore ormers. Bacterial spores are resistant to desiccation, chemicals, radiation.

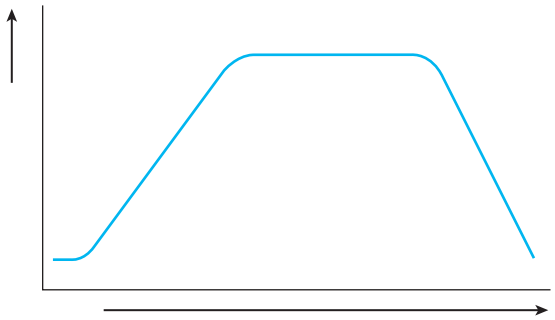
Spores ary cation ell. They y e lassified ollows

- Central: resent enter ell, uch *Bacillus anthracis*



Bacterial cell arrangements.





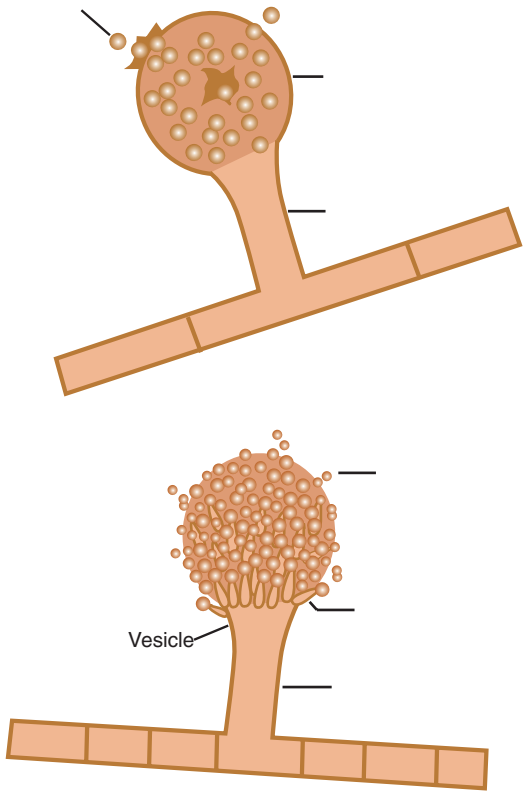
Generalized bacterial growth curve.

- Subterminal: resent ell, uch *tridium chauvoei*
- Terminal: resent ell, uch *tridium tetani*

Performing special spore may be necessary, because ndospores ually isualized odies ram-stained

Bacterial ells ontain rand eproduce ri marily y inary hen cteria olonize such ving issue ure oratory, cteria growth roceeds ough inct initial phase, which referred to phase, represents time during which bacteria are adapting their metabolism to esources ssuming media ontains propriate rowth ctors onditions for ticular cterial ecies, ollowed by xponential rowth ate rowth uring hase ften eferred oubling enera tion ime. he eneration ariable erent ecies under different environmental conditions. The exponential growth hase ontinues ntial utrients epleted, toxic e roducts ccumulate, ce ecomes ing. The olony nters ionary hich during hich umbers ells or ecrease. ariable or er ent cterial ecies. garithmic ecline phase, r ate cessarily ate rowth. ore ormation ually occurs uring

Fungi are heterotrophs, they may be parasitic or saprophytic. Most e ulticellular, xcept **yeasts** hey ontain ukaryotic cells ith ell omposed hitin. ungal rganisms consist ely ebs nder ubes led **hyphae** hich



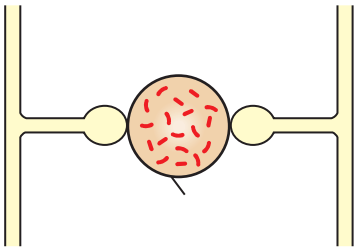
Sporangiospores and conidia, which are the two main types of asexual spores. (Courtesy Ashley E. Harmon. From Songer JG, Post KW:

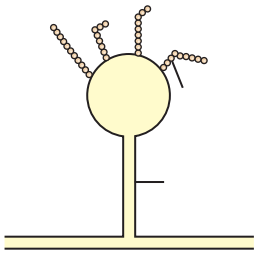
grow oward ood ces. ungi ood xternally, through elease estive nzymes, ring resulting yphae. yphae branching eb led **mycelium** yphae escribed septate r n-septate. eptate yphae ve oss-walls hyphae. The presence or absence of cross-walls identification of organism. Fungal organisms may have eproductive tructure alled ruiting ody hat roduces eleases eproductive ells led ores. ifferent roups of i roduce erent ypes ores. eproduce budding ather ore ormation.

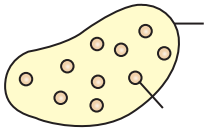
Most i ely eproductive systems. sexual ores roduced ither **sporangiospores** **conidia** . exual ores lude **ascospores** **basidiospores** **zygospores** . ungi be differentiated on of structure of hyphae resence ores.

Pathogenic rganisms egorized ollowing roups ype eproduc tive ructures resent:

1. Basidiomycetes: ushrooms lub
2. Ascomycetes:
3. Zygomycetes:
4. Deuteromycetes: wn *Fungi imperfecti,* ecause known xual ccurs







Most virologic techniques are performed specialized labora tories. They include histopathologic serologic examination, electron microscopy, attempted isolation identifi cation of virus. The veterinary technician contact diagnostic laboratory to check which facilities are available, which are preferred, whether transport medium necessary. If exotic reportable suspected, proper authorities be notified, clinical material be removed from facility.

Many of viral encountered may be diagnosed on clinical pathologic grounds. Serologic tests are available for viral Some may require paired serum are collected to weeks apart, starting during early stages of rising antibody titer indicates recent infection by virus.

Virus isolation expensive time-consuming, may provide only diagnosis after have recovered or died. However, some instances, isolation identification of virus be attempted, such to establish identity of viral previously seen practice, to discover exact agent when serologic other tests have given equivocal results, to immunologic type of virus epizootic, to verify etiologic agent public problem involved.

The isolation of virus from diseased does necessarily virus caused because many viruses persist without clinical signs of illness. Some other pathogen or condition could have been responsible for Virus isolation successful when speci mens are collected early during active infectious phase of

Viruses vary greatly with regard to their ability to remain viable tissues exudates. Contamination with bacteria greatly decreases success of attempted virus isolation. Specimens are selected on of their likelihood to contain large numbers of virus particles. Samples for virology testing must be collected aseptically, kept taken to laboratory shortest time possible.

To demonstrate presence of virus specimen, virus grown (isolated) laboratory, or virus antigens or antibodies are assayed. Unlike bacteria, which be grown on nutrient agar, viruses need living cells which to grow replicate. The tissue cells are placed into suitable glass bottle or chamber containing medium rich nutrients. The cells settle begin to grow confluent monolayer across the surface of the container. Various types of cells have been used for tissue culture of viruses. Most cells be grown vitro for some generations, but some cells divide indefinitely are used for virus isolation. These cells are called continu cell they are of single type of cell. Continuous cell such from fetal kidney, embryonic trachea, other cells, are derived from monkeys, dogs, pigs, mice, hamsters, rabbits, other The virus specimen commonly inoculated into primary culture of cells derived from species of from which specimen taken.

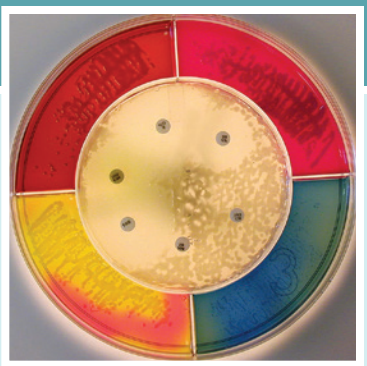
After cell culture been inoculated with virus speci men incubated, examined. If virus present, cell damage may be visible virus particles invade tissue cells. This damage referred to cytopathic effect. Different types of cytopathic effects are used to identify viruses. Some viruses cell lysis, others cells to form syncytiae (sheets) giant cells. An inclusion body another type of cytopathic effect may be seen.

Clinical signs cell culture examination may identify virus to family level perhaps to genus species level, but definitive identification requires serologic procedures are based on immunologic principles. Sometimes serologic procedures may be used on specimens directly, which saves time expense of cell culture. In-house diagnostic tests are available to detect presence of some common viral pathogens. Unit contains more details about immunologic tests used for detection of viral antigens antibodies.

Molecular diagnostic tests (e.g., polymerase chain reactions) are routinely used for identification of pathogens. These are discussed Unit

Chapter eview uestions ppendix

- Bacterial rphologic haracteristics escribed f rangement ells.
- Bacteria ary egard equirements or xygen, temperature, utrients.
- Some cteria ontain ecialized ructures spores) entification.
- Different roups roduce erent ypes ores.
- Yeasts eproduce ather ore ormation.
- Fungi e lassified erent roups type f eproductive ructures resent.
- Viral culture performed specialized reference laboratories.



After studying this chapter, you will be able to:

- List upplies eded or ollecting valuating bacterial
- Discuss ety oncerns elated obiology laboratory.
- Describe ypes vailable or uring bacteria.
- List ommonly ure characteristics
- Describe ommonly vailable dular
- Describe ure or valuation fungal

Agar
Alpha-hemolysis
Beta-hemolysis
Blood agar
Culture medium
Culturette
Differential media
Enriched media
Enterotubes
Fastidious
Gamma-hemolysis
Inoculating loops
MacConkey agar
Mueller-Hinton agar
Sabouraud dextrose agar
Selective media
Thioglycollate

Ideally practice facility have separate room away from traffic areas of clinic for microbiologic procedures. The room must have adequate lighting ventilation; able or ed raffic; ork or processing or ure ork) surfaces are easily disinfected; electrical outlets; storage space; ccess or efrigerator.

Most f oorganisms ncountered obiology laboratory re otentially athogenic, any re oonotic. ll specimens reated otentially oonotic. ety of every person working laboratory depends on strict observance septic echnique ways bserved hen transferring orking ectious ents ecimens.

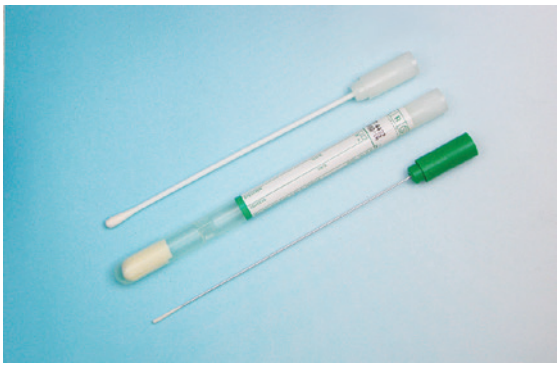
Veterinary echnicians ersonal rotective quip ment when handling patient specimens, including clean, long-sleeved, knee-length, white laboratory coat or clean, long-sleeved surgical scrubs to prevent contamination of street clothes dissemination of pathogens to general public. Disposable gloves are always worn microbiology laboratory, face y eded roduction erosol ticles likely. oratory oats eekly water strong bleach. If coat becomes soiled during daily diagnostic rocedures, emoved diately ced

eceptacle esignated or ty ll oratory oats e ogether. oratory oats be ed ith om eterinary laundry om tside oratory.

All personal protective equipment be removed before leaving oratory. eterinary echnician or r roughly efore ving oratory.

Materials have been contaminated with potentially infectious ents econtaminated efore rs, forceps, scalpel blade holders be sterilized autoclave. Potentially dous erials ubes, pipettes, roken ced propriate ontainers or osal. erials ded trash eceptacles, toclaved liminate infectious agents. Bench tops are cleaned with disinfectant ethanol r ute leach ution) eginning ork eriod. led ures reated ectant lowed ontact or utes efore leaned The urfaces quipment, uch ors refrigerators, be wiped down with disinfectant on daily ondisposable ire ops ve een ontaminated with obes diately er

Eating, drinking, smoking, handling contact lenses, apply osmetics ermitted oratory. ppropriate signage state rule. Personnel who wear contact lenses laboratory wear goggles or face shield. Long ust e ied ck ucked oratory oat. Labels istened er ather technician's tongue. No food stored laboratory; instead, ored tside oratory esignated inets refrigerators. ll ccidents eported romptly laboratory upervisor eterinarian.



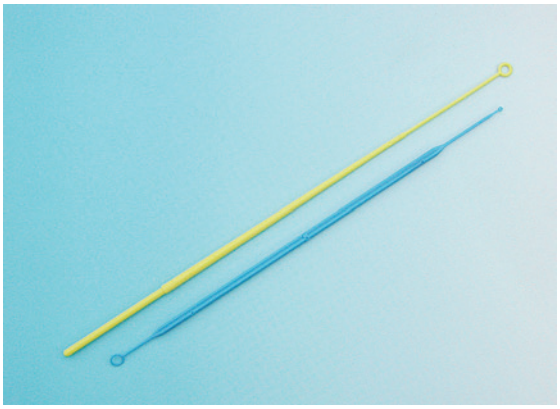


Fig. 38.3 Propane burner for sterilizing metal inoculating loops. (Courtesy

good-quality incubator capable of constant temperature umidity rimary ce quipment needed microbiology laboratory. More information about incubators vailable upplies eded for collecting preparing bacterial fungal include ollowing:

- Sterile otton-tipped
- Dull el lades
- 3- o ringes auge edles
- Sterile ndotracheal ube inary ter
- Collection ubes reservatives
- Rayon ransport uch **Culturette** , Franklin
- High-quality overslips **Inoculating loops** or wires; reusable metal or single-use posable ops rated ops
- Bunsen urner ural ropane Fig.
- Candle ar erobe
- A ariety ure luding roth
- Antibiotic ensers

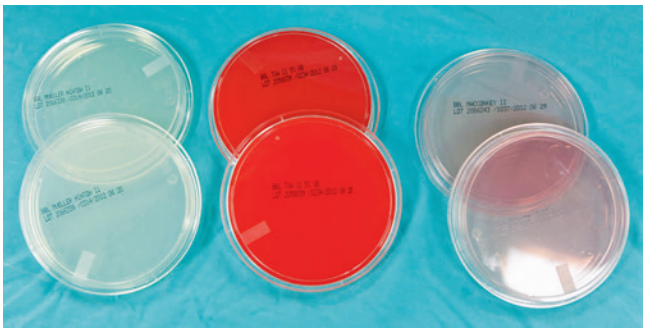
Antibiotic disks with dispenser.

- Gram eded
- Scissors, orceps, nd calpel ith lades stored 0% lcohol d erilize)
- “Discard ontaining ectant or ontaminated instruments
- Wooden ongue epressors or ecal ecimens
- Racks o ubes
- Refrigerator cold acks” nd olystyrene hipping ontainers for eference oratories

culture medium dia) erial—solid liquid—that upport rowth oorganisms. or bacteriology, ure chased ehydrated powder r repared eady-to-use for iochemical ll ommonly obtained eady repared om upply eference esearch oratories repare erilize wn media om ehydrated owder. olidifying ents preparation lude **agar** elatin. gar dried xtract ae wn arophytes. latin protein btained om issues.

For um ept efrigerated o ept way om ernal of efrigerator, ecause ontact acket eeze uin

Six general types of culture media are available: transport media, general pose **enriched media** **selective media** **ferential media** nrichment ome ure contain characteristics of more one type. There are hundreds of erent ypes ure vailable, ut verage

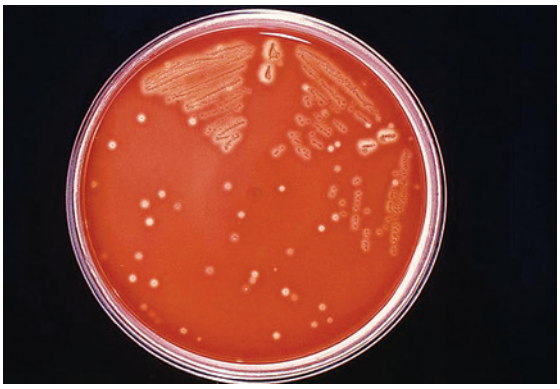


Commonly used culture media. From left to right, Mueller-Hinton agar, blood agar, and MacConkey agar.

veterinary ractice eds ew eteri nary ractices, dular ontain ultiple ypes media ure ommon. neral pose media, hich metimes eferred utrient ommonly eterinary ractice. iched formulated et equirements **fastidious** pathogens. hey utrient xtra utrients added, uch lood, rum, gg. lude lood agar hocolate ar. elective ontain ibacterial substances, uch imicrobials, kill l ut ew ypes cteria. hey cilitate ion of articular enus rom ixed noculum. **MacConkey agar** ype f lective ifferential low cteria be erentiated roups iochemical eac tions on medium. Simmons citrate differential medium. Enrichment vor rowth ticular roup rganisms. hey ontain utrients encourage rowth esired rganisms ontain inhibitory substances suppress competitors. Examples include tetrathionate roth lenite roth. ransport designed o eep obes ve ncouraging rowth eproduction. ulturette or ecimen ollection contains repared ransport ore ecific etails out some f ommonly ure resented next ctions hapter. resented be l-inclusive ozens dditional ypes available. However, many of listed here are found only e eference esearch oratories.

This nriched dium upports rowth cterial pathogens. lthough veral ypes **blood agar** e vailable, trypticase ep lood ommonly used ype. lood cts nrichment dium differential dium, ecause inct ypes molysis e etected lood ar:

1. **Alpha-hemolysis** partial hemolysis creates narrow band of greenish or slimy discoloration around bacterial colony Fig.



1977, Centers for Disease Control and Prevention.)

2. **Beta-hemolysis** complete hemolysis creates clear zone around cterial olony
3. **Gamma-hemolysis** molysis roduces hange pearance dium molysis colonies
4. Delta-hemolysis: zone of hemolysis surrounded by narrow zone of hemolysis around bacterial colony; called double-zone molysis

MacConkey eosin-methylene blue agars are selective erential acConkey ontains ystal iolet (which uppresses rowth ram-positive cteria), salts that are selective or ctose-fermenting *Enterobacteriaceae*, few other bile-salt–tolerant gram-negative bacteria. Growth or rowth acConkey or primary entification ram-negative enera. performs tion entify lactose-fermenting rganisms.

The ors acConkey ctose utral red. ctose-fermenting rganisms uch *Escherichia coli* *Enterobacter* *Klebsiella* ecies roduce om ctose grow pinkish-red colonies on medium. Bacteria erment ctose ck eptone acConkey ar, producing eaction olorless olonies. specimens or outine ion ually ured parately on both blood MacConkey agars. The examination of both blood acConkey ures ve een culated with clinical specimen yield considerable informa tion. or xample, rowth acConkey ut good rowth lood uggests ed pathogen robably ram-positive rganism.

ide ariety hromogenic ars vailable selective erential esigned rovide sufficient ormation efinitively entify cterial species. n hromogenic lows or more apid entification cteria ves costs ecause dditional iochemical eded. Some chromogenic agars are divided into sections, whereas others are le hromogenic lude veral types designed to identify certain resistant bacteria based on their color olony pearance ecific hromogenic Fig. nother ype entify erentiate *Escherichia coli* om r nteric cteria. or *E. coli* hromogenic ar, olonies *E. coli* e resent, will pear lue-green hereas nteric cteria ill be enta. on-enteric cteria ited but y row, olonies ill olorless.

Thioglycollate liquid medium used to culture anaero bic bacteria to determine oxygen tolerance of microbes. The medium ontains xygen radient, oncen trations f xygen urface erobic conditions ottom.

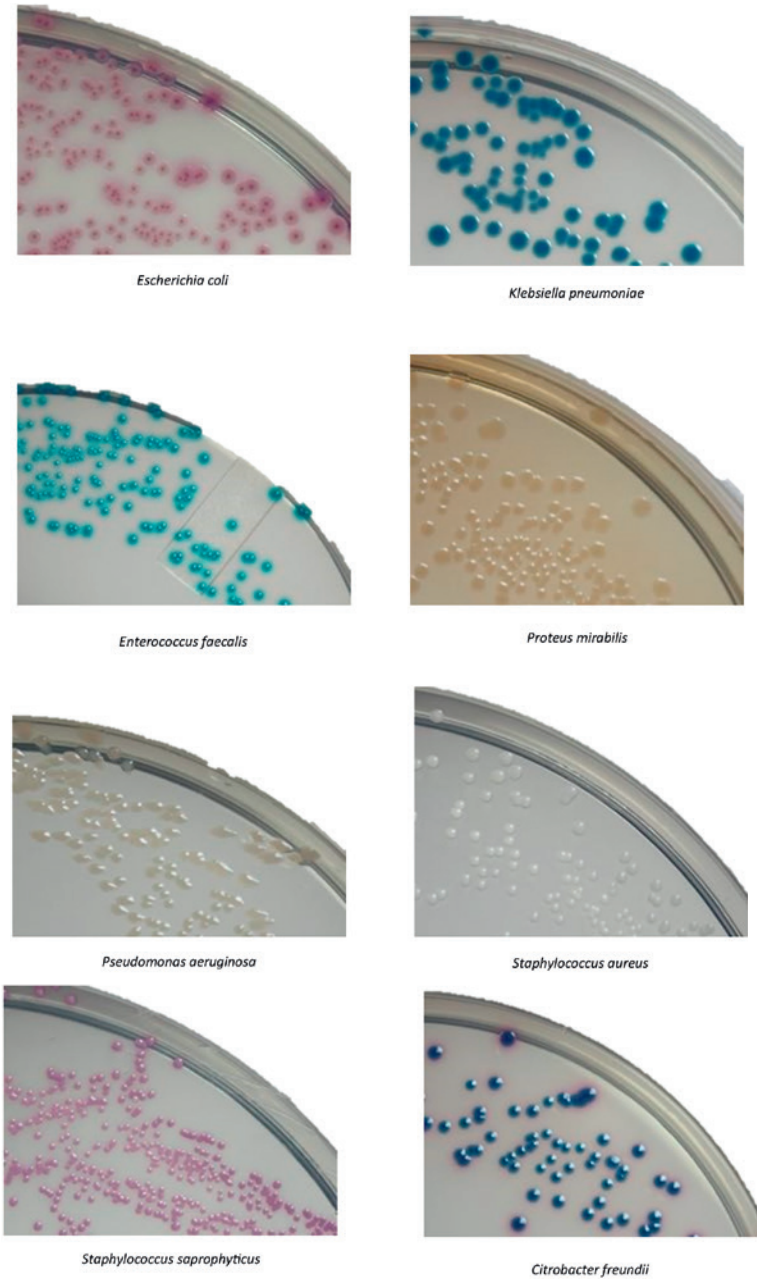
Obligate erobes ill row nly xygen-rich op yer, whereas obligate anaerobes will grow only lower part of tube. acultative erobes row oughout dium, but y ill rimarily row ube, etween oxygen-rich oxygen-free zones. The primary of glycollate broth veterinary practice enrichment media or lood ures.

Urea are streaked with inoculum incubated overnight rea dium each olor. cteria ydro lyze ea dium, roduction urns medium o olor. gative esult roduces olor change .

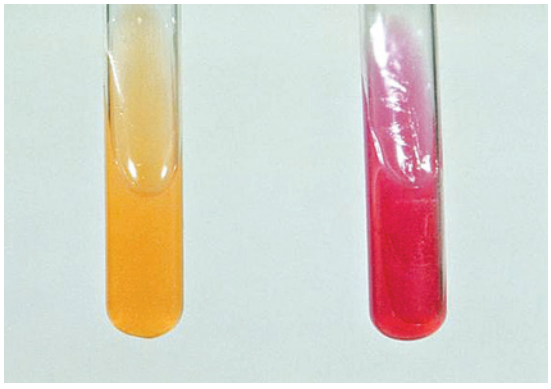
The tube of sulfide-indole motility medium inoculated with raight epth proximately are en o ithdraw ire used or ntry. ydrogen ulfide roduction ed blackening of medium. Indole production requires addi tion f ops ovac's eagent op dium. tryptophan een roken own cteria ube, ed diately orms op dium.

Simmons citrate medium differentiates bacteria according to their f rate. urface culated. cteria rate dium, eep lue olor evelops. unchanged dium reen.

Triple ugar on dium or resumptive identification nellae erentiation enteric cteria. dium ontains or stem or hydrogen ulfide roduction ell or, henol red, olors culated dium ed. ll *Enterobacteriaceae* erment lucose, resent

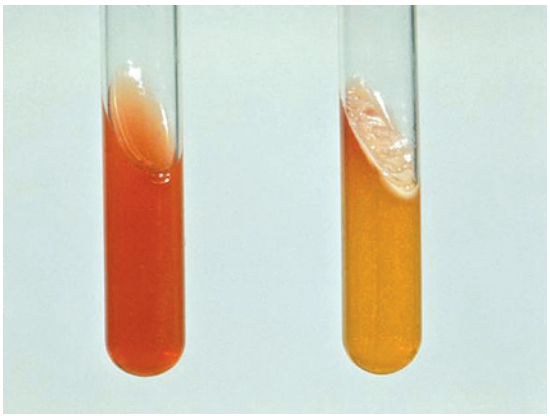


Chromogenic agar. (Courtesy of Microvet Diagnostics.)



cked referentially apidly. ly of incubation, both butt turn yellow result of cid roduction. owever, er lucose tabolized under erobic onditions rganism erment lactose r ucrose, pe everts ed on dition. he utt, er erobic onditions, emains ellow (acidic) . low eaction, riple ugar on agar ways ubes ugged with erile otton.

If the organism can ferment lactose, sucrose, or both in addi tion to glucose, lactose sucrose are then attacked with resulting acid production, medium turns yellow (acidic) throughout. Lactose sucrose are present quantities to cidic onditions hich emains ellow.



their ability to ferment glucose, lactose, or sucrose as well as to produce

result indicates no fermentation. (Courtesy Public Health Image Library,

With organisms produce hydrogen sulfide, blackening of medium partly superimposed on other reactions. The triple sugar iron be read after about hours of incubation After longer incubation, blackening tends to reach bottom of tube obscures yellow butt.

The following summarizes the reactions of *Salmonella* species triple sugar iron agar:

- Alkaline (red) (red) butt: none of sugars attacked
- Alkaline (red) acidic (yellow) butt: glucose fermentation only
- Acidic (yellow) acidic (yellow) butt: glucose attacked addition to lactose, sucrose, or both
- Blackening along through medium: hydrogen sulfide production

The triple sugar iron inoculated with single colony from selective medium with straight inoculating wire. The wire pushed down to bottom of agar, when withdrawing wire, agar streaked. The inoculating wire still contains enough bacteria to inoculate tube of lysine decarboxylase broth. During search for salmonellae, two suspicious colonies be individually tested triple sugar iron agar per brilliant green plate. The triple sugar iron tubes be incubated, with loose for to hours.

Brain–heart infusion broth useful general-purpose broth used to increase number of organisms (preenrichment) before they are plated on medium. The broth inoculated with patient subcultures are taken needed for additional testing.

For culture of blood approximately of patient's blood added to nutrient broth or to special blood culture medium be obtained commercially. Because patient's blood contains many substances are inhibitory to bacteria, adding blood directly to broth dilutes effect of natural inhibitors.

Mannitol agar routinely used, but highly selective medium for staphylococci, could be used to isolate *Staphylococcus aureus* from contaminated specimens. The medium high content contains mannitol indicator phenol red. Staphylococci are tolerant. *S. aureus* (but usually *Staphylococcus epidermidis* ferments mannitol. The resulting acid turns *S. aureus* colonies surrounding medium yellow.

In selective medium, freshly precipitated bismuth sulfite acts with brilliant green to suppress growth of coliforms while permitting the growth of salmonellae. Sulfur compounds provide substrate for hydrogen sulfide production. The metallic medium colony surrounding medium black or brown presence of hydrogen sulfide.

Atypical colonies may appear medium heavily inoculated with organic matter. This situation may be prevented by suspension of sterile of supernatant for inoculation.

The freshly prepared medium strong inhibitory action, suitable for heavily contaminated Storing poured plates for days medium to change color to green, thereby selective, with small numbers of salmonellae being recovered.

The following summarizes typical appearance of more important bacterial organisms on bismuth sulfite agar appearance of their colonies:

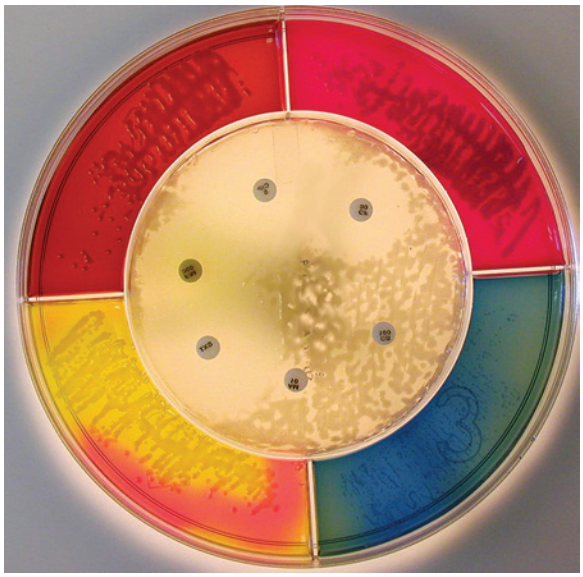
Salmonella typhi: black "rabbit eye" colonies, with surround black zone metallic sheen after hours; uniformly black after hours of incubation

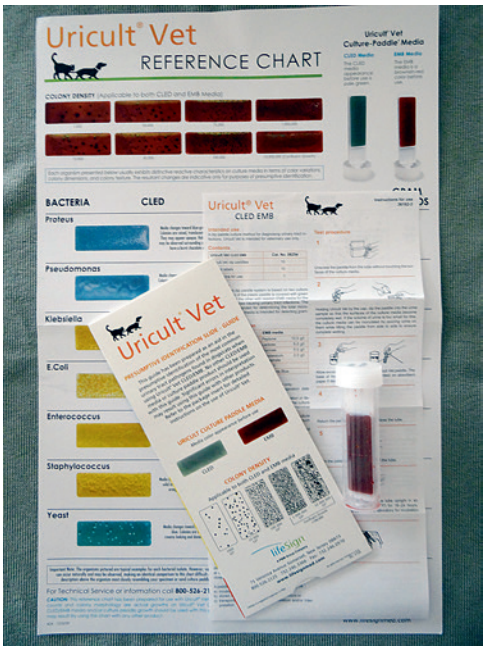
- Other *Salmonella* species: variable colony appearance after hours (black, green, or clear mucoid); uniformly black colonies seen after hours, often with widespread of medium pronounced metallic sheen
- Other organisms (coliforms, *Serratia* *Proteus* species): usually inhibited but occasionally dull green or brown colo with metallic sheen or of surrounding medium

Mueller-Hinton agar general-purpose medium primarily used for performance of agar diffusion antimicrobial sensitivity test. The chemical composition of the media does not interfere with diffusion of antimicrobials through agar.

Both of media are used specifically for culture of fungi yeasts. Bismuth–glucose–glycine–yeast agar commonly referred to "biggy." Dermatophyte test media found veterinary clinics composed of **Sabouraud dextrose agar**

Several modular culture systems are available for veterinary practice laboratory. The Bullseye (HealthLink, Jacksonville,





HealthLink, Jacksonville, FL.)

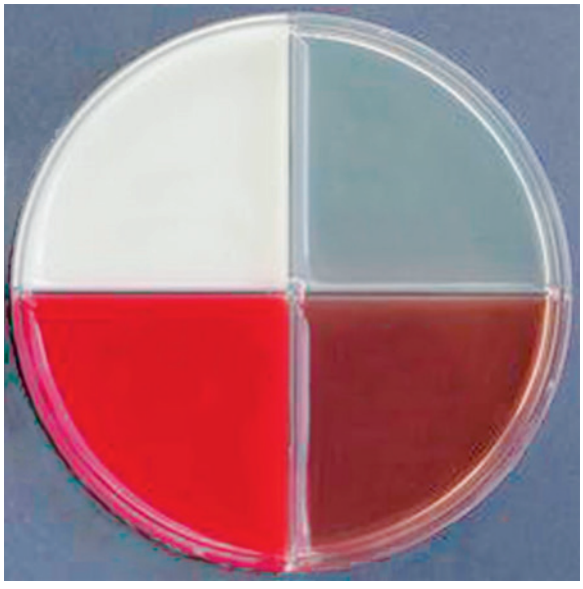
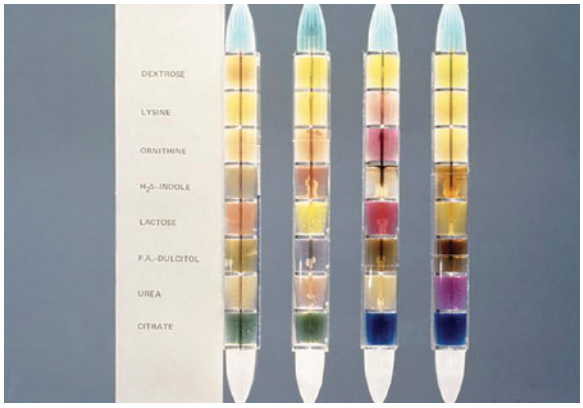


Fig. 38.11 Spectrum CS plate showing gram-positive, gram-negative,



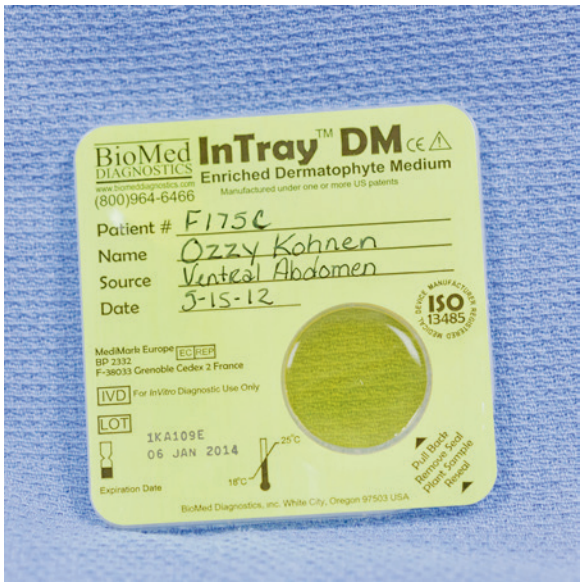
different agar preparations. (Courtesy Public Health Image Library,

Fig. Spectrum (Vetlab Supply, Palmetto Bay, . stems ulti-chambered contain oth lective n-selective ullseye plate ontains entral ueller-Hinton or sensitivity testing. ipslides" or paddle" media such Uri-Cult (Orion iagnostics) ools or inary tract infection screening. They consist of two-sided agar paddle ched ew-top ube. They e de ariety ombinations, ommon ve ither acConkey cystine lactose electrolyte–deficient agar. fter incubation, olony ount erformed, olor compared ith hart or resumptive entification. ositive cultures meet quantitation criteria for be sent to outside laboratory for confirmation susceptibility testing.

Commonly used modular media include the Bullseye

Enterotubes BD, ranklin akes, Fig. 8.13 re ype of commercially available microbiology test incorporates multiple ypes esigned rovide or differentiation of enteric bacteria on of their biochemi eactions ends elatively xpensive, y ly ustified umbers microbiology erformed ariety ecies.

Several products are available for culturing of dermatophytes. The ost ommon tandard ermatophyte est edium DTM),



monly used dermatophyte test medium.

which ontains or urns ed resence ermatophytes ell imicrobial ents bacterial growth Fig. Rapid sporulation medium or enhanced sporulation media with color indicators be d onjunction ccelerate formation f acroconidia sed or dentification onfirmation. formulations are available plate tube formats. Standard abouraud extrose ill romote lier formation f croconidia, ut ontains olor or.

Dermatophyte test media contains Sabouraud dextrose

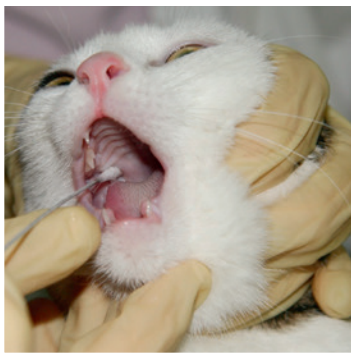
Some ures equired oratory or uality ontrol purposes. arious rocedures upplies nitored for uality ccuracy, luding ibacterial usceptibility tests, dia, iochemical ertain or entification, uch one eta-hemolysis *S. aureus* cyclic adenosine monophosphate test. selection of control organisms btained edia ontain fermentable ugars, uch rypticase ar, uitable for ewer rganisms, uch *S. aureus* *Enterobacteriaceae.* he cteria culated into ube dium ubcultured proximately very nths.

Streptococcus, Pasteurella, Actinobacillus species quickly on ure reptococci ept ube ooked roth ubcultured proximately very eeks. *Pasteurella Actinobacillus* ecies emain iable ed approximately erile lood ube ored eep eeze r wer. therwise, two genera be subcultured on blood agar approximately every days. Control cultures may be kept room temperature ew-capped ubes ut referably efrigerator which educes tabolic ate rganisms.

Chapter eview uestions ppendix

- Equipment upplies eded erform biology esting ractice oratory lude tor, ollection erials, ure supplies.
- Culture edia an btained ubes lates. ube edia e
- A ariety ure vailable, ut eterinary practice oratories equire nly ew ypes.
- Commonly ure ludes lood ar, ac Conkey ar, ueller-Hinton ar, ermatophyte media.
- A variety of modular systems are available contain more one type of culture media to help with identification of rganisms.

Sample Collection and Handling



After studying this chapter, you will be able to:

- Discuss eneral elines or ollection bacterial
- List thods ollect or microbiology esting.
- Describe thods ollecting or evaluations.
- List escribe ects ollection or ecific ariety

General Guidelines,
Collection of Viral Specimens,
Submission
Key Points,

Aspiration
Culturette
Imprint
Swabbing
Transport media

Samples or obiologic valuation ollected uickly, do require specialized materials or equipment for proper evaluation. Specimens are commonly collected by various methods, including **aspiration** **swabbing** **Imprints** of tissues or external lesions provide suitable spiration enerally ollect om low rgans ladder) xternal ecific techniques epend ype cation on ody. areful ention ptic echnique critical o chievement nostic-quality esults. ore information about aspiration, swabbing, imprints located nit

The specific choice of collection method depends on loca tion of lesion on animal's body well specific type of esting esired. amples hat re mmediately rocessed usually be collected sterile cotton However, suitable method of collection, because contamination risk h, otton obial rowth. xygen be trapped fibers, recovery of anaero bic cteria ely. ayon cron referred. delays rocessing xpected, ayon **transport media** (e.g., **Culturette** must be used to preserve quality f

The ecimen lected ontain rganism causing he roblem. ormal ora and ontaminants ay om plicate ollection ubsequent erpretation results. Better results will be obtained specimens are collected from sites would normally be sterile, because infections are likely o e redominant rganism. od examples e ollected stocentesis) ct pustules. ecal mselves ell obiology esting ecause umber om mensal condary rganisms ypically opulate uch exposed eas.

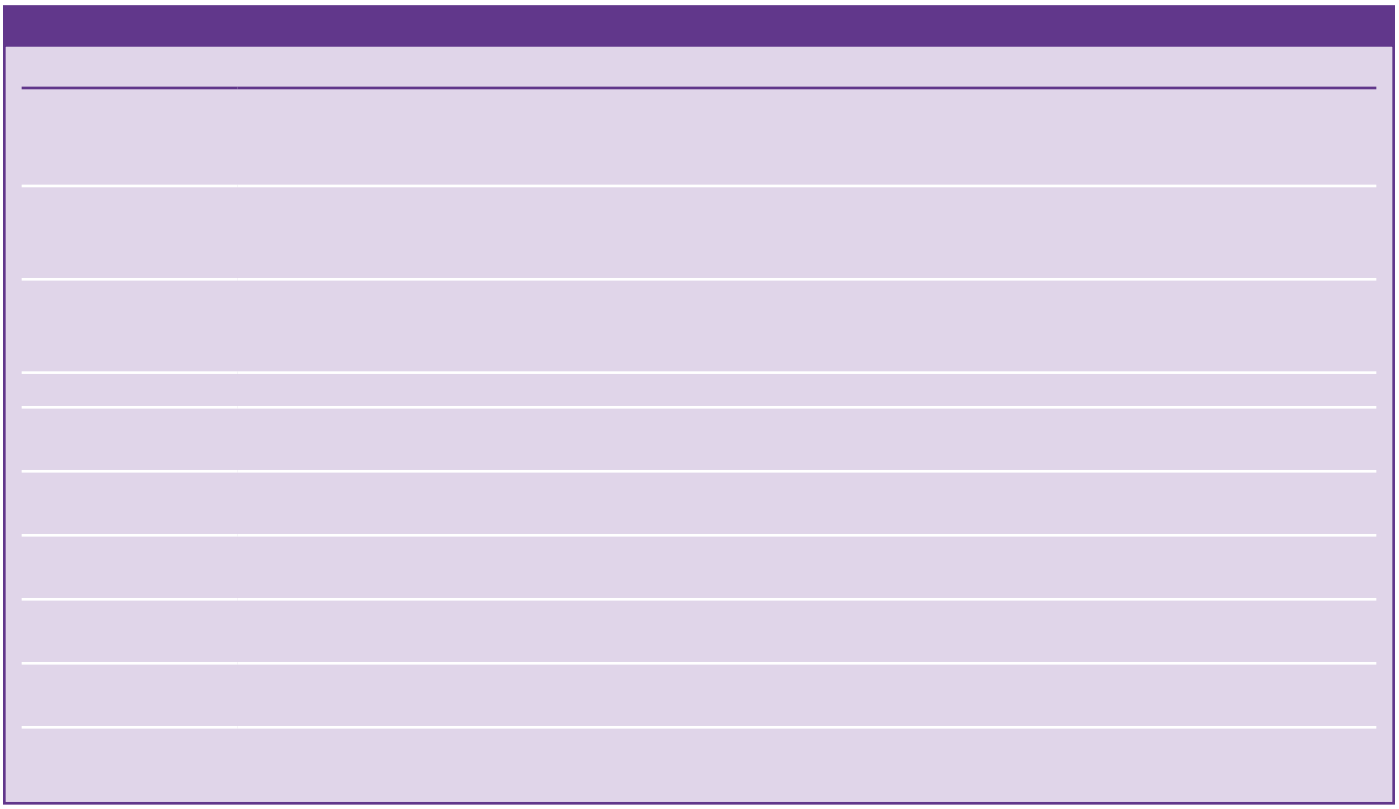
Photo om *Consultations in feline internal medicine,* ol ouis, aunders.

The ollowing eneral elines ply roper ecimen collection:

1. A omplete ory ufficient btained to lp lect rocedures propriate isolate y rganisms resent. equired include owner's clinic's address, phone number. Species, age, sex, number of affected or dead, duration of problem, major signs bserved luded. entative rganism uspected, reatment iven, type of laboratory investigation required be included ecord.
2. The specimen must be collected aseptically. Specimen contamination ommon nostic ure. importance of aseptic collection of microbiologic specimens e veremphasized. ollected soon ossible er nset
3. Multiple ecimens ept parate void contamination. ractice ntial or estinal eci mens ecause ra rmally resent re.
4. The ecimen ontainer eled, ecially oonotic condition uspected, uch abies, ptospirosis, brucellosis, quine ncephalitis. issues om uspected zoonoses be submitted sealed, leak-proof, unbreakable ontainer.
5. Adequate en. btaining esults uickly xpense ccuracy ounterproductive.

Table summarizes collection guidelines for microbiology ecimens.

When ollecting ecimens or ermatophyte ure, lesion to remove some of surface contamination collect ecimens om eriphery. roken



From Songer JG, Post KW: *Veterinary microbiology: bacterial and fungal agents of animal disease,*

y ely ontain iable rganisms. airs ucked om oothbrush e d btain ollect with oothbrush, btain oothbrush rush uspected or utes. airs isible ristles er rushing.

Table 9.2 ummarizes ample ollection uidelines or ungal testing.

Samples for dermatophyte testing are usually obtained

Viruses e ften resent haryngeal cretions early the acute stage of respiratory diseases. Mucosal apings rather of secretions be taken. Sterile wooden tongue epressors or ucosal apings. ttempted isolation from blood may be considered for generalized catarrhal which tend to have viremic stage. Poxviruses are ften emonstrated lectron oscopy om early vesicular lesions sometimes from early lesions.

Specimens be selected for indirect studies, such serologic, hematologic, histologic, bacteriologic examinations. Viral are often complicated by pathogenic bacteria act condary vaders, hich urn iral ection into serious Specimens for histopathologic examination onsist ctions issue ced diately formalin. Sections for histologic examination must never be frozen, ecause issue tifacts to erentiate om gic rocess.

Tissue for attempted virus isolation 2-inch cubes ontain rmal issue, ossible. Mucosal apings btained ead erile screw-capped ontainers or ollection, separate ontainer or ch eterinary echnicians must strict aseptic technique label containers carefully.

Specimens be refrigerated when possible, because virus iters ecrease emperature ecimens are o e elivered irology oratory they y e ored cked oolant cks ice olystyrene-insulated ton or ment. delay ill e re eezing shipping on dry ice desirable, except for specimens of suspected parainfluenza irus, hich egrity of iruses reserved ecimens shipped tight ontainers revent ntry bon dioxide o ontainer. arbon xide om wer iruses.

Small ces issue, ecal erial, re served ials led lycol ored irus transport dium vailable ommercially iagnostics, Mississauga, Canada). Because viruses vary their longevity, eference oratory ontacted or ecommenda tions egarding propriate ransport dium procedure.

Fecal erials ften ubmitted or lectron microscopic examination. fixative (e.g., buffered neutral formalin) be added to maximum fixative-to-sample atio revent verdilution irus particles.

For ine proximately sterile container. irus transport medium be used. The specimen ust ept hilled rive oratory within ollection; rwise, ped frozen.

If lood amples ave een ollected or erologic xamination, they ollected rocessed escribed

Chapter eview uestions ppendix

- Samples for microbiologic evaluation be collected quickly, do require specialized materials or equipment.
- Careful ention ptic echnique ro duction f nostic-quality esults.
- Microbiology ollected bing, tion, rint, iopsy, ariety echniques.
- Samples or ermatophyte esting ually ollected plucking om uspected
- Normal flora contaminants may complicate col lection ubsequent erpretation esults.

Staining Specimens

After studying this chapter, you will be able to:

- List ommonly or obiology specimens.
- Describe omponents ram procedure.
- Describe rocedure or erforming ram
- Describe otassium ydroxide hen valuating bacterial
- List escribe rocedures or ecific

Gram Stain,
Procedure,
Interpretation,
Potassium Hydroxide Test,
Ziehl-Neelsen Stain,
Giemsa Stain,
Specialized Stains,
Quality Control,
Key Points,

Acid-fast stain
Capsule stain
Endospore stain
Flagella stain
Giemsa stain
Gram stain
Lactophenol cotton blue
Potassium hydroxide
Simple stain
Ziehl-Neelsen stain

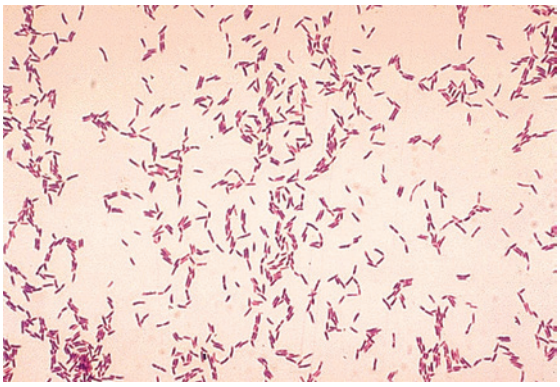
ariety f vailable or reparation cterial al ommonly Gram **Ziehl-Neelsen** cid-fast) amples en directly om atients re ften ram tained efore ample cultured. Information obtained from direct may help to etermine uitability ecimen or entification, redominant rganism ed ecimen, pro priate dium or ure, propriate ibacterials for nsitivity esting. or ram Ziehl-Neelsen (acid-fast) stains are available commercially. Com mercially repared utions equire ering precipitate forms. **Simple stains** such crystal violet or methy lene blue, are typically used for yeasts. **Lactophenol cotton blue** d onfirm entity rganisms. any other ypes vailable or obiology, ut are erformed nly eference esearch oratories. Standard matology or valuation of cteria. ematology ill olor all cteria ormed lements pear bacteria, lead to misclassification of organisms.

Gram egorize cteria ram ositive gram negative on of cell wall structure. The **Gram stain** procedure requires primary mordant, decolorizer, ounterstain. rdant ubstance ye structure. n rimary cterial cell l. epending ufacturer, rimary usually solution of crystal violet, mordant Gram's iodine solution, ecolorizer ither cetone, ounterstain ither

TECHNICIAN NOTE

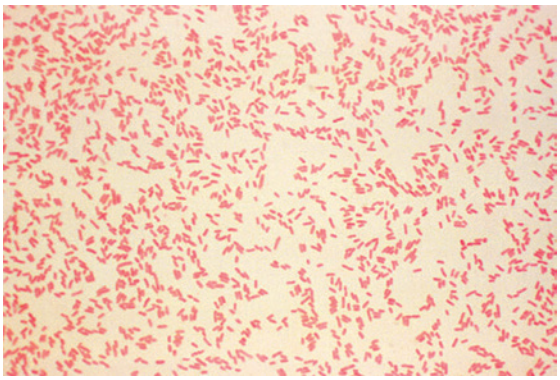
The plied eci mens y e olled htly nto ouching erile wire o ne olony ually ufficient btain enough bacteria for application to slide. The colonies

Gram staining kit. (Courtesy B. Mitzner, DVM.)



bacteria.

Atlanta, 1977, Centers for Disease Control and Prevention.)



Typical staining pattern of gram-negative
(Courtesy Public Health Image Library, PHIL#6711, Atlanta, 1980, Centers

be oung ure), ecause er olonies ield roper esults, cteria ften ecome excessively ecolorized.

Bacterial om ently ed op of er r btained om inoculated roth, wo hree oopfuls re pread nto he lide. ed ectly nto uch from issue egardless ecimen transferred nto ette, ire), be en estroy rganisms.

The oplet ncircled encil o er fter rial ied ed through ee ecimen The echnician eful verheat temperature may be tested on back of slide eel ut revents from reserve ell rphology, kills cteria enders ermeable roce dure ontains ep-by-step ram rocedure. The ecolorizer ep itical ep rocess. sible to over-decolorize or under-decolorize, yielding ambiguous or rroneous esults. ote ariations different est lways onsult erials rovided ufacturer.

Heat fixing before Gram staining prevents the sample

Bacteria etain ystal iolet–iodine omplex purple e led ram-positive rganisms ystal iolet olor ed safranin r lassified ram-negative rgan . rphology cteria ortant

Determining ram eaction ortant ep entification rocess. erforming rocedure rop erly erpreting esults orrectly equire ractice. ensure roper uality, wn ontrol) ram-positive ram-negative rganisms nce week with each new batch of These control organisms may e ept rowing oratory.

Gram-positive bacteria appear purple when they are

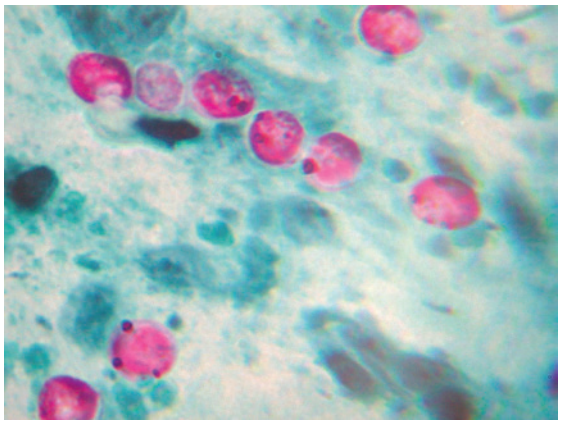
Sometimes organism may both gram positive nega tive, which called gram-variable reaction. This may occur result of excessive decolorization, overly thick smear, excessive ion, ures, oor-quality

If ram-variable eaction ccurs, uick heck reaction with **potassium hydroxide** (KOH) test. The pro cedure or ollows:

1. A loopful (or two, necessary) of KOH solution placed on e.
2. A enerous uantity urface rowth emoved om culture ransferred op
3. The ecimen irred op op; loop n ed wly ently. fter utes irring ually conds), ram-negative organisms develop mucoid appearance produce sticky strand when drop lifted with loop. If organisms are ram ositive, ure ys mogeneous oes orm rand hen ed.
4. The reaction recorded gram negative sticky strand ucoid ormed) ram ositive icky strand r ucoid ormed).

o ution or valuation fungal Several versions of procedure are performed. Some dd thyl ulfoxide eed activity of KOH, whereas others involve heating slide. Additional components may involve counterstaining with India r omanowsky

This rimarily etect rganisms *Mycobacterium Nocardia* ecies. umerous ypes e vailable, onfigured or use n he eterinary ractice aboratory. cid-fast tains ontain several solutions, including primary typically carbol hsin; cid-alcohol ecolorizer; ounterstain such thylene lue. ied ed by ecimen ough primary od ed over ooled or minutes insed er. to ecolorize or utes ed olor gone, insed ounterstain dded, n insed er ied. gents uch luded rocedure allow enetrate ells uch *Myco bacterium*. The subsequent addition of acid alcohol removes If removed, organism acid fast" appears ed, hereas n–acid-fast oorganisms lue Fig.

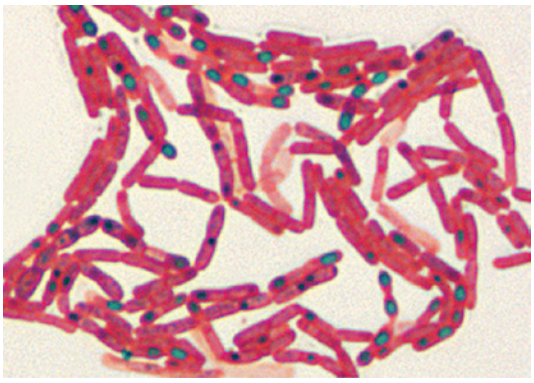


DVM, Avian and Exotic Animal Medical Center, Miami, FL.)

Giemsa stain d o etect ochetes ickettsiae well o emonstrate *Bacillus anthracis* morphology of *Dermatophilus congolensis*. The fixed absolute thanol or utes ied. dipped uted or utes. may e xtended ed esults. or *Borrelia anse rina*, ently ed overed or utes. insed, ied, xamined or plish-blue–stained cteria.

Flagella stains capsule stains endospore stains fluorescent are available, but they have limited application average eterinary ractice oratory. uorescent end to e uite xpensive, rimarily or en tification f *Legionella Pseudomonas* ella ually contain crystal violet, they are used to detect characterize bacterial ility. end mewhat xpensive or small eterinary ractice oratory. ther thods be d o est ility lude op reparation ility apsule or etection genic cteria. ll cteria ontain capsules e genic. owever, genic cteria contain apsules. apsule tains ften equire he se right-field hase ontrast oscopy.

Bacterial spores contain protein coats of keratin are resis to normal procedures. Endospore detect resence, cation, ores, erentiation cteria. ospore one er ure re ecause ore formation occurs during logarithmic decline phase. The pro cedure involves addition of malachite green to specimen on e counterstained ores pear dark lue reen, emainder cterial ell being ed ores ee from ells.



Veterinary microbiology: bacterial and fungal agents

Each ime linical pecimen tained, ample rom ontrol culture erifies uality rocedures erials. ecialized ram control es ommercially vailable.

Chapter eview uestions ppendix

- The Gram common procedure per formed obiology oratory.
- Gram requires primary mordant, decolor izer, ounterstain.
- Gram-positive organisms appear purple when viewed under oscope, ram-negative rganisms ed.
- Samples ram-variable eactions valuated OH est.
- Flagella capsule endospore fluorescent e rimarily eference oratories.
- The Ziehl-Neelsen stain is used to identify acid-fast organisms.

After studying this chapter, you will be able to:

- Describe eneral quence hen entifying bacteria.
- Describe uadrant reak thod culation.
- Describe rocedure or culation tubes.
- Differentiate etween resumptive entification definitive entification.
- Discuss ects ion ure
- List olony haracteristics valuated cterial colonies.
- Describe thods or ure erobes.

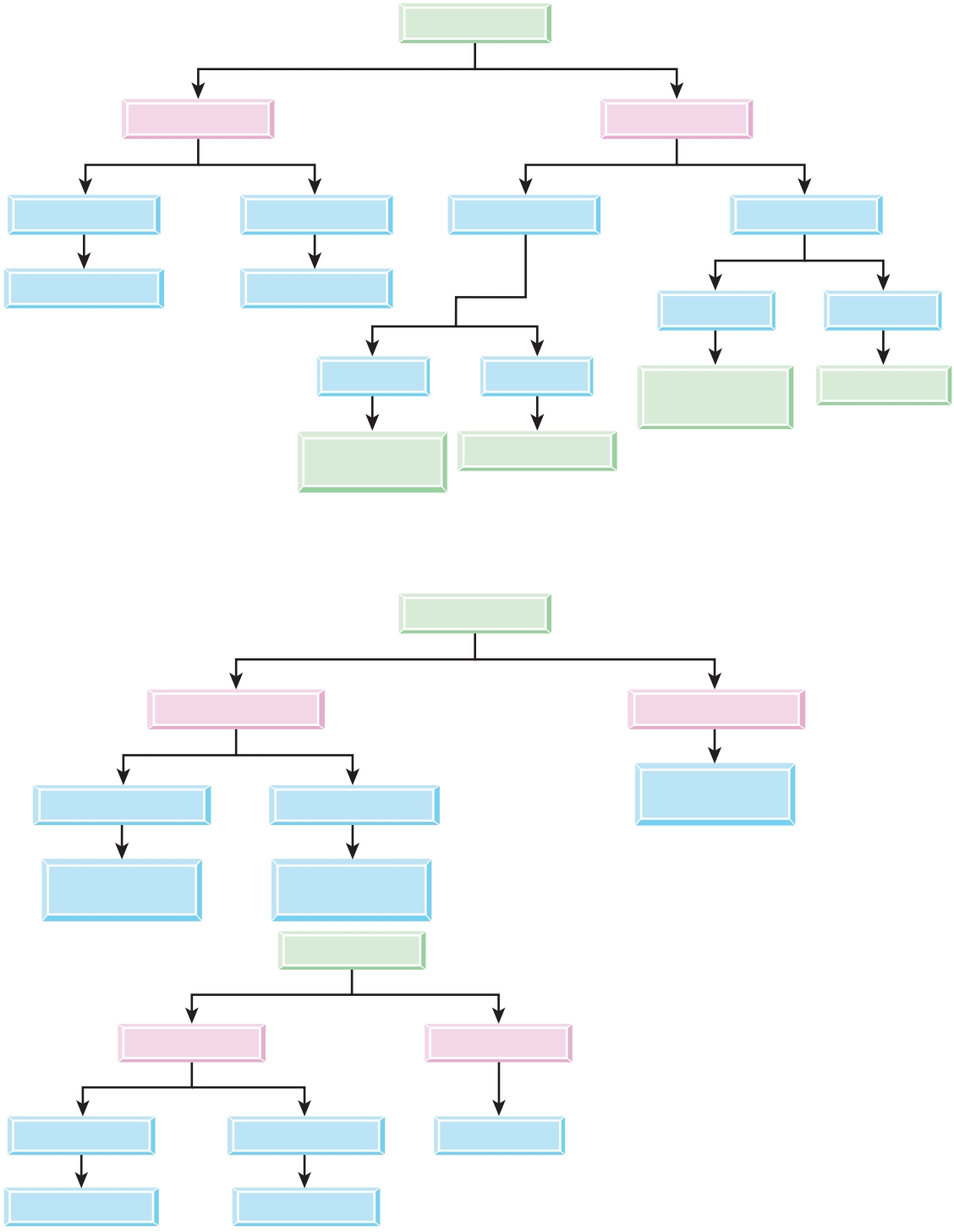
Inoculation of Culture Media,
Streaking ulture
Inoculation
Incubation of Cultures,
Colony Characteristics,
Culture of Anaerobes,
Key Points,

Candle jar
Filamentous
Incubation
Mucoid
Presumptive identification
Quadrant streak
Rhizoid
Slant tube
Undulate

systematic approach needed for identification of genic bacteria. Procedure shows typical sequences used to process microbiologic specimens. The practice laboratory develop w harts or epresent teria en ften ell erentiate cteria. here ften veral ptions to etermine cterial ecies. flow hart or erentiation obes. Specimens reaked nto rimary dium, uch blood agar MacConkey agar. The plates incubated for to hours and then examined for growth. Suspected pathogens on incubated plate be further identified regarding their genus or species with of flow chart. Determining genus f genic rganism ften ossible ure haracteristics. eferred presumptive or tentative identification. Definitive identification usually equires dditional iochemical esting. ortant to e eterinarian ecision egarding ial reatment **presumptive identification** dditional esting ect rapeutic ptions. comparatively ew rganism entified genus level with degree of certainty. Table summarizes identifying characteristics of common bacterial pathogens veterinary species. Table summarizes bacterial pathogens of veterinary importance, species are affected, resultant r ecimens equired or Appendix Bacterial Pathogens of eterinary Importance, con ummary haracteristics roduced microbial gens en irds.

Each practice laboratory should develop flow charts

Most ram-positive ram-negative rganisms row blood ar. ram-positive rganisms ually row MacConkey gar, ut his gar upports he rowth ost ram-negative organisms. Selection of colony from routine blood agar plate preferable to selecting from MacConkey agar. The danger rent ubculturing om lective dium uch acConkey ited rganisms resent



Examples of flow charts that are used for the differentiation of bacteria.

Typical Sequence of Testing of Microbiology Specimens

Collect specimen.

Common Bacterial Pathogens in Veterinary Specimens

Cyclic adenosine monophosphate; triple sugar iron agar.

Veterinary Importance

ocolonies dvertently be olony erest.

Samples that are collected with a sterile swab can be directly

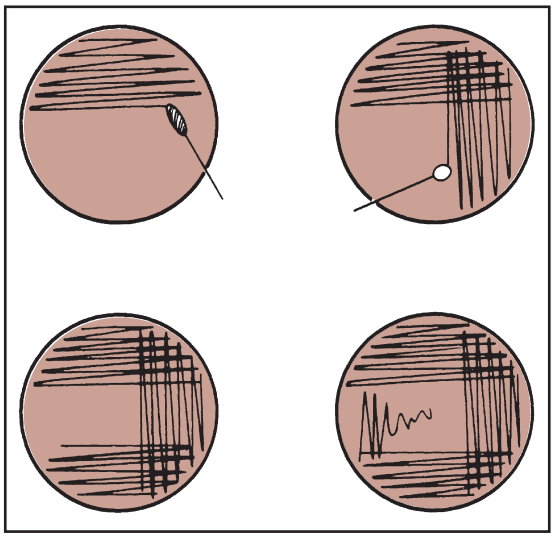
Method for Isolating Bacteria

Care must be taken to prevent contamination when inoculating media handling specimen. septic (sterile) technique must be used all times. Before obtaining from cadaver or xcised rgan, urface rgan issue flamed spatula before cut open for collection. Culture plates e ept losed culating emoving olony specimens or esting. hen ransferring om tube, ube ck ough efore er transfer of material avoid putting down cap. Instead, etween ers. hen culation op ire, ce ortion ire ork oward ontaminated Placing contaminated end into first could result ering cteria, esulting erosol ontamination. When ecimen ollected uantity well-mixed culated dge erile cteriologic op. ome oratories presterilized ods or reaking ecause unsen burners are always available glass rods be autoclaved. Disposable culating ops ires vailable.

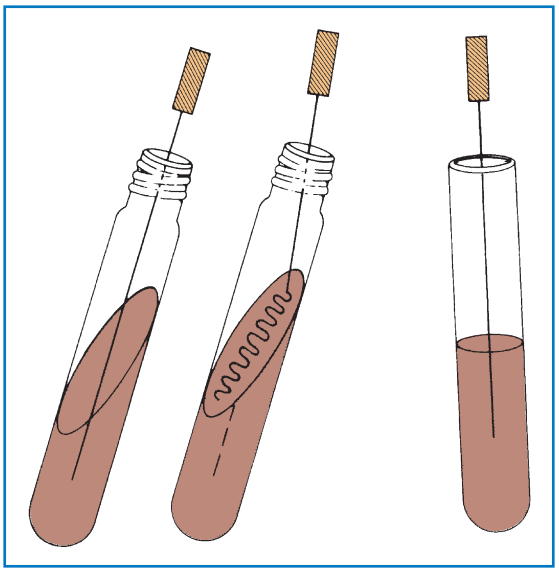
specimen ially een ollected erile streaked ectly nto

The preferred method of streaking agar plate **quadrant streak** thod rocedure oal hen method to obtain well defined isolated bacterial colonies for further testing. Fig. shows primary streak or well" f he late. he acteriologic oop ay ay ot flamed ooled efore reaks wn *B, C,* f e. epends imated umber bacteria resent ecimen. ops—one which ooling eing ractical echnique.

Each reaked verlapped nly nce ice void depositing xcessive umbers cteria therwise,



The quadrant streak method for the isolation of bacteria. (From



Clinical textbook for veterinary technicians,

se a sterile bacteriologic needle to remove a small amount of the bacterial

the needle directly into the center of the agar, and push the needle

resultant colonies are discrete isolated. Isolated colo ypically row wn he of ntire ortant, reak kept lose ogether lude reaks ossible, care en o verlap reak veral ypes of olonies row ch olony ubcultured nto separate rocedure epeated ure of ete ed olonies btained.

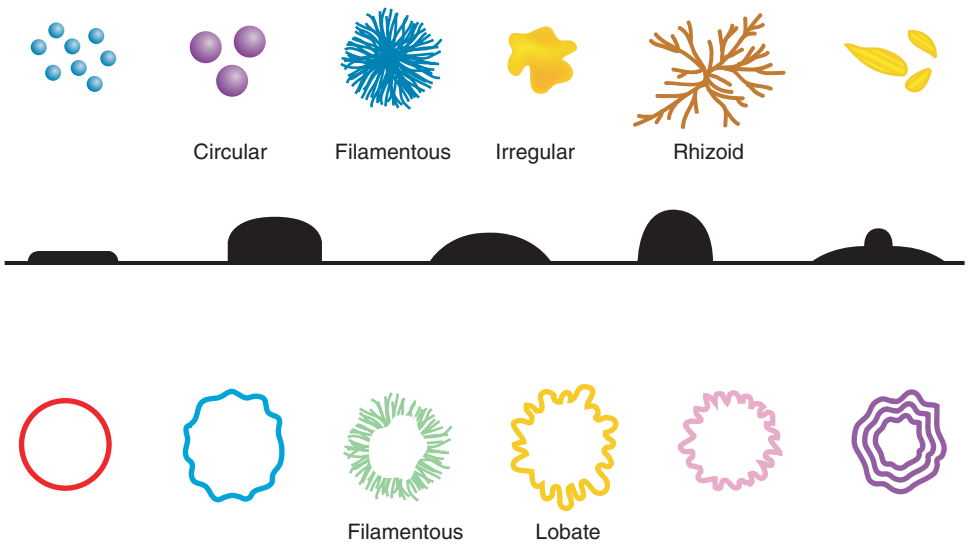
If ar nly urface inoculated, utt urface culated. inoculate nly urface raight ire used o btain olony cteria om rimary ion plate. he urface **slant tube** reaked To culate utt utt stabbed ith culating ire efully withdrawn rtion urface n reaked rocedure Enough cteria ire culate urface, even er utt een bed. ube's replaced osely.

TECHNICIAN NOTE

For pathogens invade internal organs of the ptimal rowth emperature sually ear 7° or ome gens, gens ermatophytes), nvi ronmental organisms, optimal growth temperature lower. Care en or emperature hich ptimal emperature, ecause cterial growth or genic cteria ccur ove temperature.

Incubation time epends the generation time individual bacterial ecies ype dium hich growing. or outine ultures, lates hould ncubated or hours, ith xamined er ion. Organisms uch *Nocardia* ecies y bation efore olonies isible. ure inverted uring ncubation hat oisture oes ot ollect surface of agar, which may clumping of colonies.

Some gens equire bon xide or rowth culture mosphere. **candle jar** y e d or pose. The es ced ar, op of es, on leaving ecreased xygen eased bon dioxide jar's atmosphere. (This does create anaero bic ondition.) ed or then hecked or rowth. rowth ccurs, reincubated or rechecked or rowth. er oratories ve ors tomatically nitor emperature, bon xide oxygen vels, umidity.



Bacterial colonies may be described on the basis of their form, elevation, and margins.

An xperienced echnician ecognize veral cteria f ross bservation olonies. arious olony characteristics, including following, may help to identify bacterium volved

- Size limeters escribed oint, dium, large)
- Pigment
- Density paque, ransparent)
- Elevation aised, onvex, oplike)
- Form (e.g., circular, irregular, **rhizoid filamentous undulate**
- Texture (e.g., glassy, smooth, **mucoid** buttery, brittle, sticky)
- Odor ent, eet)
- Any molysis eta,

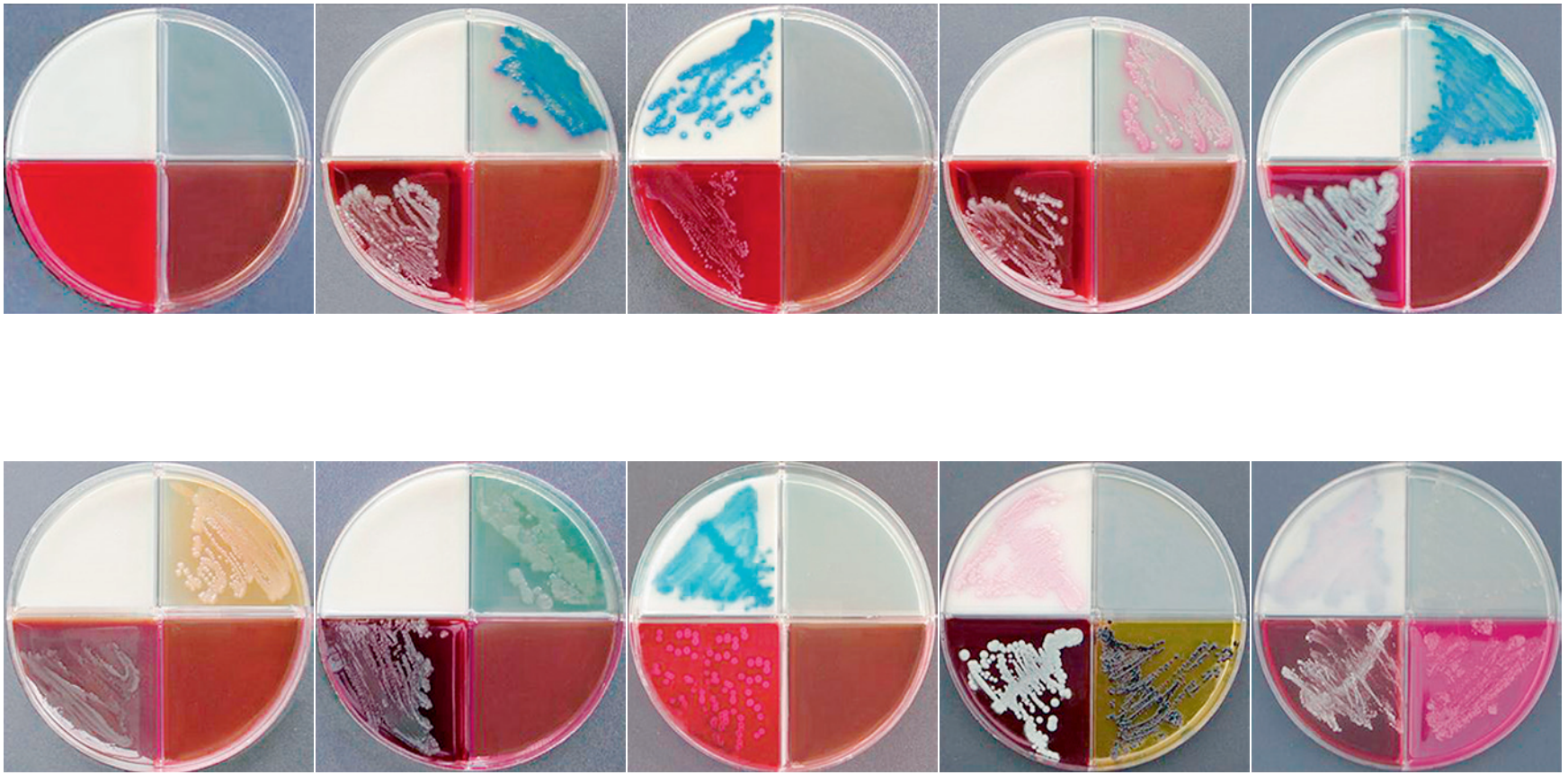
Many of the modular stems are provided detailed color charts entify cterial ecies part n ir olony rphology

Because erobes urvive xposure or utes, ollection or erobic ure cceptable. referred erobic ecimens lude blocks f issue h-cube losed, sterile ontainer ell xudate ollected erile syringe, ith xpelled edle ugged rubber stopper or bent backward on itself. Specialized anaerobic specimen ollection stems vailable.

Specimens ured on ossible er lection. The specimen inoculated onto blood agar plate into glycollate roth. lood anaerobe ar, hich rovides erobic nvironment uring incubation. self-contained system, such Pack (Oxoid, Columbia,

Conditions n hich he solation naerobes ay ignifi include soft-tissue abscesses, postoperative wounds, peritoni tis, septicemia, endocarditis, endometritis, gangrene, pulmonary infection, oot ep, ed anaerobe tiologic ent, tner synergistic relationship with another bacterium. For example, liver abscesses seen slaughter otherwise healthy feedlot commonly ield erobe *Fusobacterium necrophorum* erobe *Actinomyces pyogenes.* or onditions volve *Clostridium chauvoei, Clostridium septicum, Clostridium novyi, Clostridium sordellii,* oratories rescent antibody echnique or ecimens lude ected muscle ontains one row.

Chapter eview uestions ppendix



Spectrum CS interpretation guide. Modular culture systems usually have interpretation guides that are based on colony morphology. (Courtesy Barry Mitzner, DVM.)

- A systematic approach needed for proper evaluation of cultures.
- The quadrant streak method used to isolate pure culture.
- Slant tubes be inoculated on surface, butt area, or both areas.
- High-carbon-dioxide environments be achieved with candle jar.
- Cultures are incubated initially examined after to hours.
- Presumptive identification often be achieved with evaluation of colony morphology.
- The evaluation of colony morphology includes colony size, density, pigmentation, elevation, form, texture, odor well determining presence of any hemolysis.

Antimicrobial Sensitivity Testing

After studying this chapter, you will be able to:

- Discuss ions or erforming imicrobial sensitivity esting.
- Describe rocedure or erforming imicrobial sensitivity est thod.
- Describe rocedure or uring one inhibition nificance urement.
- Discuss thods erform olony counts.

Antibiotic Resistance,
Antimicrobial Sensitivity Testing,
Agar Diffusion Method,
Reading ones nhibition,
Interpretation one izes,
Control anisms,
Limitations
Minimum Inhibitory Concentration,
Limitations
Colony Count,
Key Points,

Antimicrobial disks
Antimicrobial susceptibility test
Bauer-Kirby technique
Beta-lactamase
Direct sensitivity testing
Indirect sensitivity testing
McFarland suspension
Minimum inhibitory concentration
Zone of inhibition

Antibiotic esistance een led orld's pressing roblems. ntibiotic tance ccurs hen obe hanges utates educes liminates ffectiveness ugs, hemicals, or r ents esigned revent ections. early every ype f cteria ecome ronger esponsive to ibiotic reatment hen eded. ntibiotics or it rowth usceptible cteria. ometimes cteria urvives ecause ility utralize escape ffect ibiotic. cterium multiply replace all bacteria were killed off. Exposure to antibiotics therefore provides selective pressure, which makes urviving cteria re ely ddition, bacteria ere usceptible ibiotic acquire resistance through mutation of their genetic material or by cquiring ces ode or esistance roper ties om cteria. odes or esistance be rouped ransferable ckage. that acteria an ecome esistant any ntimicrobial gents because f ransfer ce ome cteria develop ility utralize ibiotic efore harm, others rapidly antibiotic out, still others hange ibiotic ck ect function f cteria.

These antibiotic-resistant bacteria quickly spread to other even family members threaten community with w rain ectious re cure re xpensive reat. or eason, ibiotic resistance among CDC's USDA's top concerns. ntibiotic resistance er uffering or with have common infections, once easily treatable with antibiotics. icrobes evelop esistance ecific common misconception becomes resistant to specific drugs. However, microbes, become resistant o ugs.

When rganism ugs, reating infections ecome ven ossible. ith ection ertain ection way, difficult-to-treat infection be spread from one to r, ven ection d o rious ility ven

The ost ommon utation orm of ntimicrobial resis tance volves xtended-spectrum **beta (** -Lactamases known penicillinase) are enzymes produced by me cteria rovide ultiresistance -lactam i biotics. hese ibiotics ypically reat ections with road ectrum ram-positive ram-negative bacteria lude enicillins, ephalosporins, ephamy -Lactamase provides antibiotic resistance by breaking antibiotics' ructure. -Lactam ibiotics ve ommon element their molecular structure: four-atom ring known -lactam. hrough ydrolysis, ctamase roduced bacteria cleaves -lactam ring, deactivating antibacterial properties f dication. ommonly *Escherichia coli, Klebsiella* p., *Proteus mirabilis* ut e found ram-negative ods. rganisms serious infection are common source of nosocomial infec tion. They are often resistant to other classes of antimicrobials (e.g., glycosides, roquinolones).

When cteria ed om ient, imicrobial sensitivity est erformed etermine cterium's susceptibility esistance ecific imicrobial ugs.

The nsitivity esults low eterinarian hoose propriate imicrobial reatment or ient. The pecimen sed or ntimicrobial usceptibility esting ust be btained om efore reatment egins. et erinarian egin reatment efore btaining usceptibility results ut hange re propriate hen esults vailable.

TECHNICIAN NOTE Results of antimicrobial sensitivity testing allow for the

The agar diffusion test commonly performed method for imicrobial usceptibility esting. er have been impregnated with antimicrobials. This quantitative test equires urement itory one give imate imicrobial usceptibility. oncentra tion f ug hosen orrelate rapeutic levels f reated iffusion thods are ommon lude ood istration thod; dized usceptibility method, hich dified **Bauer-Kirby technique** International ollaborative udy dized echnique. The ommonly thod ibiotic usceptibility testing auer-Kirby echnique.

Although ultures an erformed he lassic anner vidual ueller-Hinton dular ure systems r ecialized re ommonly veterinary ractice ome rganisms, uch streptococci, row ufficiently ell ueller-Hinton agar for test to be read. In Mueller-Hinton agar us lood owever, ving eparted from dized thod, itory one be erpreted tion. or one or novobiocin ler dium ontains lood. streptococci ill usceptible enicillin.

upply f ibiotic eded orresponds ibiotics ommonly dosages ften eded. imicrobial ontent ed able epre sentative f etracyclines epresentative ulfonamides cessary ecause henomenon cross-resistance. For example, bacterial species resistant to ne f etracyclines, ually mbers of roup.

Antimicrobial disks always be kept refrigerator hen eing eplaced on ossible after tdated ver otency antimicrobial be monitored with control organisms of wn nsitivity erns. enser er clear verlays or uring itory ones equired. Disk dispensers be obtained from manufacturer roducts erchangeable. noculation of thioglycollate or trypticase soy broth tube recommended time plates are streaked. If culture results rove onclusive, roth ure tside oratory or onfirmation.

Indirect sensitivity testing equires olony taken om ure ubcultured roth incubated to achieve turbidity matches standardized **McFarland suspension** Fig. 42.1 The broth suspension then applied o ueller-Hinton op over e venly. btain roth ution, urfaces of ee r our olonies ouched erile op. The op ced roth uspen sion. f cteria en om urface olony, there elihood en ontaminant growing olony. ore olony bacterial species always be taken, because single colony may epresent ariant usceptibility ern different om ent rain. orrect ensity important ect etting eproducible esults. lawn" cells rows er culation venly ributed. Extremes oncentration voided.

to the Antibiotics Being Tested

From McCurnin DM, Bassert JM: *Clinical textbook for veterinary technicians,*
Modified from National Committee for Clinical Laboratory Standards document M31-A2, Table 2, pp 55-59, 2002.
*Ampicillin is used to test for susceptibility to amoxicillin and hetacillin.
Cephalothin is used to test all first-generation cephalosporins, such as cephapirin and cefadroxil. Cefazolin should be tested separately with the gram-negative enteric organisms.
Clindamycin is used to test for susceptibility to clindamycin and lincomycin.
Oxacillin is used to test for susceptibility to methicillin, nafcillin, and cloxacillin.
Available as an infusion product for treatment of bovine mastitis during lactation.
Tetracycline is used to test for susceptibility to chlortetracycline, oxytetracycline, minocycline, and doxycycline.
**Trimethoprim/sulfamethoxazole is used to test for susceptibility to trimethoprim/sulfadiazine and ormetoprim/sulfadimethoxine.

The plication uted ectly to ueller-Hinton eferred **direct sensitivity testing** lthough recise ect esting, easonable results be expected when only one organism present. nti biotic susceptibility be interpreted with caution when culture ws ultiple rganisms.

The imicrobial ced culated surface ith enser erile orceps ve een flamed ooled etween ch loser dge ufficiently separated om ch void verlapping **zone of inhibition** nless ere ced dispenser, second sterile to gently press antibiotic into agar. The plates are incubated aerobically y ced or utes after cing culated ar. noculated

The use of calipers to measure the zone of inhibition.

e verted efore ced or avoid ondensation ollecting urface ar. plates ept cks ewer, ecause enter ck nger each ion temperature.

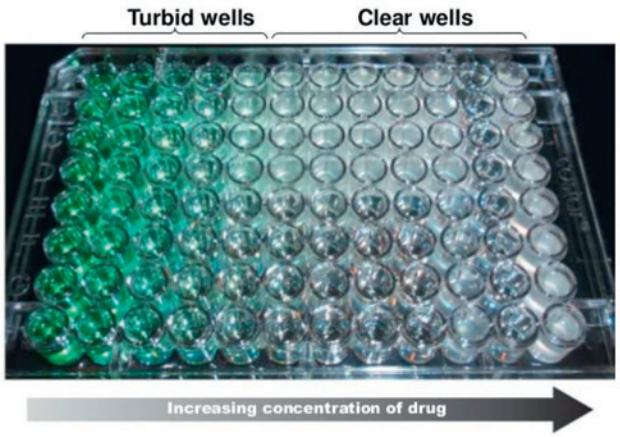
Whether testing performed by direct or indirect method, antibiotic susceptibility must be determined by physical surement of inhibitory zones Fig. That measurement n ompared hart itory ones etermine elative esistance cterium ibiotics eing tested e able

The plates be read after constant period, satisfac torily after overnight incubation .e., 18 to 24 hours). Prolonged incubation er one ition antimicrobials r, zones hard to read. If rapid results are imperative, diameters of ones ition ead er incubation. esults onfirmed eading again after overnight incubation. The diameter of each inhibition zone luding ter ured om underside f ers, ransparent uler, template. he ones ured ecorded millimeter. ueller-Hinton lood een one e ead om op urface, e emoved.

Table ommonly imicrobials gives uggested erpretation one standardized thod. one vided major categories: resistant or susceptible to particular antimi crobial agent. The latter category subdivided into intermediate susceptibility susceptible. For predictive purposes, resistant organism ely espond rapy ug. susceptible rganism usceptible rdinary oses he nti microbial. Intermediate susceptibility organism usceptible rdinary hen oncentrated ine issues. imicrobial or treatment f stemic ections osage The one ive fficacy antimicrobial. ome ugs, uch ancomycin olistin, do eadily ough ive ition zones, even when test organism fully susceptible. Therefore, direct comparisons with zone diameters produced by unrelated antimicrobials de.

Susceptible eference rganisms uch *Staphylococcus aureus,* American ype ulture ollection *Escherichia coli,* American Type Culture Collection be tested regu larly, referably allel ch ch **antimicrobial susceptibility tests** hese ontrol rganisms heck such actors rowth-supporting apability edium, potency of antimicrobial other variable condi tions ect esults.

The A thod esigned or apidly rowing cteria. aution eded or erobes w-growing rganisms or which iteria or erpretation one ters ve not et been stablished ith ertainty. eneral, one am eters e mewhat er or quivalent itory concentration w-growing rganisms ompared rapid rowers.



Microwells with varying concentrations of antimicrobials used for determination of MIC

Some rare strains of staphylococci are resistant to methicillin and other penicillinase-stable enicillins. The outine test nnot be elied n etect rains, ut etected incubation of additional susceptibility test plate contains methicillin reduced zone diameter or zone surrounding methicillin on plate been bated presumptive evidence of methicillin resistance.

thod thod or nsitiv ity esting etermine **minimum inhibitory concentration** f imicrobial. west concentration ecific imicrobial growth f iven cteria. hoice ecific oncentra tion f imicrobial ection, breakpoint of antimicrobial. Breakpoint refers to dilution of antimicrobial where specific bacteria begins to show resistance. Other considerations for specific antimicrobials include ient haracteristics, uch ecies, verall ell ossible ffects, frequency oute dministration.

To erform he est ith gar iffusion method, aper isks or strips with varying concentrations of chosen antimicrobial are ced n eshly culated ure ed. Measuring ones ition ch will lp eterinarian hoose propriate oncentration of dication iven ient.

Alternately, be determined microwells with multiple known concentrations of antimicrobial being tested Fig. standard suspension of of bacteria prepared from pure colony, suspension inoculated into ells. umber west oncentration of antibiotic inhibits growth of given strain of bacteria. The breakpoint range of dilutions differ by drug cterial ecies. herefore, omparing erent antibiotics based solely on numerical value but on how from breakpoint, site of infection, other considerations. For example, assume strain of *E. coli* or xicillin

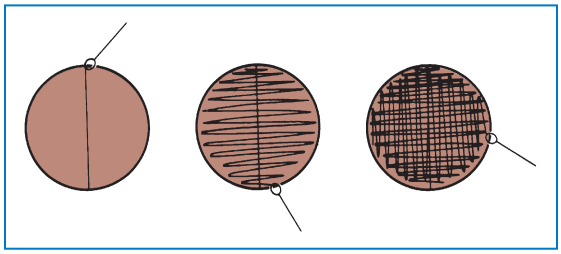


Fig. 42.4 The procedure for inoculating media for semiquantitative bac

for cephalexin. For dilutions for amoxicillin, this rain of *E. coli* is dilutions away from breakpoint. For cephalexin, rain *E. coli* two utions way om reakpoint. o, number, rain *E. coli* re usceptible xicillin cephalexin. The further away from breakpoint organism ill row, re ffective ibiotic ill

In rder or imicrobial ent ection, drug ust e et ection concentration infection site greater of infecting bacterium. Sometimes concentration of bacteria ection reater outine testing. he enerally or -producing isolate hen hallenged reater t higher concentration of bacteria, drug overcome increased oncentration resent. ther ctors may ontribute ures, uch oncentra tion f ug ection ompared ecting cteria oncentration ys above ll rganisms enus ecies *E. coli, Klebsiella* spp., *P. mirabilis* be tested for These are ommon rganisms xhibiting roduction; however, rganisms ve een roduce In nitoring valuating ffectiveness of imicrobial rapy, onsideration iven possibility may negatively impact effective treatment.

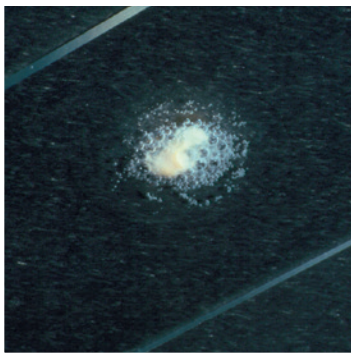
The presence of pathogenic bacteria does not necessarily indicate infection. or rmal onsidered sterile, l umbers rganisms ccasionally even after collection by cystocentesis. colony count on cultured urine upport inary ract infection. olony ounts an erformed treaking lood agar or another nonselective agar plate with calibrated loop contains of urine Fig. fter incubation, all colonies are ounted ultiplied etermine umber colony-forming er liliter lthough they nly rve elines, umbers more CFUs for collected by cystocentesis more or ollected ter. oided

ecommended or ure) ve
more ogs re
o e eemed

Chapter eview uestions ppendix

- Antibiotic esistance ccurs hen obe hanges utates me y educes liminates ffectiveness drugs, chemicals, or other agents designed to cure or prevent infections.
- Bacteria roduce -lactamases -lactam antibiotics.
- Antibiotic nsitivity esting erformed etermine resistance r usceptibility acteria pecific ntimicrobials.
- The application of undiluted directly to Mueller-Hinton e eferred ect nsitivity esting.
- Indirect nsitivity esting equires olony taken from culture plate, subcultured broth media, incubated o chieve urbidity ches dized cFarland uspension.
- Antimicrobial are placed on inoculated agar surface with isk ispenser terile orceps hat as een amed ooled etween ch
- For nsitivity ter ch ition one (including ter ured om underside f ers, ransparent ruler, r emplate.
- The MIC be determined ensure ffec tive antimicrobial will be chosen used appropriate dosage.
- The resence genic cteria oes cessarily indicate ection.
- A olony ured upport nosis inary ract ection.

Additional Testing



After studying this chapter, you will be able to:

- List escribe thods or esting ility bacteria.
- List ommonly erformed iochemical
- Describe ommonly erformed iochemical
- Describe rocedure or erforming alifornia Mastitis est.

Motility,
Indole Test,
Catalase Test,
Coagulase Test,
Oxidase Activity,
Acid Production From Glucose,
California Mastitis Test,
Immunologic Examination,
Key Points,

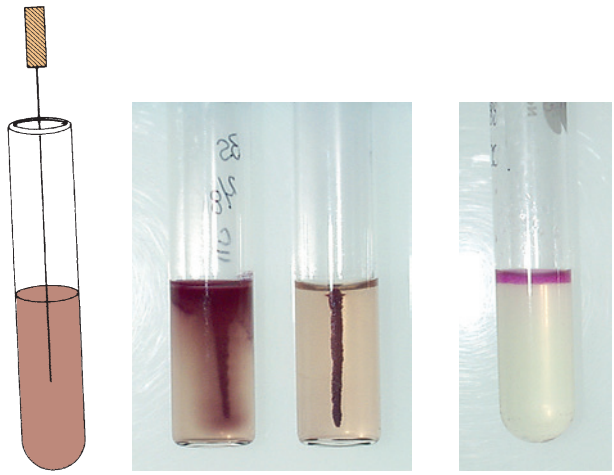
California Mastitis Test
Catalase
Coagulase
Hanging drop
Indole
Kovac's reagent
Motility media

The presumptive identification of bacteria often gives veteri narian enough information to develop diagnostic treatment owever, rganisms ther erentiated to ecies vel equire dditional esting or ositive identification. artial ist ommonly erformed ests ollows.

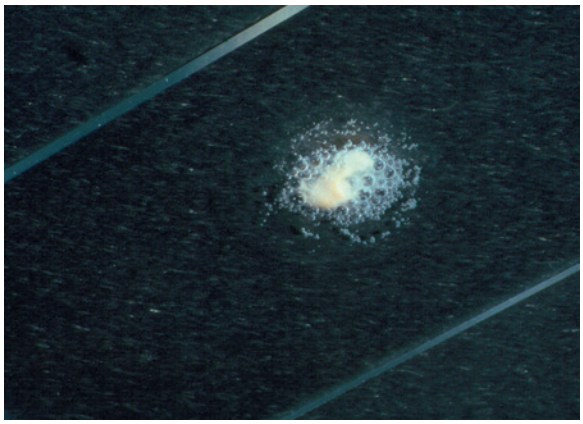
Several methods are commonly used to test motility: **hanging drop** rep, et rep, **motility media** For wet prep, oung roth ure derately uspension of cterial de ew liliters utrient broth ed or oom emperature. opful f ure ced oscope er overslip xamined bjective cteria bviously vidual ells ving backward r orward ells, er een obtained. Brownian movement be mistaken for true motility. Brownian movement shifting of cells or particles, with vement elative ch r.

Wet preps tend to evaporate rapidly under microscope, some bacteria may appear obviously motile provided with dditional iquid. he anging rop rep an liminate hese problems. Special slides are available contain concave depres sions center. The slide be cleaned with alcohol wiped y. etroleum lly ced concave area of slide just before To prepare hanging drop rep, ce op cterial uspension overslip invert coverslip onto concave area of slide. Press overslip own htly etroleum lly concavity. The suspension will be left hanging upside down into ell f oncave reparation oes end dry t uickly, bserved or ly eriod.

If rganisms nmotile oscopic tion, ility wo ubes ility medium ulfide ility dium) inoculated. ne ube ncubated 7° nd he ther oom temperature or rowth estricted inoculation organism nonmotile. Diffuse growth throughout dium cterium To erpret esults, ubes ood culated ubes ompared culated one. he dium uitable or ility esting organisms produce hydrogen sulfide medium, because



Kovac's reagent added to detect the production of indole. (From McCurnin D, Bassert J: *McCurnin's clinical textbook for veterinary*



A positive catalase test is indicated by the production of bubbles

lackening esults ility to ead. he roviding esults or **indole** est. he est valuates ility rganism produce indole. **Kovac's reagent** added to incubated tube. The eagent urns ed cteria roduce

Although indole test be performed Kovac's reagent ube escribed lier, re ommonly performed here veral thods or er forming olony ked from e ure otton op ovac's reagent added to positive result indicated by develop ment f ed olor olor evelopment indicates gative lternately, veral ops reagent plied ce er er, op cterial olony ubbed nto eagent-saturated area. Kovac's reagent suitable for testing anaerobic bacteria. Other eagents erobic aerobic bacteria. The color change may differ depending on specific eagent hosen. evelopment lue olor ositive est eagent.

The test performed on gram-positive cocci small gram-positive bacilli. It tests for enzyme which acts on hydrogen eroxide roduce ater nd xygen. mall mount of olony om lood ced oscope slide, op eagent ydrogen eroxide) added. f he olony atalase ositive, as ubbles re roduced Fig. ubble roduction gative esult.

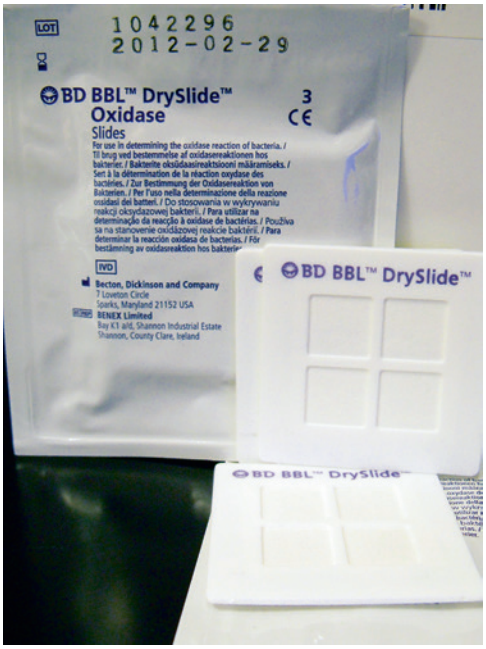
No blood agar be transferred with colony, because blood ar roduce htly ositive eaction. ositive reaction may occur mixed colony sampled one with both ositive gative rganisms rowing together). The plate must be carefully streaked to obtain isolated colonies. Staphylococci be used catalase-positive controls, reptococci gative ontrols.

The **coagulase** est erformed ositive, ram-positive occi. *Staphylococcus aureus* roduces oagulase, hich enzyme coagulates Two versions of test are available: slide coagulase test tube coagulase test. The coagulase est erentiate oagulase-positive *S. aureus, Staphylococcus intermedius,* oagulase-negative *Staphylococcus* ., *Staphylococcus epidermidis Staphylococcus saprophyticus*

The ube oagulase yophilized chased from medical supply been diluted according to ufacturer's ections. pproximately ced est ube culated opful rganism cultured n ninhibitory dium, uch lood ar. tube incubated at 37° and ead urly for hours. gative reaction ed ormation, hereas ositive reaction ed esult emains gative, ed or ead.

The e oagulase ommercially vailable screening est etects urface-bound oagulase lump factor. More of coagulase-producing staphylococci possess clumping factor. loopful of staphylococci from colony st mulsified op er ution ield k uspension. op abbit then dded irred erile op. ositive eaction indicated y lumping ccurring conds.

The xidase est epends resence tochrome **oxidase** cteria. op etramethyl-p-phenylenediamine added o ce er er etri er er



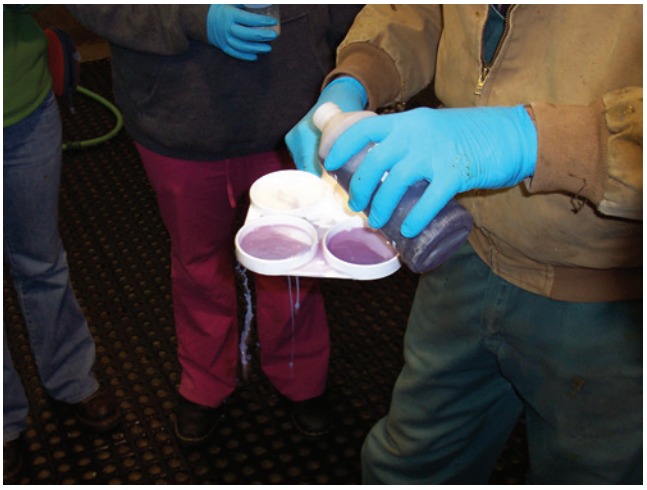
DrySlide oxidase test.

must e ut urated. repared eagent are vailable or erforming liminate ed or eagent ling. rt reak made n er er repared od nd f asteur ette een ent The plied entle ubbing ction. nickel–chromium ire cteriologic op because y races on ive ositive esult.

In positive test, reagent reduced to deep purple color within seconds. If color-change reaction takes longer esult egarded gative. eagent tends o e ecomes olored e ded ecomes *Pseudomonas aeruginosa* be used oxidase-positive control, *Esch erichia coli* e d xidase-negative ontrol. slide vailable or

tube of glucose peptone broth contains tor culated ed or or fastidious organisms such *Streptococcus* species, approximately ops f erile rum dded eptone er medium, r rowth ccur.

Mastitis may be caused by bacterial or mycotic organisms. Several laboratory ests vailable itis, luding **California Mastitis Test** (CMT), somatic cell count, culture. quick check to detect bacteria be performed by reparing itic fixed ram thylene lue. common oorganisms volved itis *S. aureus,*



grew-Bohling K: *Large animal clinical procedures for veterinary techni*

Streptococcus agalactiae, Streptococcus uberis, E. coli, Corynebac terium ecies, *Pseudomonas aeruginosa.*

The ualitative eening cow-side" el ormation hen test eagent eacts matic ells. ell of the milk creases, the gelling action increases. Therefore, the test rovides ect ure ell egree of el ormation ored gative; race;

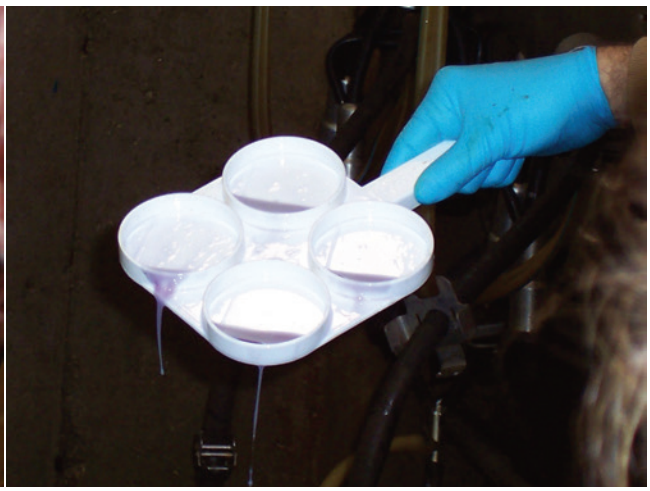
To perform CMT, approximately of placed each of four on paddle, equal of eagent dded ddle ently otated for proximately conds ern eagent. ore ned or ch uarter according to chart of grading interpretation Table

Precautions onsidered hen erforming ollows:

1. DNA matic ells eteriorates on o e d oratory kept refrigerated, but for more hours. Unrefriger ated ested ccurately er out
2. White blood cells tend to migrate with Therefore, thorough efore esting ntial.
3. CMT eaction ored conds er starts. eaker eactions de reafter.

Only ositive entified direct matic ell ured culated lood acConkey then incubated for hours. Milk are incubated ultaneously. ures growth er ion, ubculture de lood acConkey om ed he ubcultured riginal ure are ed or ut resumptive identification rganism de colonial rphologic haracteristics, ill ollowed by confirmatory tests (e.g., triple sugar iron, lysine, methyl red, rate, oagulase,

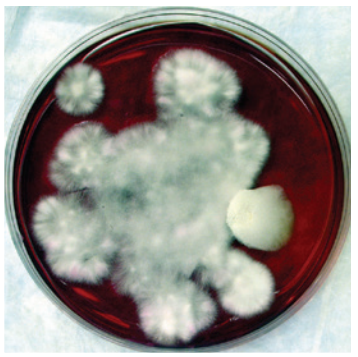
Polymorphonuclear leukocyte.



Numerous immunologic tests are available for identification of bacterial pathogens, particularly obligate intracellular bacteria. See nit or re ormation out unologic esting.

Chapter eview uestions ppendix

- Additional esting metimes eded or efinitive identification cteria.
- The ility cteria valuated et ing op rep, ility
- Commonly performed biochemical tests performed on bacterial oagulase,
- The alifornia astitis ommonly cow-side" test etect itis.



After studying this chapter, you will be able to:

- Describe rocedure or reparing ermatophyte cultures.
- Describe rocedure or oscopic valuation dermatophyte ures.
- Describe ood's
- Discuss ure thods or ndermatophytes.
- List haracteristics eterinary ortance.
- List eneral haracteristics stemic rphic fungi.
- Describe oscopic pearance specimens.

Dermatophyte Testing,
Fungal Cultures,
Key Points,

Dermatophyte test medium
Potassium hydroxide
Ringworm
Sabouraud agar
Wood's lamp

Supplies eded or ollection xamination fungal uch or cterial samples—specifically, orceps, ctophenol otton lue **potassium hydroxide** ution, ure **dermatophyte test medium** **Sabouraud agar** **Wood's** y rove lpful, ellophane to ollect ertain ypes or ysis.

Ideally, parate oom or or fungal culture, because fungal spores may contaminate plates e d or cteriologic ure. or vertently ontaminated ores, erior be bed roughly thyl propyl one owl f er ced bottom f or or mpty for s, oor ut,

TECHNICIAN NOTE A separate incubator for fungal samples will minimize

Most al ents ortance eterinary practice e taneous ycotic rganisms wn dermatophytes. rganisms ften eferred **ringworm** i ecause haracteristic y ften ected er matophytes re aprophytic ycelial-forming ungi hat ossess keratolytic roperties low vade hair. ome ents ect eings.

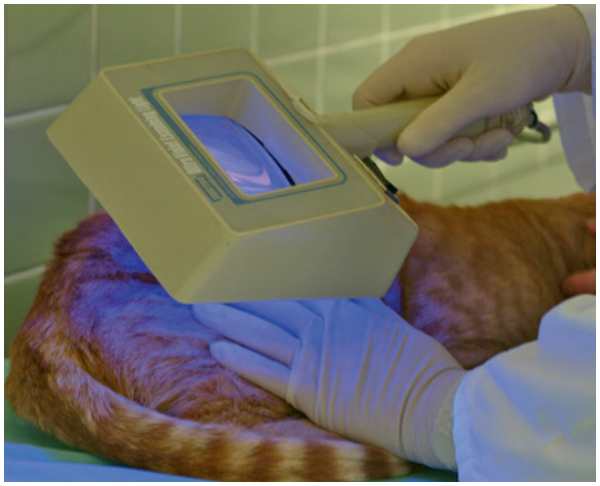
The dermatophytes comprise more three dozen different organisms n he axonomic enera *Microsporum* *Trichophyton* he ommonly en ecies *Microsporum canis, Microsporum gypseum,* *Trichophyton mentagrophytes.* er matophytes lassified hich ely opophilic onfined beings), zoophilic (parasites of geophilic (normally existing ee-living rophytes wn geophilic species, five may occasionally be opportunist pathogens, only one, *M. gypseum,* commonly lesions These eophilic ecies ertain because y erentiated om oophilic ecies.

approximately ogs, ection
ue o *Microsporum canis*

Most dermatophytes will grow on outside of In some they be visualized microscopically after mounting them otassium ydroxide ombination KOH thyl ulfoxide liminates ed o rovides re learing or ermatophytes, ew ucked from eriphery uspect ced with or drops of clearing solution. cover glass applied, e med ently fter to utes, lobular throspores ched e isible ositive esult.

ood's raviolet ce used o een uspect or ermatophyte ection ut esults om ogs r mselves—can xamined er ood's airs ected ecies *Microsporum* y resce ple-green er Wood's kened oom uorescence only vident proximately volving *M. canis,* apparently epending hether eached right growth stage to produce fluorescence. lack of fluorescence with ood's examination does rule out possibility of ingworm ection. lways low ood's or utes efore

Several products are available for culturing dermatophytes. The common standard which contains indicator

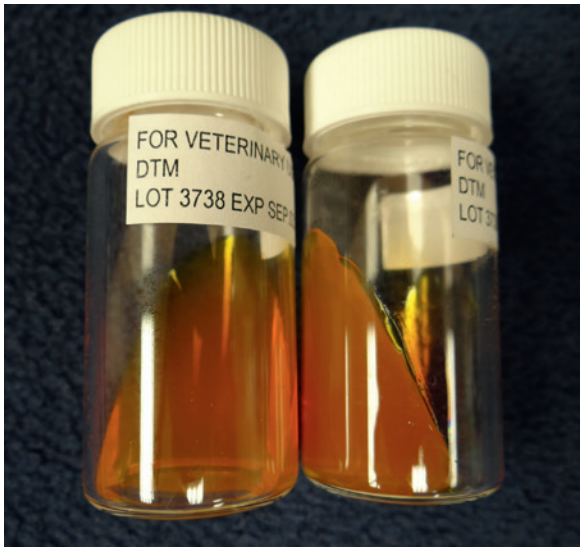


turns ed resence ermatophytes. available tube formats Fig. variety of plate con figurations. late onfigurations end llow or asier ampling of colonies. Rapid sporulation medium or enhanced sporulation olor ors conjunction ccelerate ormation of croconidia or entification onfirmation (DermatoPlate, etlab Supply, Palmetto Bay, Standard Sabouraud dextrose agar will promote earlier formation of croconidia, ut ontains olor or.

When ollecting ecimens or ermatophyte ure, lesion to remove some of surface contamination collect ecimens om eriphery. roken y ely ontain iable rganisms. ecimens tially elow urface Fig. ncubate ure oom emperature or plate cover loosened observe daily for growth. first n f olor hange, erform et rep ungi-Tape (Scientific Device Laboratory, Des or clear cellophane tape ctophenol otton lue onfirm resence of pathogenic forms. Remember, presence of red coloration r eal oloration

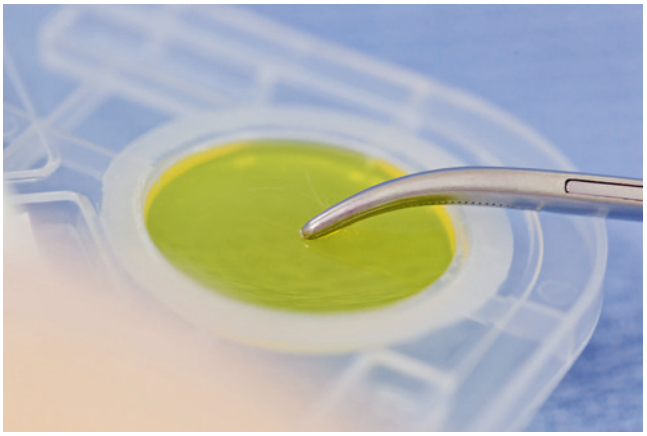
Green fluorescence of the hairs around a skin lesion on a

clinical techniques,



Dermatophyte test media in a tube format.

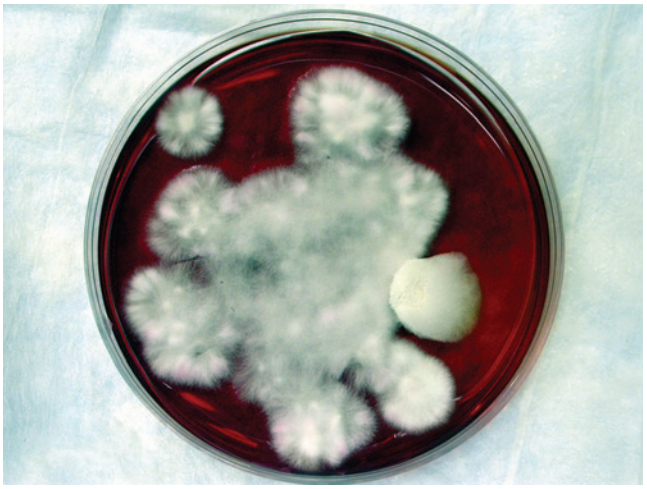
containing standard DTM and enhanced sporulation media. (Courtesy

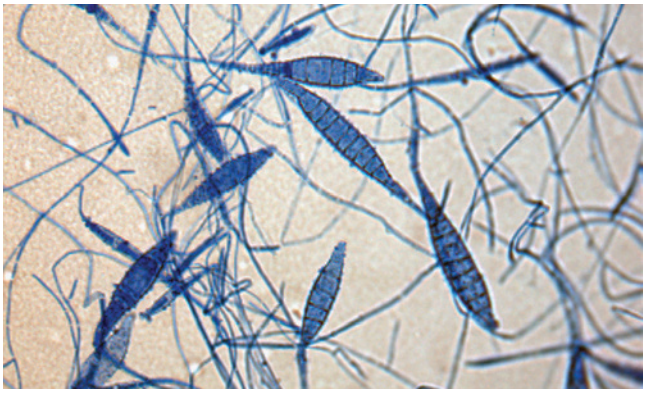


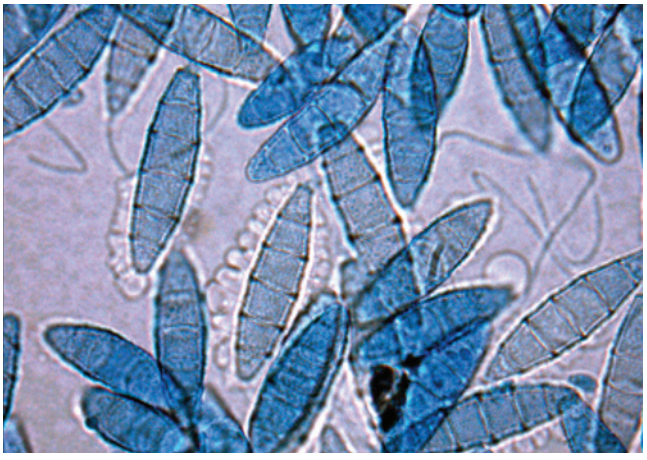
The sample is placed onto the media and pressed slightly into

diagnostic f ermatophyte ection. cterial ontami npathogenic er ertain onditions, ositive olor eactions. herefore, supported y oscopic xamination

Confirm all dermatophyte infections by verifying the







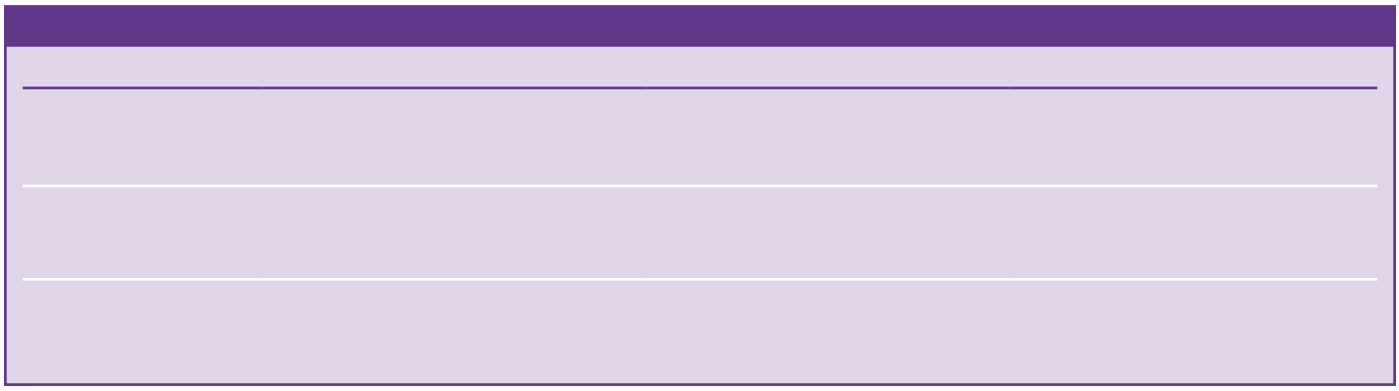
color change. The red color should develop as soon as colony growth
organisms as viewed

Cultures of nondermatophytes are usually streaked out on blood agar r abouraud extrose ar, one or cteria. or fungal rganisms roduce ores, ures prepared y tting ar om enter abouraud dextrose ar erile el. uare size taken from edge of colony to be subcultured. This square de ugly ar. material for subculture from edge of fungal colony always advisable, ecause ycelia enter olony may e iable.

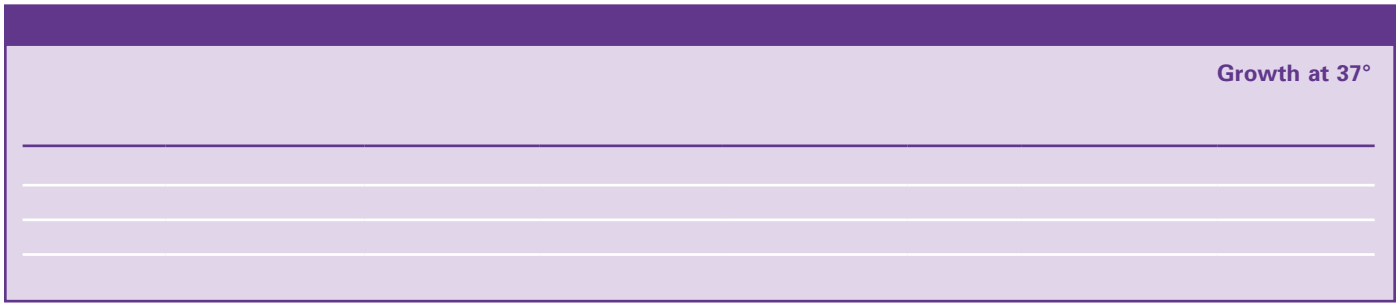
Fungi vade issue row ody emperature his emperature or ion rimary ures of ndermatophytes ontaminant rophytic species. he xception xamination ecimens

for dimorphic fungi, such *Blastomyces* *Histoplasma* species. These rganisms row hen ody emperature ultures ed, allel, both emperatures. haracteristics stemic rphic fungi f eterinary ortance iven able

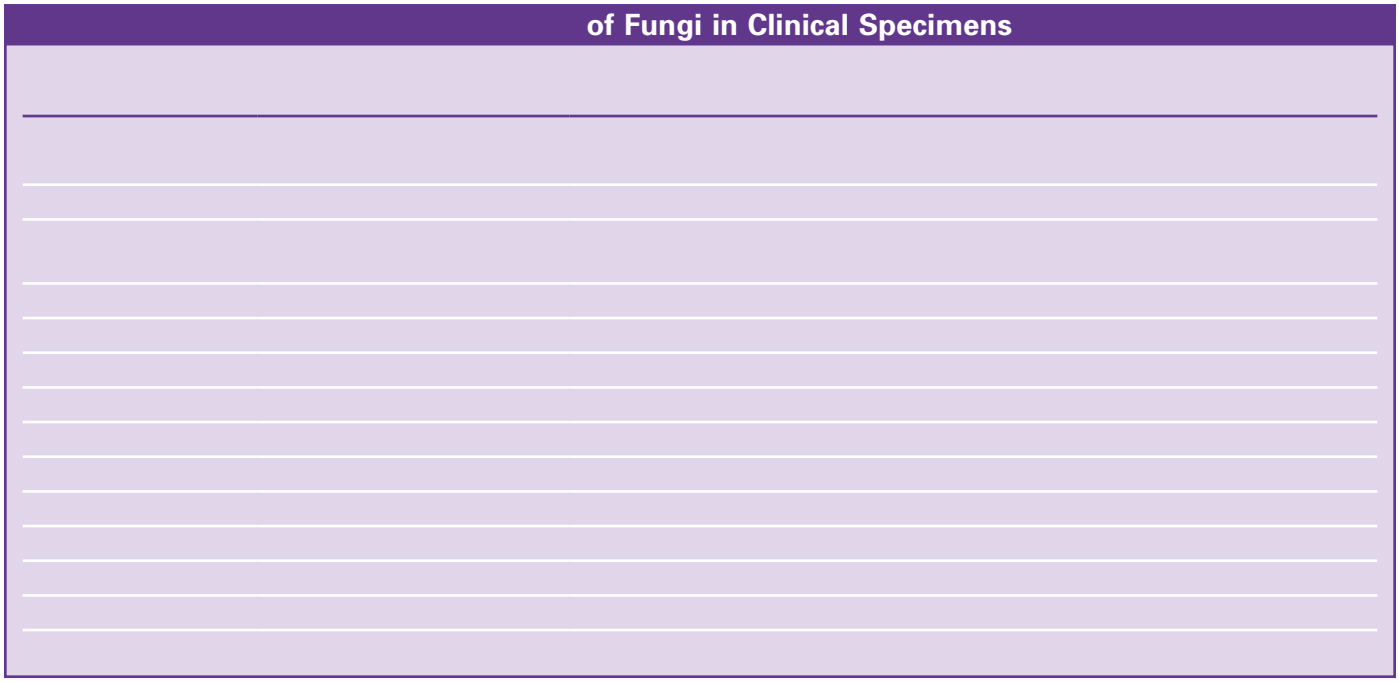
Because many pathogenic fungi, such *Candida albicans* *Aspergillus fumigatus,* e iquitous, issue ctions wing invasion may be needed for definitive diagnosis of mycotic



From Songer JG, Post KW: *Veterinary microbiology: bacterial and fungal agents of animal disease,*



From Songer JG, Post KW: *Veterinary microbiology: bacterial and fungal agents of animal disease,*



From Songer JG, Post KW: *Veterinary microbiology: bacterial and fungal agents of animal disease,*

infection. casionally, ycelial pear blood agar plates were prepared for bacteriology. These fungi or yeasts, of course, may be contaminants, but some of original specimen hould ubmitted or stopathologic xamination ormalin. xamination et reparation for al lements able ummarizes characteristics ommon eterinary ortance.

After ion, ures xamined entify types f ores resent. one out hes f ellophane ressing enter e, icky own, nto enter olony. tape, ith yphae uiting dhering ced with icky-side own oscope op f ctophenol otton lue cts own overslip. reparation xamined er power or with high dry lens, necessary. dditional informa tion out oscopic pearance cated Table

Chapter eview uestions ppendix

- Supplies are needed for collection examination of fungal are much used for bacterial
- Most ents ortance eterinary practice e taneous ycotic rganisms wn dermatophytes.
- Fluorescence with ood's only evident approxi mately volve *M. canis.*
- The commonly encountered dermatophytes are *M. canis, M. gypseum, T. mentagrophytes*
- Dermatophyte upported oscopic examination.

Unit Outline
Chapter 45: Nematodes,
Chapter 46: Cestodes, Trematodes, and Acanthocephalans,
Chapter 47: Protozoa and Rickettsia,
Chapter 48: Arthropods,
Chapter 49: Sample Collection and Handling,
Chapter 50: Diagnostic Techniques,

The objectives for this unit are:
List the common internal parasites of domestic animals.
List the common external parasites of domestic animals.
Discuss the life cycles of common parasites of domestic animals.
Describe the treatment and control strategies for common parasites of domestic animals.
Describe the procedures that are used to diagnose parasites.

Parasitology is the study of organisms that live in (internal parasites, endoparasites) or on (external parasites, ectoparasites) another organism, the host, from which they derive their nourishment. Parasitism is a type of symbiotic relationship. Symbiosis involves two organisms living together, and there are three types: (1) Com mensalism: One organism benefits, the other is unaffected; (2) Mutualism: Both organisms benefit; and (3) Parasitism: One organism benefits, the other is harmed.

The organism that the parasite lives in or on is called its host. The host may be a definitive host that shel ters the sexual, adult stages of the parasite, or the host may be an intermediate host that harbors the asexual (immature) or larval stages of the parasite. There are also paratenic hosts or transport hosts for some para sites, in which the parasite survives without multiplying or developing. Parasite life cycles can be simple with direct transmission, or they may be complex and involve one or more vectors. A vector can be mechanical or biological. Mechanical vectors transmit the parasite, but the parasite does not develop in the vector. Biological vectors serve as intermediate hosts for the parasite. The term life cycle refers to the maturation of a parasite through various developmental stages in one or more hosts. For a parasite to survive, it must have a depend able means of transfer from one host to another and the ability to develop and reproduce in the host, ideally without producing serious harm to the host. This requires the following:

A mode of entry into a host (infective stage)

The availability of a susceptible host (definitive host)

An accommodating location and environment in the host for maturation and reproduction (e.g., the gastro intestinal, respiratory, circulatory, urinary, or reproductive system)

A mode of exit from the host (e.g., feces, sputum, blood, urine, smegma), with dispersal into an ecologically suitable environment for development and survival

Parasites have a wide distribution within host animals. They can have a negative impact in a number of ways, including the following:

Injury on entry (e.g., creeping eruption)

Injury by migration (e.g., sarcoptic mange)

Injury by residence (e.g., heartworms)

Chemical or physiological injury (e.g., digestive disturbances)

Injury due to host reaction (e.g., hypersensitivity, scar tissue)

Internal parasites, called endoparasites, live within an animal. These parasites derive their nutrition and protection at the expense of the infected animal, which is called the host. The various internal parasites have many different life cycles. Each parasite's life cycle is distinctive. It is composed of various developmental stages, all of which may occur within the same host or separately within sequential hosts. Endoparasites of domestic animals include unicellular protozoans, trematodes (flukes), cestodes (tapeworms, with their associ ated metacestode stages), nematodes (roundworms), and acanthocephalans (thorny-headed worms). A few arthropods (e.g., horse bots) are endoparasites. Ectoparasites usually live on or in skin surfaces or feed on them. Ectoparasites infest the skin or external surfaces of animals and produce an infestation on the animal.

The host that harbors the adult, mature, or sexual stages of a parasite is called the definitive host. The dog is the definitive host for *Dirofilaria immitis;* adult male and female heartworms are found in the right ventricle and pulmonary arteries of the dog's heart. The host that harbors the larval, immature, or asexual stages of a parasite is called the intermediate host. The mosquito is the intermediate host for *immitis;* the first, second, and third larval stages of *D. immitis* are found within the mosquito.

The life cycle of most parasites has at least one stage during which the parasite may be passed from one host to the next. Diagnostic procedures frequently detect this stage; therefore, it is referred to as the diagnostic stage. The diagnostic stage of a parasite may leave the host through excreta (e.g., feces, urine), or it may be transmitted from the bloodstream to its next host by an arthropod (e.g., a mosquito). The microfilarial stage is the diagnostic stage of *D. immitis;* the female mosquito takes in the microfilariae during a blood meal.

The diagnosis of endoparasitism is one of the most frequently performed procedures in the veterinary clinical setting. An accurate diagnosis of endoparasitism is based primarily on the veterinarian's and the technician's awareness of parasites that are prevalent in the immediate geographic area or ecosystem. However, because of the far-ranging mobility of owners and their pets in the twenty-first century, residence in or travel to another geographic region should also be considered when endoparasitism is among several differential diagnoses.

Heavily parasitized animals often show clinical signs that are suggestive of the infected organ system. Depend ing on the affected organ system, these signs may include diarrhea or constipation, anorexia, vomiting, blood in the stool, or fat in the stool. Parasitized animals are frequently lethargic and display an unthrifty appearance that is characterized by weight loss or stunted growth, a dull hair coat, dehydration, or anemia. The animal may also experience coughing or labored breathing.

Internal parasites of domestic animals comprise several types of organisms that live internally in animals, that feed on their tissues or body fluids, or that compete directly for their food. These organisms range in size from being too small to be seen with the naked eye (microscopic) to being more than 1 ngth. Parasites also vary with regard to their location within the host and the means by which they are transmitted from one host to another. Because of these diverse variations, no single diagnostic test can identify all endoparasites.

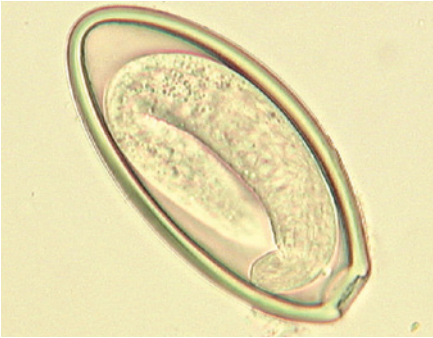
The time elapsed between initial infection with a parasite until the infection can be detected with the use of common diagnostic procedures is called the prepatent period. The best example of this concept is trying to diagnose hookworm disease *(Ancylostoma caninum)* in a 1-week-old puppy via the observation of eggs on fecal flotation. This attempted diagnosis is not helpful, because the minimum time from infection until adult hookworms are present in the bowel and begin to produce eggs (prepatent period) is 12 days. The astute veterinary practitioner uses fecal flotation results but also the puppy's history, clinical signs, and other labora tory tests (e.g., blood values) to arrive at a specific diagnosis of ancylostomiasis (infection with hookworms).

Classification of Parasites. Parasites of domestic animals are found in the kingdom Protista and the kingdom Animalia as well as in a large number of phyla in those kingdoms. There is some variation with regard to the classification schemes of different references, and organisms are often reclassified when new information about their biochemistry is obtained. In Appendix G, the box entitled "Kingdom: Animalia (Animals)" contains a summary of the taxonomic classifications of common parasites of domestic animals.

The majority of information in this unit is related to parasites of companion and farm animals. Lists of the major parasites of exotic species are located in Appendix E

Zoonoses. Zoonoses are diseases that can be transmitted between animals and humans. Veterinary technicians are responsible for educating clients about preventing infection with zoonotic parasites. Parasites of zoonotic significance include protozoans, trematodes, cestodes, nematodes, and arthropods. Commonly encountered zoonotic parasites are summarized in Appendix H entitled "Zoonotic Internal Parasites."

For additional sources for this unit see the Resources Appendix at the end of this textbook.



After studying this chapter, you will be able to:

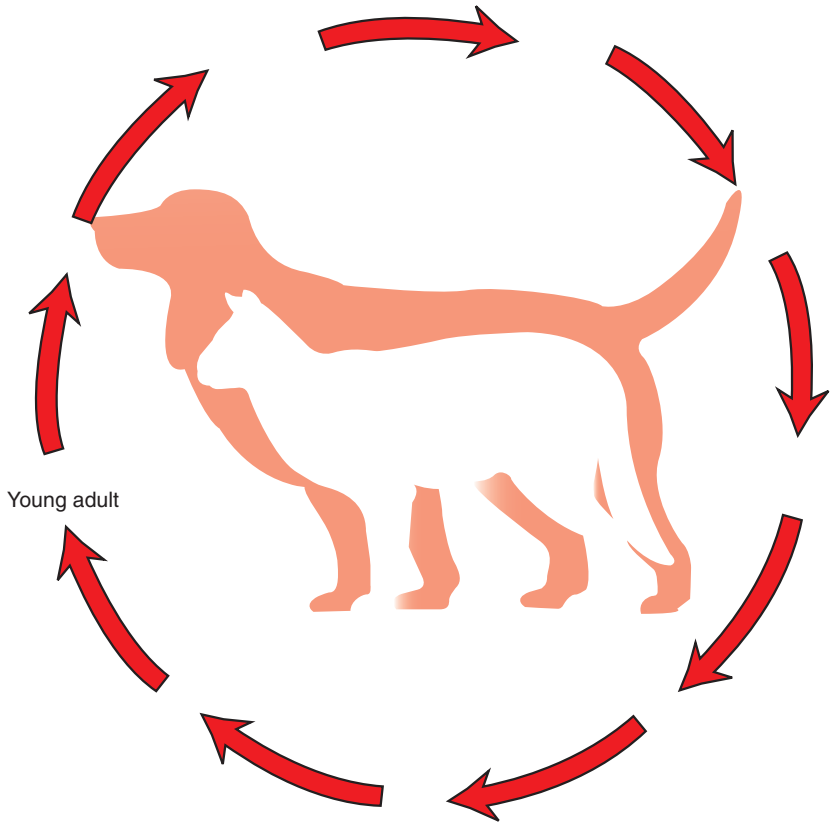
- Describe eneral haracteristics matodes.
- Describe eneralized cle matodes.
- Differentiate etween ect ect cles.
- List ommon ecies ect omestic
- List ommon ecies okworms, ungworms, whipworms ect omestic
- Discuss cle tworm.

Phylum Nematoda,
Ascaroidea scarids),
Strongyloidea,
Trichostrongyloidea,
Rhabditoidea *Strongyloides* p.),
Metastrongyloidea,
Trichuroidea *Trichuris* p., *Eucoleus* p., *Trichinella spiralis*
Oxyuroidea,
Spiruroidea,
Dracunculoidea,
Dioctophymoidea,
Filaroidea,
Key Points,

Ascarid
Cuticle
Definitive host
Direct life cycle
Endoparasite
Indirect life cycle
Intermediate host
Microfilaria
Nematode
Parthenogenetic
Prepatent period
Pseudocoelom

Organisms phylum Nematoda are commonly called round worms ecause lindrical ody **Nematodes** e multicellular. They possess body wall composed of external, acellular, protective layer called **cuticle** cellular layer beneath ticle led ypodermis; yer ngitudinal somatic muscles function locomotion. The digestive tract reproductive organs of roundworms are tubular, they are suspended body cavity **pseudocoelom** The digestive tract is traight ube hat uns he ength he ody rom he outh to matodes ve parate female organisms. The reproductive organs are tubular, but y e ypically nger ody oil intestinal ract orm. ematodes ve rvous stem xcretory stem ut espiratory stem.

The e cle matodes ollows ern consists f veral evelopmental gg, val stages are wormlike appearance, sexually mature adults. he ective gg ontains va, ee-living va, va **intermediate host** transport cle onsidered **direct life cycle** intermediate cessary or evelopment ective stage. If intermediate required for development to



infective cle onsidered **indirect life cycle** ransmission efinitive harbors xually ure dults) ccur ough estion, penetration of infective larvae, ingestion of intermediate r eposition ective vae nto ermediate

After nematode gains entry into new development to dult ccur cation, y ccur er xtensive ration ough ody definitive The diagnostic stages of parasitic nematodes are typically found feces, blood, sputum, or urine. Most parasitic nematodes estinal racts espective definitive but some are found lungs, kidney, urinary bladder, or heart. Table summarizes nematode parasites of eterinary ecies.

The ollowing onomic uperfamilies matodes significant eterinary

1. Ascaroidea
2. Strongyloidea
3. Trichostrongyloidea
4. Rhabditoidea
5. Metastrongyloidea
6. Trichuroidea
7. Oxyuroidea
8. Spiruroidea
9. Dracunculoidea
10. Dioctophymoidea
11. Filarioidea

Toxocara canis, Toxocara cati, Toxascaris leonina are **rids** of dogs These roundworms are found small intestine f ogs orld. ll puppies ens resented eterinary examined or obust matodes dult ascarids y ary om hen they e ually ightly oiled ggs *Toxocara* species are spherical, with deeply pigmented center rough, pitted ter ll ggs *T. canis* e o ter, hereas *T. cati* e ler nly o ter ggs *T. leonina* are herical void, nsions y m. These ggs ve ter ll yaline ground-glass" entral ortion. ws haracteristic vum *T. leonina.* he **prepatent period** *T. canis* o ys, whereas *T. leonina* ys.

Toxocara canis, Toxocara cati, and *Toxascaris leonina*

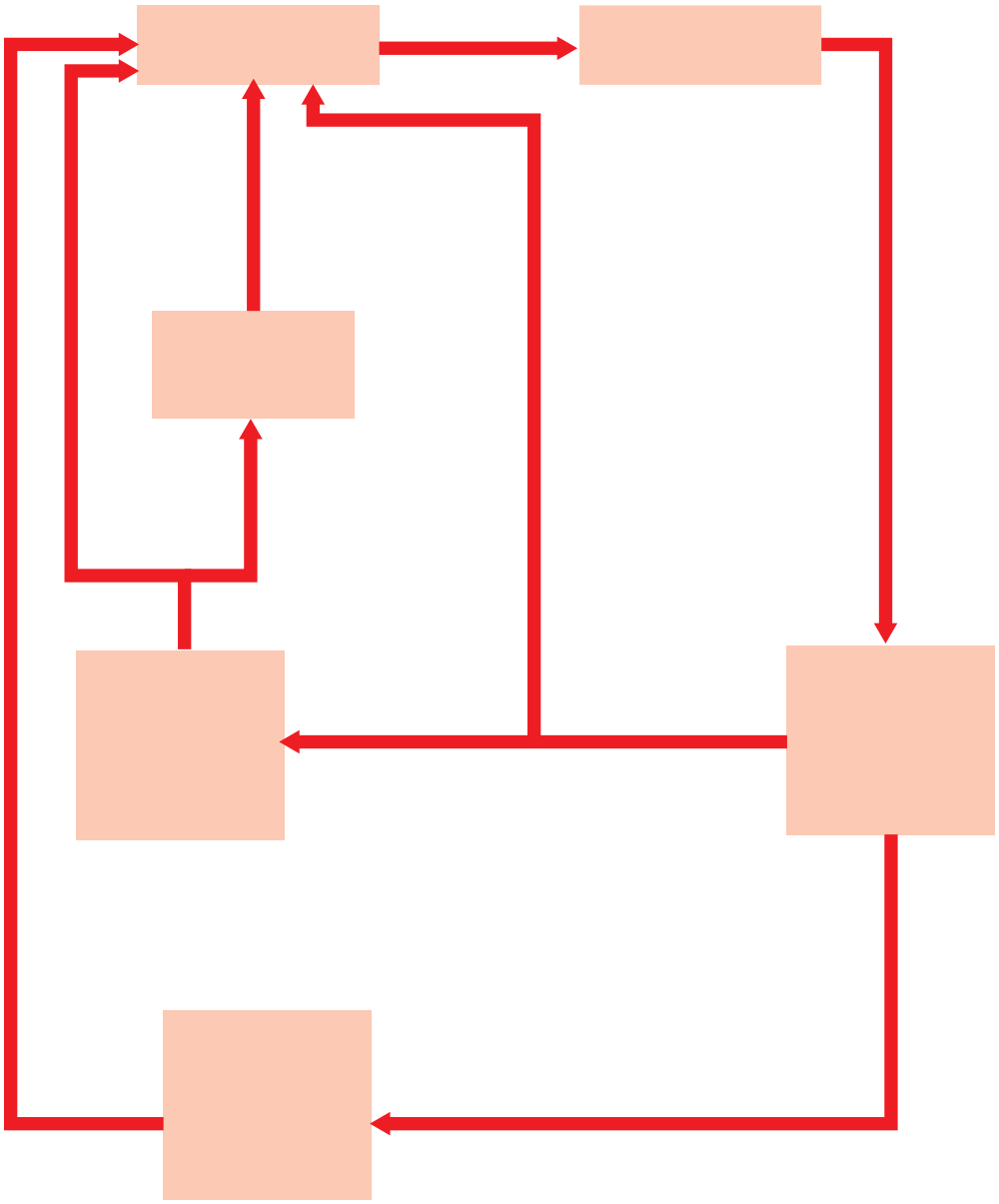
Selected Nematodes of Veterinary Species

Common Name

Continued

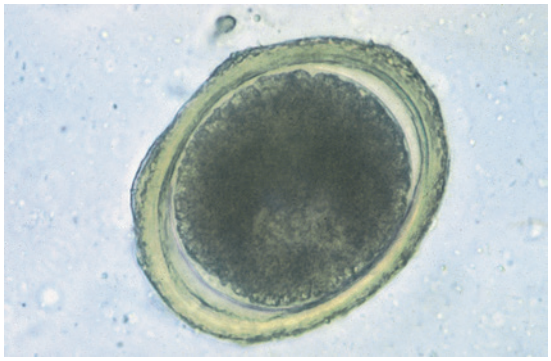
Selected Nematodes of Veterinary Species—cont'd

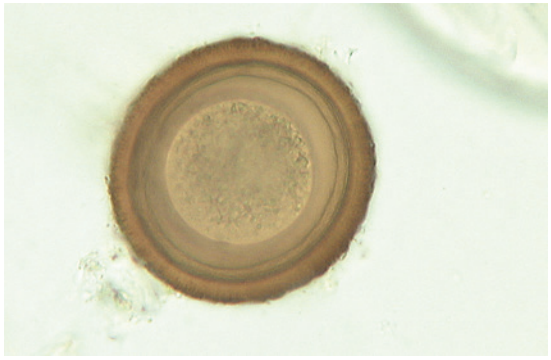
Common Name



Parascaris equorum ften led equine "equine roundworm." It found small intestine of horses, particularly oals. repatent eriod ys. Eggs recovered from feces of young horses are oval brown. The ll kened, ly ranular urface. ggs measure o ter. enter gg contains ne ells ggs ecovered easily ith ecal ion.

Toxocara (Neoascaris) vitulorum transmitted via trans mammary oute ves. orm, ggs ve k, ed ll. *Ascaris suum,* ine intestinal roundworm, largest nematode found within small ntestine igs. he ggs ay ecovered ith tandard fecal ion. hey val olden rown, albuminous ll ears rominent rojections. ggs measure o y o .





the equine ascarid

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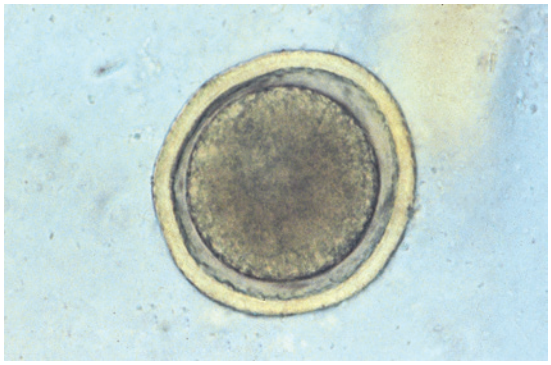
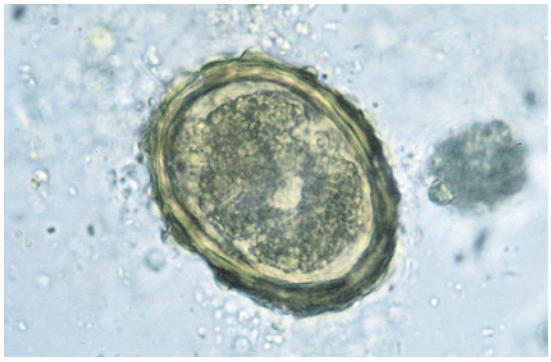




than those of *Toxocara canis,* measuring only 65 m to 75 m in diameter. (From Hendrix CM, Robinson E: *Diagnostic parasitology for veterinary*

m. (From Hendrix CM,

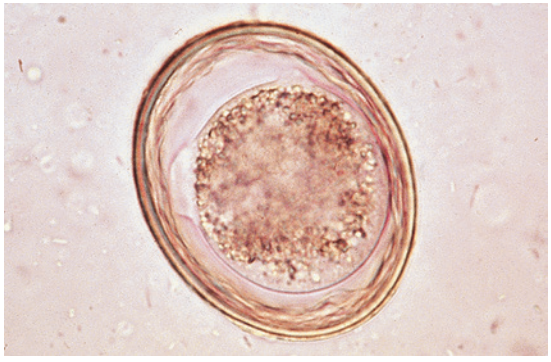
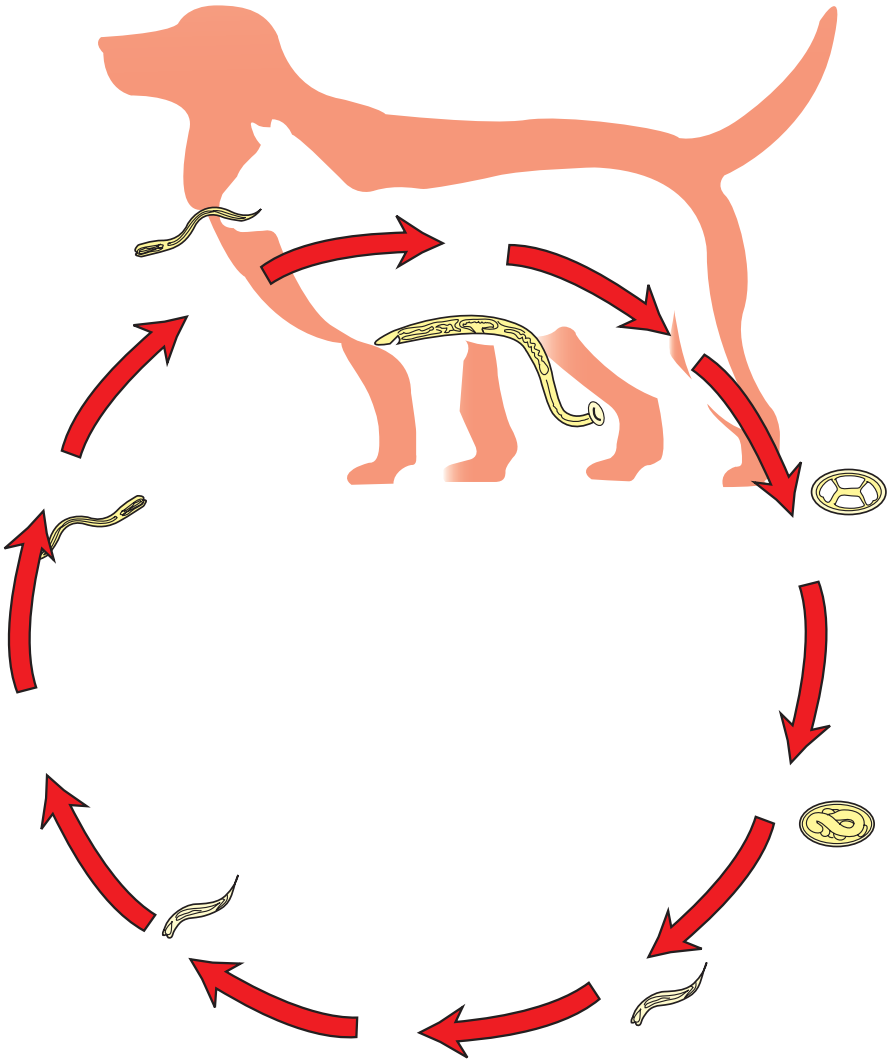


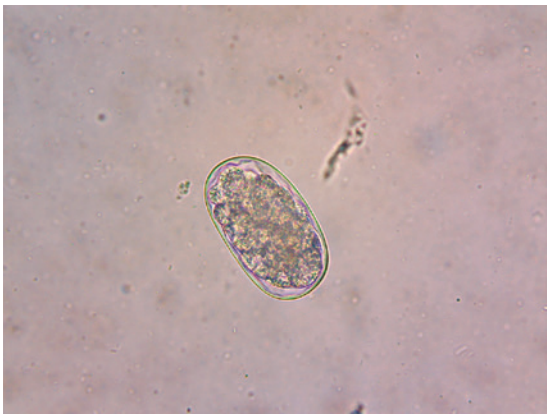
Fig. 45.6 Characteristic ovum of *Toxascaris leonina.* These eggs are

(From Hendrix CM, Robinson E: *Diagnostic parasitology for veterinary*

Ancylostoma caninum, okworm; *Ancylostoma tubae forme,* feline hookworm; *Ancylostoma braziliense,* feline hookworm; *Uncinaria stenocephala,* northern hookworm, are mall intestinal ematodes. Hookworms re found throughout world, they are common tropical subtropical reas orth merica. ookworm nfection, hich produce vere young kittens and puppies, can be erious roblem ennels nd atteries. he repatent eriod depends n ecies okworm oute ec tion Fig. *Bunostomum phlebotomum* hookworm of ruminants, roduces richostrongyle-type ggs.

The ggs f okworm ecies val llipsoid. hey have ontain ells hen passed eces. ecause ggs vate apidly the external environment (i.e., early hours after feces are d), eces eded or worm ections. ggs *A. caninum* e o by o . *A. tubaeforme* e to by to Those of *A. braziliense* are y *U. stenocephala* e to y o hese ggs ually ecovered with ecal ion.





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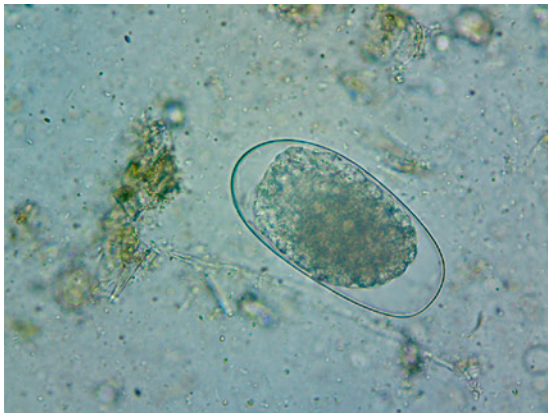
Strongyles matodes asitize estine of horses. They are typically divided into two types: large stron gyles small strongyles. The small strongyles comprise several genera ary egard genicity. strongyles luded *Strongyloidea,* y are genic rongyles. *Strongylus vulgaris, Strongylus edentatus, Strongylus equinus* are large stron gyles .

Regardless of whether **endoparasites** are small strongyles or large strongyles, their eggs are virtually identical. Identification to ecies vel ccomplished ecal ure identification of larvae. Strongyle eggs are often observed standard fecal flotation. They contain to 16-cell morula, y ure proximately o y to hen haracteristic ggs ecal flotation, bservation ecorded strongyle-type va" rather ticular ecies rongyle.

Oesophagostomum dentatum, he nodular orm wine," found large intestine of swine. The prepatent period days. he ggs richostrongyle ype; ords,

are val, lled ggs. hey ontain ells, measure y hese ggs ecovered standard fecal flotation. with bovine trichostrongyles, definitive diagnosis made only by fecal culture larval identification.

The ovine richostrongyles omposed veral enera of matodes omasum intestines f nera roduce trichostrongyle-type ggs re *Bunostomum, Cooperia, Chabertia, Haemonchus, Oesophagostomum, Ostertagia, Trichostrongylus*

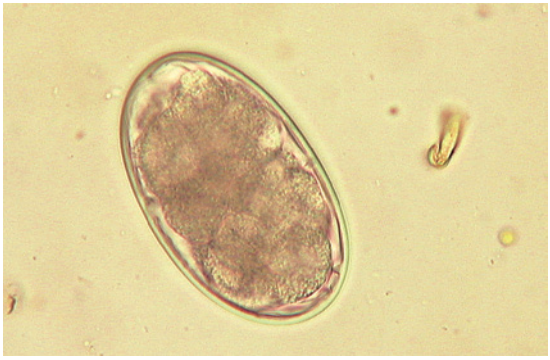


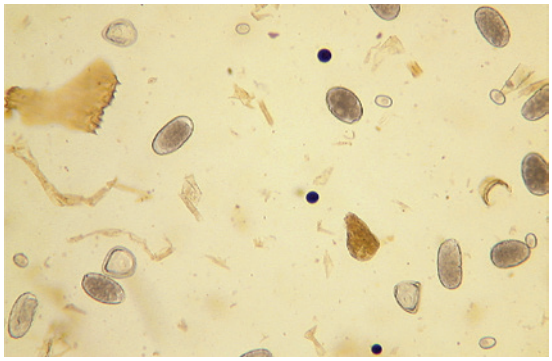
These seven genera others) produce oval, thin-shelled eggs. They ontain re ells, o long. ome va entified espective genera; however, identification usually because mixed infections f ovine richostrongyles uite ommon.

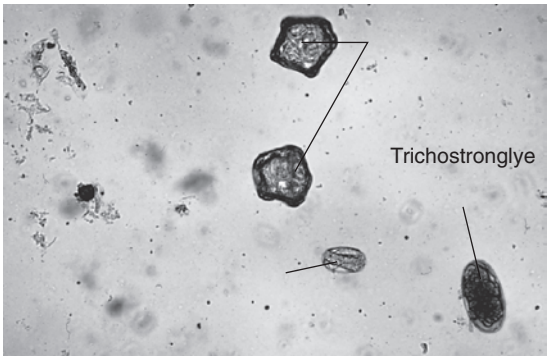
Upon entification haracteristic ggs, ecord richostrongyle-type gg never be recorded by individual genus The identification of enus ecies ually erformed nly ecal culture val entification.

Nematodirus species *Marshallagia* species are bovine trichostrongyles; however, their eggs are much larger of enera ntioned reviously. heir ggs richostrongyle y. ws ggs *Nematodirus* ecies. n ecal ion, ggs *Nematodirus* ecies e o y o they have tapering ends four to eight cells. The eggs of *Marshallagia* species are large to by o hey ve allel ounded y ontain ells.

Dictyocaulus ecies e ungworms *(Dictyocaulus viviparus),* ep, oats *(Dictyocaulus filaria).* dults e found bronchi of *Dictyocaulus arnfieldi,* equine ungworm, ronchi ronchioles horses, mules, donkeys. The prepatent period varies with







Characteristic trichostrongyle-type ova of the bovine trichostrongyles. These oval, thin-shelled eggs contain four or more cells. They measure 70 m long. Some of these ova can be identified by their

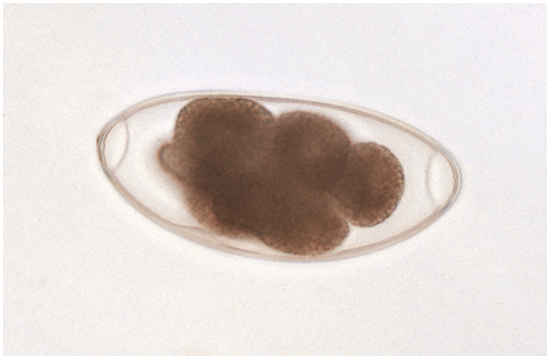
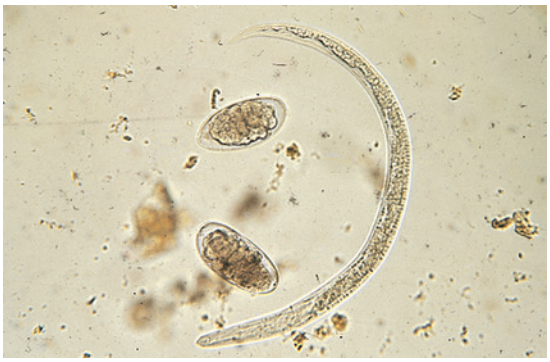


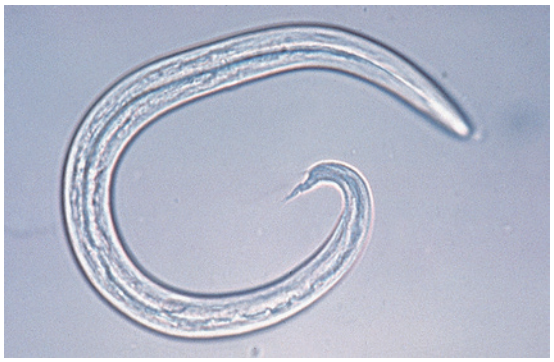
Fig. 45.13 Characteristic large ova of the species. (From

Diagnostic parasitology

for veterinary technicians,



species (cattle



the "hair lungworm"

species, but approximately days. The prepatent period for equine lungworm to days. Eggs are usually coughed swallowed. They hatch intestine, where they produce larvae ecovered eces.

Larvae f *D. filaria* ve rownish ood ranules intestinal ells, lunt ticular b. hey are 550 o ng. The larvae of *D. viviparus* also have brownish ood ranules estinal ells, ut have raight ck ticular b. larvae e o ngth

Hyostrongylus rubidus referred to red stomach worm" of ine. he ggs richostrongyle ype val, thin-shelled ggs). hey ontain re ells, measure o y o hese ggs be recovered with fecal flotation. with bovine trichostrongyles, definitive diagnosis be made only with fecal culture larval identification. repatent eriod proximately ys.

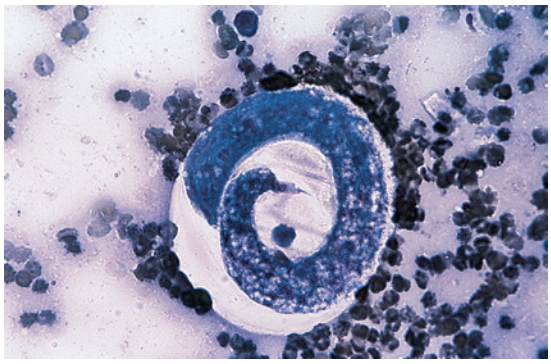
Ollulanus tricuspis feline trichostrongyle." This parasite usually associated with vomiting It commonly identified y xamination 's omitus secting r ompound oscope.

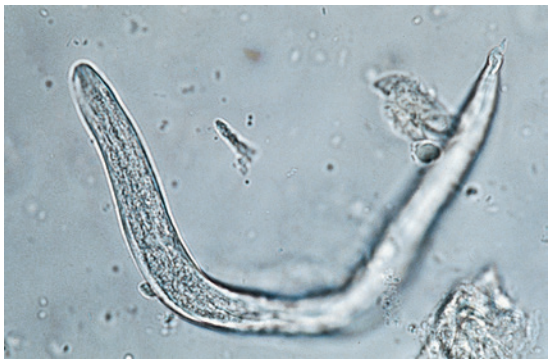
Strongyloides stercoralis, Strongyloides tumefaciens, Strongy loides papillosus are often referred to estinal threadworms." These nematodes are unique; only **parthenogenetic** female emale ggs ithout opulation

parasitic arasitic emales produce eggs, and, dogs, these eggs hatch the intestine and release st-stage vae. ws asitic dult females, ggs, nd rst-stage arvae *Strongyloides* pecies. he larvae e o ng. hey ve habditiform (club-shaped) phagus lub-shaped orpus, narrow median isthmus, bulb. The prepatent period o ys. *Strongyloides westeri* ften eferred "intestinal eadworm" rses. emales roduce vated ggs ure o y o m. The eggs are usually recovered with fecal flotation of fresh feces. The repatent eriod ys. *Strongyloides ransomi,* intestinal threadworm of pigs, found within small intestine of pigs. These females produce larvated eggs measure to y o ggs e ually ecovered fecal flotation of fresh feces. The prepatent period to days.

Muellerius capillaris ften led ungworm." dults are ound ithin ronchioles, ommonly dules ung enchyma ep oats. ggs evelop ungs **definitive host** st-stage vae coughed , lowed, eces. hey o ng. he val ulating orsal

Adult *Protostrongylus* ecies ccur ronchioles of ep oats. gain, ggs evelop ungs





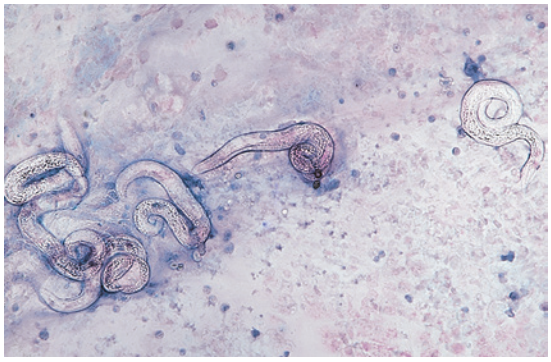
the feline lungworm. (From Hendrix CM, Robinson E: *parasitology for veterinary technicians,*

definitive st-stage vae oughed lowed, eces. vae to ng. his matode's val ulating tip, ut cks orsal aermann echnique to nose ungworm ection

Metastrongylus apri, ine ungworm, ronchi ronchioles val, k-walled eggs ure y ontain vae. ggs recovered ith ecal ion ion dium specific ravity re ecal dimenta tion echnique. repatent eriod proximately ys.

Filaroides (Oslerus) osleri, Filaroides hirthi, Filaroides lungworms," rachea, lung enchyma, ronchioles espectively. The larva to long, short, S-shaped *Filaroides* ecies e matodes their st-stage vae diately ective or definitive No period of development required outside of iagnosis volves haracteristic vae with ecal ion aermann echnique. shows unique infective larvae of *F. osleri.* Nodules of *F. osleri* e ually ifurcation rachea, here they be observed by endoscopic examination. The prepatent period *F. osleri* proximately eeks.

Aelurostrongylus abstrusus feline lungworm. The adults live n he erminal espiratory ronchioles nd he lveolar ucts, where y orm gg dules. ggs parasite are forced into lung tissue, where they hatch to form characteristic st-stage vae proximately long. ch va ed end orsal . haracteristic vae ecal tion r aermann echnique etermine resence. Recovering larvae with tracheal possible Fig. he repatent eriod proximately ys.



recovered with tracheal washing. (From Hendrix CM, Robinson E: *Diagnos*

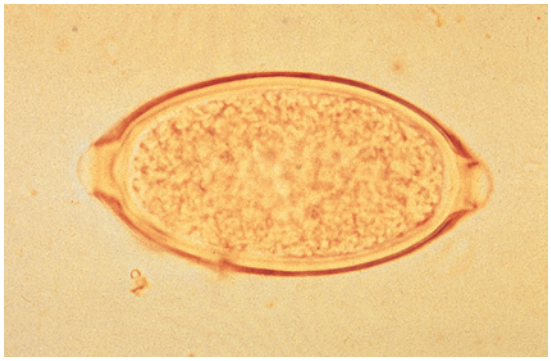


Fig. 45.20 Characteristic ovum of *Trichuris vulpis.* (From Hendrix CM,

Trichuris vulpis, hipworm, *Trichuris campan* *Trichuris serrata,* eline hipworms, eside cecum olon espective hipworms are common, but feline whipworms are rare North merica diagnosed only sporadically throughout world. hipworms derive ir om ct dults ve filamentous nd gg worm escribed richinelloid richuroid. yellow-brown, mmetric ll ugs The ggs e mbryonated vated) hen ggs *T. vulpis* e o y o . shows haracteristic gg *T. vulpis.* he repatent eriod *T. vulpis* o ys.

The ggs f *T. campanula* *T. serrata* y e on fused with of *Aonchotheca putorii, Eucoleus aerophilus, Personema feliscati,* which are parasites of feline stomach,

respiratory ract, nd rinary ystem, espectively. he ggs *T. campanula* verage o y o hen examining cat's feces for feline trichurids, veterinary techni ware udoparasites; ggs richurids or apillarids requently arasitize utdoor at's rey ia osts such e, abbits, irds. ggs richurids capillarids ss naltered hrough at's astrointestinal system, emaining ntact nd nembryonated nd hus ppearing to ect eline

Trichuris ovis infects cecum colon of ruminants. Eggs of ovine hipworms ure o y o m. *Trichuris suis* ine hipworm. ggs orcine whipworms ure o y o he prepatent eriod ys.

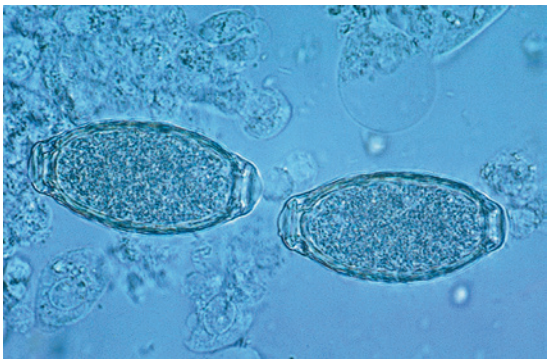
Aonchotheca putorii ommonly eferred astric capillarid of It once known by former *laria putorii.* This capillarid frequently parasitizes mustelids, such ut een eported matodes are arely eported orth merica. ggs *A. putorii* are y onfused richinelloid matodes section about feline whipworms). Their eggs are to by o y xhibit tlike urface to f ggs *Eucoleus aerophilus,* per espiratory capillarid. The eggs of *A. putorii* are dense delicate f *E. aerophilus,* y e rganized ngitudinal formation. They have flattened sides, they contain one- or two-cell mbryo gg.

Eucoleus aerophilus (Capillaria aerophila) capillarid nema tode ound rachea ronchi ogs The prepatent period approximately days. In standard fecal flotations, eggs of *Eucoleus* species e often confused with those of *Trichuris* whipworms). ggs *E. aerophilus* re maller han whipworm ggs o y o re broadly rel-shaped, hter olor. gg rough ter urface tted pearance. *Eucoleus böehmi* ound vity ontal ogs. ggs are ler ve other ter urface *E. aerophilus.* ts ll ed pearance. asite be etected ecal ion.

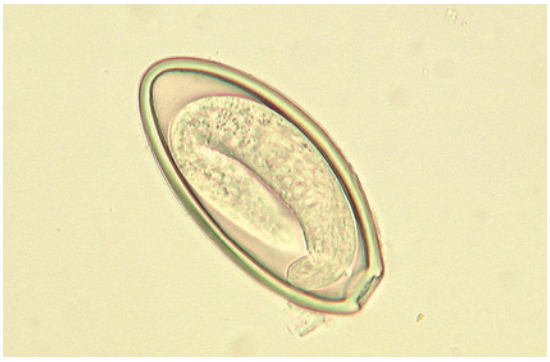
Pearsonema (Capillaria) plica *Pearsonema (Capillaria) feliscati* e matodes inary ladder ogs respectively. heir ggs eces on taminated with urine. The eggs are clear to yellow color, they measure o y o y ve flattened ipolar ugs heir ter urface roughened. These eggs may be confused with of respi ratory gastric capillarids with of whipworms.

Trichinella spiralis found many species of carnivores omnivores, ut ften ciated aw ercooked pork. nimals luding eings) ecome ected *T. spiralis* hen y ective val uveniles)

The larvae mature into adults host's small intestine within ew eeks, emale orms ive irth vae.



the urinary capillarid. (From Hendrix



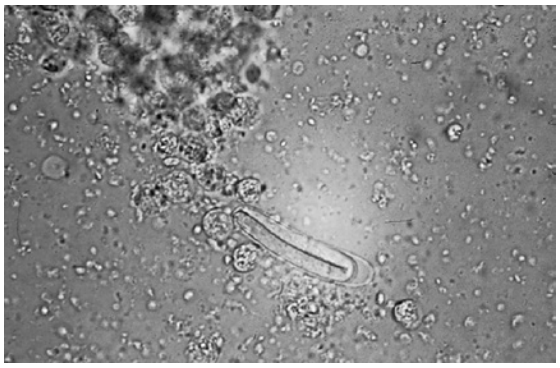
the pinworm of horses. (From Hendrix CM, Robinson E: *Diagnostic parasitology for veterinary*

The er ertilizing emales, emales after roducing vae. vae nter loodstream ventually 's usculature.

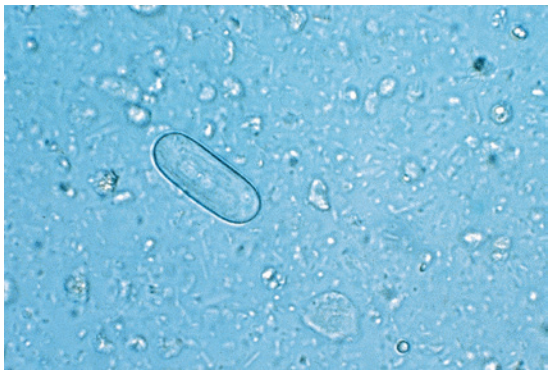
muscles, larvae mature into infective encysted larvae. The next ost ecomes nfected hen ats hese rvae. richinosis probably best known parasite human beings contract from ing aw ercooked ork. ually etected proper ection. ecent tbreaks richinosis nited ve een raced ork roducts om pigs ve een ected ve een rivately slaughtered.

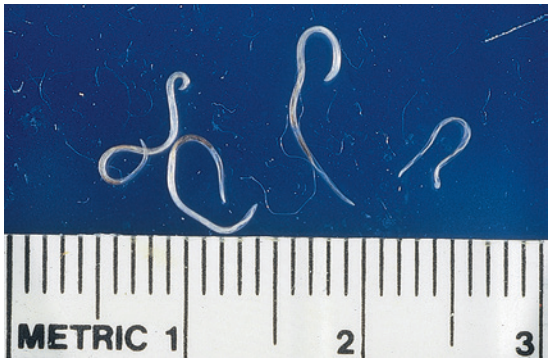
Most animals become infected with trichinosis after

Oxyuris equi worm rses. dult orms found ecum, olon, ectum. dult orms are ften bserved rotruding om rse's dult female orms ch ggs icky, elati material produces pruritus infected horses. The activity f emale orms ritating roduces ching. Eggs ecovered om eces. ggs by ith ll. hey erculated slightly ened vated he repatent eriod proximately



species





species (eyeworms) from the conjunctival sac

Diagnosis volves haracteristic ggs scopic examination of cellophane tape impressions or by scraping urface

Enterobius vermicularis uman worm, oes asitize ogs evertheless, et ften falsely iminated ractitioners ediatricians ce f worm ection hildren.

Habronema pecies nd *Draschia megastoma* re ematodes hat are found stomachs of horses. *Habronema microstoma H. muscae* ccur n omach ucosa, eneath layer f ucus; *D. megastoma* ften ciated thickened, rous dules omach ucosa. vae of both may parasitize lesions condition known summer res." repatent eriod proximately days. vated ggs vae ecovered fecal ion. ggs enera longated, walls, ure o y o Fig.

Thelazia californiensis eyeworm" of dogs dult parasites ecovered om onjunctival lachrymal uct. xamination chrymal cretions reveal ggs st-stage vae. *Thelazia rhodesii Thelazia* e eyeworms" ep, oats. shows dult *Thelazia* onjunctival ow.

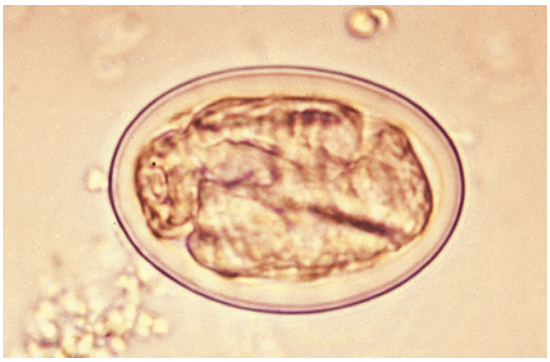
Thelazia lacrymalis eyeworm of horses throughout world. dult asites ecovered om onjunctival sac lachrymal duct. Examination of lachrymal secre tions y eveal ggs st-stage vae.

Spirocerca lupi, esophageal worm, nematode often forms nodules (granulomas) esophageal wall of dogs Occasionally may be found nodules stomach of dult orms eside eep dules xpel their ggs ough penings ranuloma. ggs are d umen phagus then eces. k-shelled ggs o y o y ontain va hen are aid. hese ggs ave nique aper-clip hape Fig. 5.25). Eggs e ually bserved ecal ion, recovered hen omitus een ubjected ecal flotation rocedure. adiographic ndoscopic xamination may eveal haracteristic ranulomas phagus within omach. repatent eriod

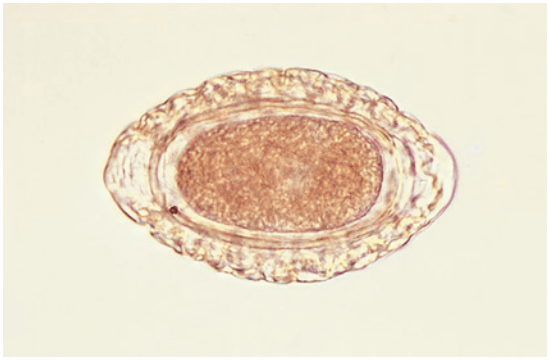
Physaloptera ecies e omach orms ogs Although they occasionally are found lumen of stomach or l estine, *Physaloptera* ecies e ually mly attached to mucosal surface of stomach, where they suck blood. t site, nematodes may be viewed with endo scope. heir onsists lood issue erived om host's astric ucosa. heir chment ontinue leed after asite etaches. omiting, rexia, ry stools y bserved ected

The dults eamy hite metimes ightly oiled, y e ng. hey ften ecovered et's omitus, onfused roundworms. uick erentiate asites to reak pen dult ecimen ecimen pens to e emale, eleased ggs oscopically. eggs *Physaloptera* ecies e k-shelled, mbryonated hen eces. ggs o y o y ontain va hen are Fig. shows characteristic ovum of *Physaloptera* species. ggs ually ecovered ecal tion utions ecific ravity re The repatent eriod ys.

Ascarops strongylina nd *Physocephalus sexalatus* re he thick stomach worms" of porcine stomach. Both of nematodes



species. (From Hendrix



Characteristic ovum of *Dioctophyma renale*
Diagnostic parasitology

produce k-walled, vated ggs ecovered fecal ion. ggs ecies ggs *A. strongylina* e o y y ve thick lls urrounded mbrane roduces irregular tline. ggs *P. sexalatus* e o by o he repatent eriod or ecies approximately ys.

Dracunculus ecies e ommon asites ogs, other arnivores. he ife ycle equires opepod ntermediate definitive becomes infected after ingestion of opepod.

Dioctophyma renale giant orm" ogs. largest f arasitic ematodes requently nfects he ight idney of dogs gradually ingests renal parenchyma, leaving only ule y. ggs ecovered entrifuga tion xamination diment. hey harac teristically rel-shaped, ipolar, ellow-brown. gg's shell ed pearance. ggs ure o by o . *D. renale* y ccur eely within peritoneal cavity. hen location, eggs are d o xternal nvironment. repatent eriod proximately eeks.

Stephanurus dentatus, ine orm, y, eters, erirenal issues heir ggs strongyle ype val lled), ontain cells, ure o y o m. Eggs y e ecovered om dimentation. The repatent eriod xtremely proximately nths.

Dirofilaria immitis, tworm, or asite stem omestic United States. This nematode parasitize ferrets. Adult heartworms are found within right ventricle, monary tery, ranches tery. site ften ecovered ariety errant uch brain, erior hamber ye, ubcutaneous The repatent eriod ogs proximately approximately to months The life cycle of *D. immitis* requires mosquito intermediate to be transmitted from o dults ve ight en tricle nary tery, here bstruct lood vasculature. emale dults emale produces **microfilariae** he ofilariae eleased host's loodstream, here ested eeding emale mosquitoes. ofilariae row uito until y each ective fter ecome ective, they nter uito eeds. hen they e vae rate ough various ody issues vae row ecome dults other

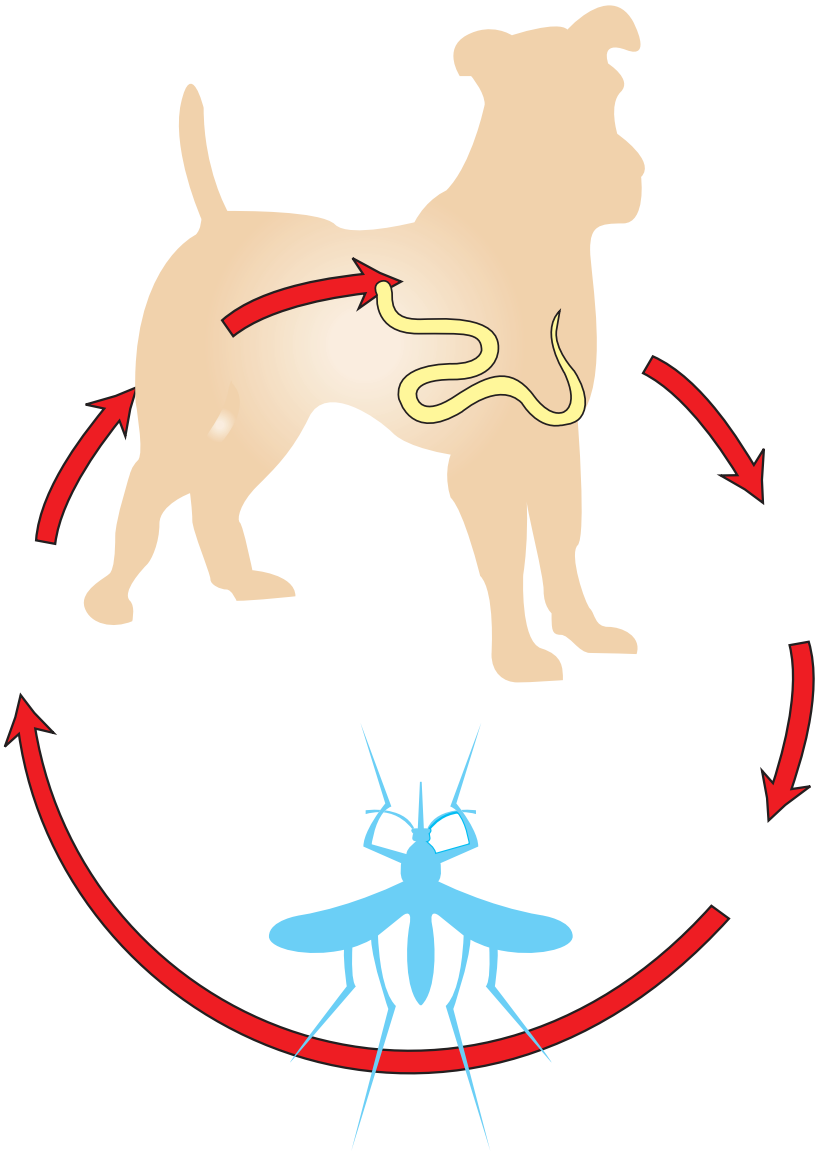
TECHNICIAN NOTE infection

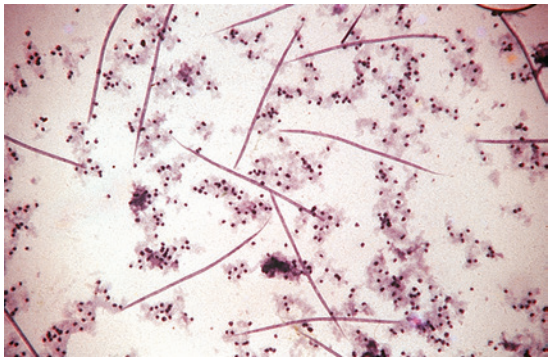
In microfilaremic dogs, diagnosis be made by observing microfilariae lood veral concentration echniques dified nott's commercially available filter techniques Figs. Microfilaremia commonly detected hen present, umbers ofilaria ersist or nly ew weeks. nfection *Dirofilaria* re ommonly nosed commercially available immunodiagnostic tests detect resence igen om dult emale orms.

subcutaneous filariid of dogs, *Acanthocheilonema (Dipetalonema) reconditum,* also roduces crofilariae the peripheral blood. he ofilariae npathogenic matode be erentiated om *D. immitis*

The ofilariae *Onchocerca cervicalis,* equine filarial parasite, have been incriminated recurrent ermatitis, eriodic phthalmia, rses. Adults ve amentum uchae, emales roduce microfilariae migrate to dermis. Biting of genus *Culicoides* e ermediate

Setaria cervi s he abdominal orm" ttle. *Setaria equina* ominal orm rses. dults ee eritoneal vity. ofilariae o





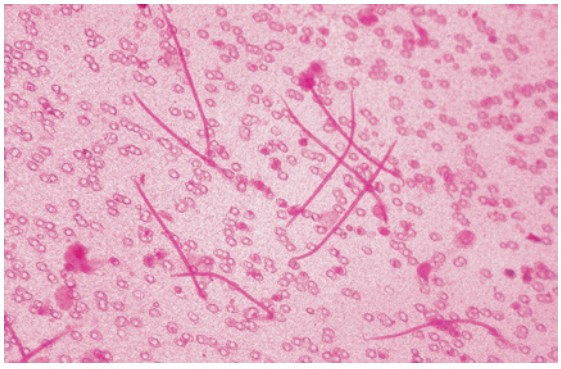


Fig. 45.30 Microfilaria of *Dirofilaria immitis* from a peripheral blood sample subjected to the modified Knott's test. (From Hendrix CM,

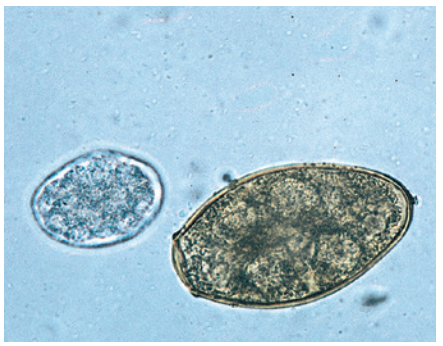
ng. iagnosis onfirmed emonstration microfilariae lood

Elaeophora schneideri, arterial orm," common otid teries ep estern western United States. Microfilariae are long thick, luntly ounded anially, ered ly. hey found ually laries orehead face. ial ermatitis en ce, oll egion, eet f ep.

Diagnosis involves observation of characteristic lesions identification of microfilariae The satisfactory of diagnosis to macerate piece of warm xamine erial or ofilariae er proximately s. n ep, ofilariae are, found n he kin nfected nimals. ostmortem xamination may be necessary to confirm diagnosis. The prepatent period eeks re.

Chapter eview uestions ppendix

- The life cycle of nematodes consists of several developmental stages: egg, four larval stages, sexually mature adults.
- Infective stages of nematodes may involve egg contains va, ee-living va, va ermediate r ransport
- A e cle onsidered ect ermediate cessary or evelopment ective
- Organisms ect cles equire ermediate or evelopment ective
- Transmission of nematode parasite to new definitive ccur ough estion, enetration ective larvae, estion ermediate eposition infective larvae into or onto by intermediate
- Nematode asites eterinary nificance mbers of onomic uperfamilies.
- Common nematode parasites of dogs include *Toxo cara* p., *Ancylostoma* p., *Trichuris* p., *D. immitis.*



After studying this chapter, you will be able to:

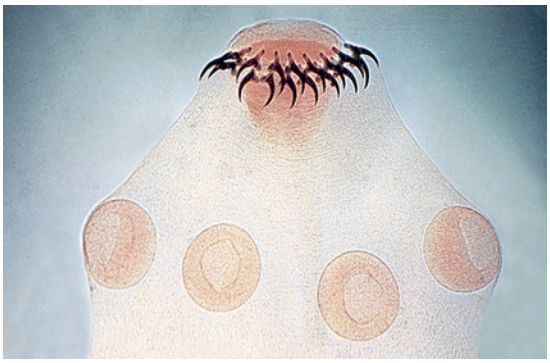
- Differentiate etween rue eworms pseudotapeworms.
- Describe eneral haracteristics ucestodes.
- Describe eneral haracteristics udotapeworms.
- Describe cle *Dipylidium caninum.*
- Describe pearance ggs ommon eworm species.
- Describe oonotic otential estodes.
- Describe eneral haracteristics rematodes.
- Discuss cle *Fasciola hepatica.*

Bothria
Cercaria
Cestode
Coracidium
Hexacanth
Metacercaria
Miracidium
Proglottid
Redia
Rostellum
Scolex
Sporocyst
Strobila
Trematode

Phylum yhelminthes ludes **trematodes** **ces todes** These are flatworms lack body cavity. The taxonomic class includes cestodes contains two subclasses. Members of ubclass ucestoda eferred rue eworms, whereas mbers ubclass otyloda eferred pseudotapeworms. The phylum canthocephala includes thorny-headed orms. ommonly ncountered parasites ompanion ractice.

The life cycle of tapeworms always indirect, involves one r o ermediate ermediate arthropods, omestic tive r ermediate or eworms, The larval stages of some tapeworms are found domestic e led ladderworms, ecause esemble filled cks ultiple oleces. hen ested definitive bladderworms are released from tissue of intermediate develop into adult tapeworms within estive ract efinitive ome estodes ve larval orms odies rocercoid, rocercoid, etrathyridium). omestic ecome ected val eworms estion estode egg ocercoid.

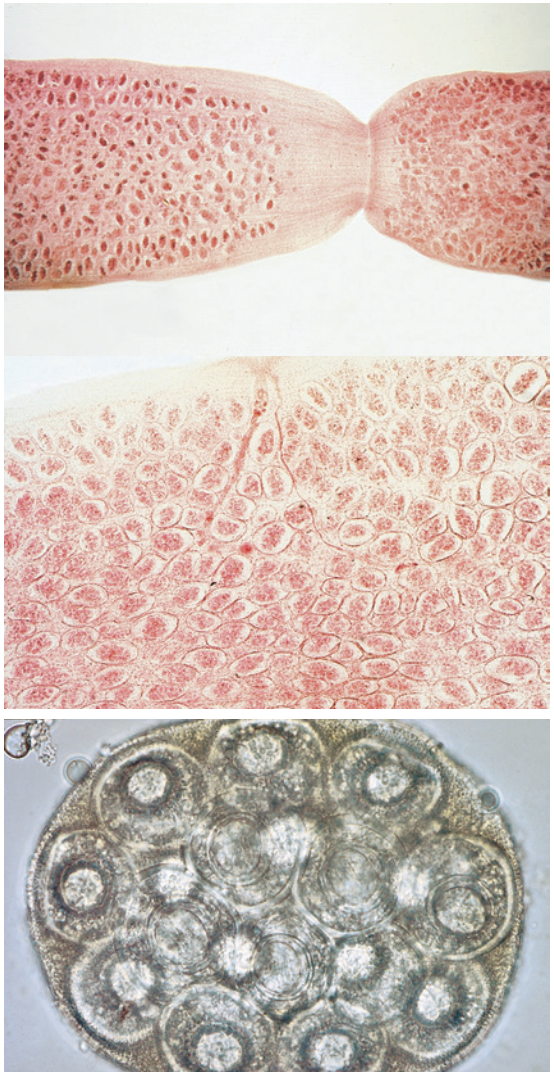
The true tapeworms are multicellular organisms lack body cavity. Their organs are embedded loose cellular tissue (paren chyma). he ody eworm orsoventrally flattened, onsists ee egions. **(scolex)** dified chment rgan ears muscular uckers cetabula. uckers med hooks. There may be snout **(rostellum)** on head, which e ed etractable. ostellum med with oks rt ck



Details of the scolex of the canine taeniid. Note the four suckers

parasitology for veterinary technicians,

Dipylidium
on canine feces. In the fresh state, these proglottids resemble



Each egg packet may contain up to 30 hexacanth embryos.

undifferentiated issue, nd his ollowed he ody **(strobila)** The ody omposed gments **(proglottids)** erent stages of maturity. Those ck are immature, these are ollowed xually ure roglottids ravid segments ontain ggs. ravid roglottids reak t f ody efinitive eces ew roglottids ontinually ormed om ferentiated tissue of neck. Cestodes lack digestive tract, nutrients are absorbed directly through body wall. The prominent organs cestodes are organs of reproductive system. Both female reproductive organs occur each individual roglottid eworm. ross-fertilization self-fertilization ce. estodes ve rvous system xcretory stem.

Tapeworms are dorsoventrally flattened and contain

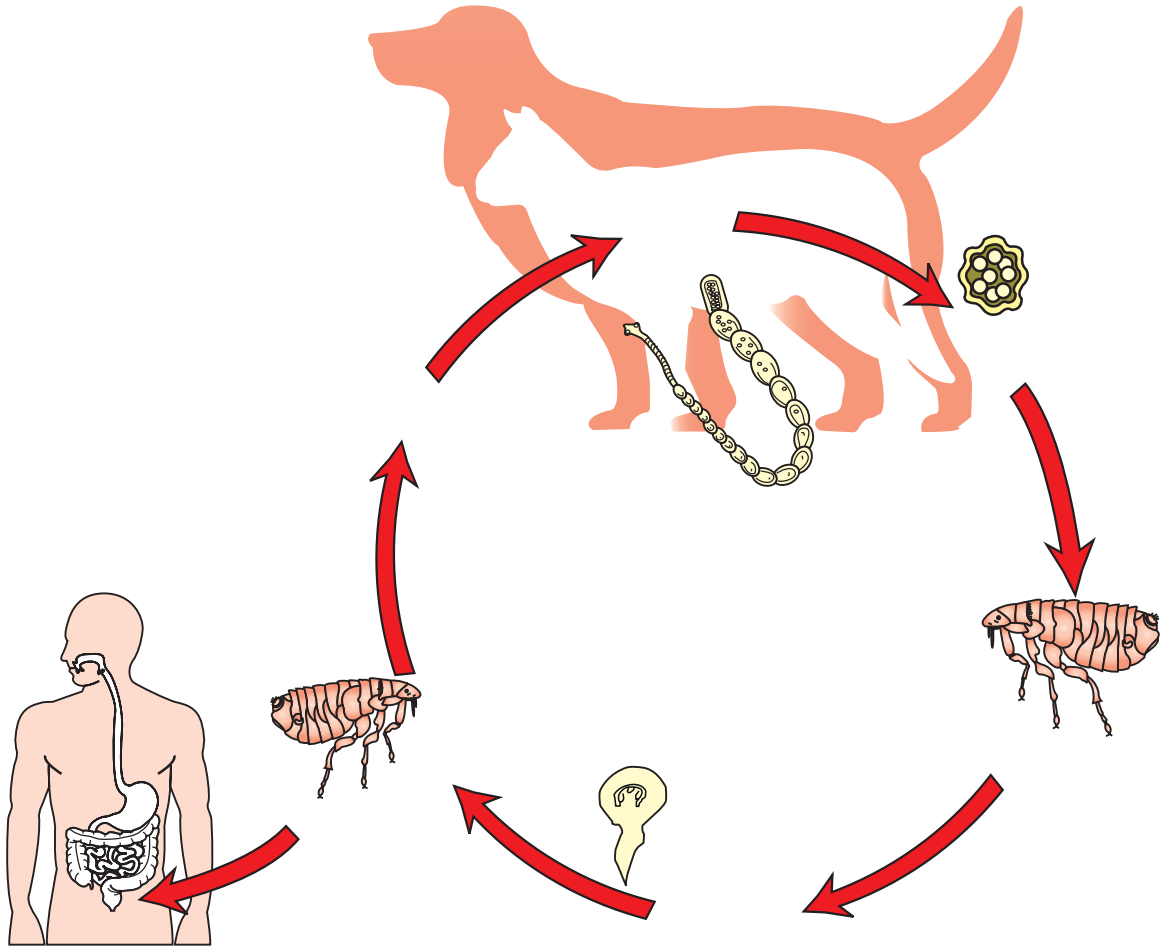
The cestode egg contains fully developed embryo, which hooks three pairs embryo or oncosphere) Fig. n ravid roglottids ct eces, ither ly upture release eggs. The eggs must then be ingested by interme diate here evelop tacestode metacestode (larval) stage may be form of cysticercus, coenurus cyst, hydatid cyst, or tetrathyridium. The definitive becomes infected after ingestion of intermediate contains metacestode stage. The juvenile tapeworm then emerges from metacestode stage, attaches to of small estine, egins roduce robili.

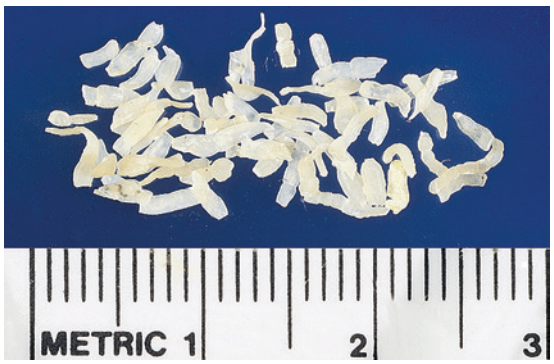
The udotapeworms ructure rue tapeworms except for fact their reproductive organs genital ores entrally cated ather erally cated. The rgans chment rgans wn **bothria** which are located on lateral aspect of scolex. The eggs f udotapeworms perculated, ually released rom he terus nd assed he eces. he gg ontains mbryo eferred **coracidium** hich eleased hen egg makes contact with water. The coracidium then ingested by microscopic aquatic crustacean, then develops into stage led rocercoid. ustacean ventually ested by r hibian, evelops tacestode stage rocercoid anum) usculature of The definitive becomes infected after ingesting cond ermediate

Common Name

Table ummarizes estode asites eterinary species.

Dipylidium caninum ommon eworm l estine og og ecomes infected y esting ected hich ermedi ate ften ontain asite's ective sticercoid stage . eworm erminal, ravid proglottids, hich ually eces, et's coat, or bedding of If fresh proglottids of *D. caninum* are teased or roken open, they may reveal usands of unique gg ckets, ch hich ontains xacanth embryos e roglottids *D. caninum* ten dry out external environment. they moisture, they shrivel esemble ooked rains ice reconstituted er, ied roglottids ually their ormer er-seed pearance. repatent eriod *D. caninum* o ys.





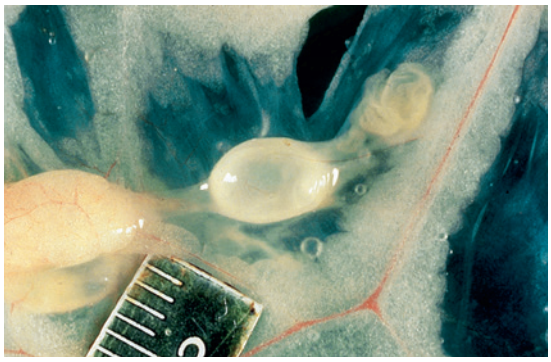
(From Hendrix CM, Robinson E: *Diagnostic parasitology for veterinary*

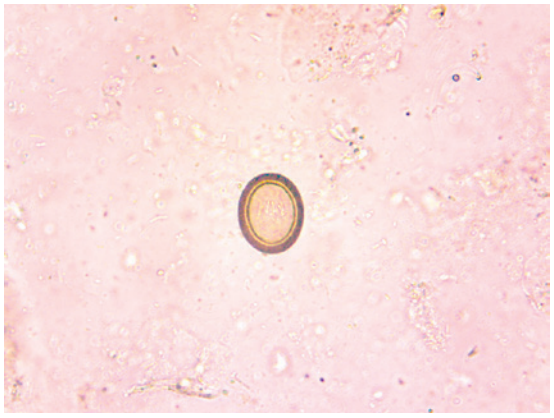
Dipylidium caninum is the most common tapeworm

Taenia pisiformis, Taenia hydatigena, Taenia ovis e eniids. *D. caninum, Taenia* eworms pear ile, erminal, ravid roglottids eces, et's oat, r edding proglottids have single lateral pore located along midpoint of ither f dges pposed ouble-pore tapeworm). ogs ecome ected esting sticercus-infected ermediate ermediate *Taenia pisiformis* e abbits *Taenia hydatigena* *Taenia ovis* volve uminant ermediate

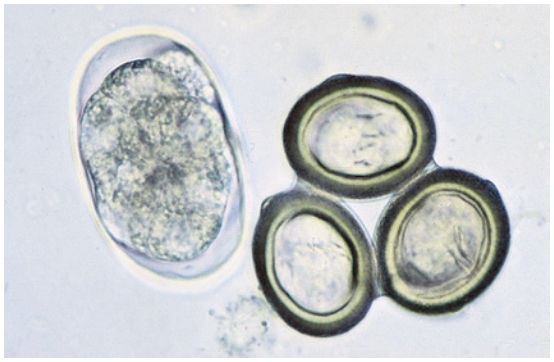
As ith *D. caninum,* esh roglottids eased broken open, they may reveal of hexacanth embryos. The roglottids *Taenia* ecies xternal environment esemble ooked rains ice. econsti tuted with water, they too usually assume their former single-pore appearance. If gravid proglottids of *Taenia* species are recovered from dog's or cat's feces, proglottid be torn open or macerated op ution eveal haracteristic ggs er ompound oscope.

The eggs of taeniid tapeworms are slightly oval. They are to by to diameter *(T. pisiformis),* to y o ter *(T. hydatigena),* o y o *(T. ovis).* Eggs of *Taenia* species contain single oncosphere with three pairs of hooks. The oncosphere hexacanth embryo. Fig. shows unique





is usually attached to the greater omentum or other abdominal organs of (From Hendrix



(From Hendrix CM, Robinson E: *Diagnostic parasitology for veterinary*

features f eniid eworm. ggs f *Echinococcus* *Multiceps* ecies. *Taenia taeniaeformis* *Hydatigera taeniaeformis* led feline eworm" feline eniid." eworm bserved equently lowed oam prey n gg eworm o ter, ontains nco sphere with three pairs of hooks. The oncosphere often called hexacanth embryo. with eggs of taeniids, eggs e *Echinococcus* ecies.

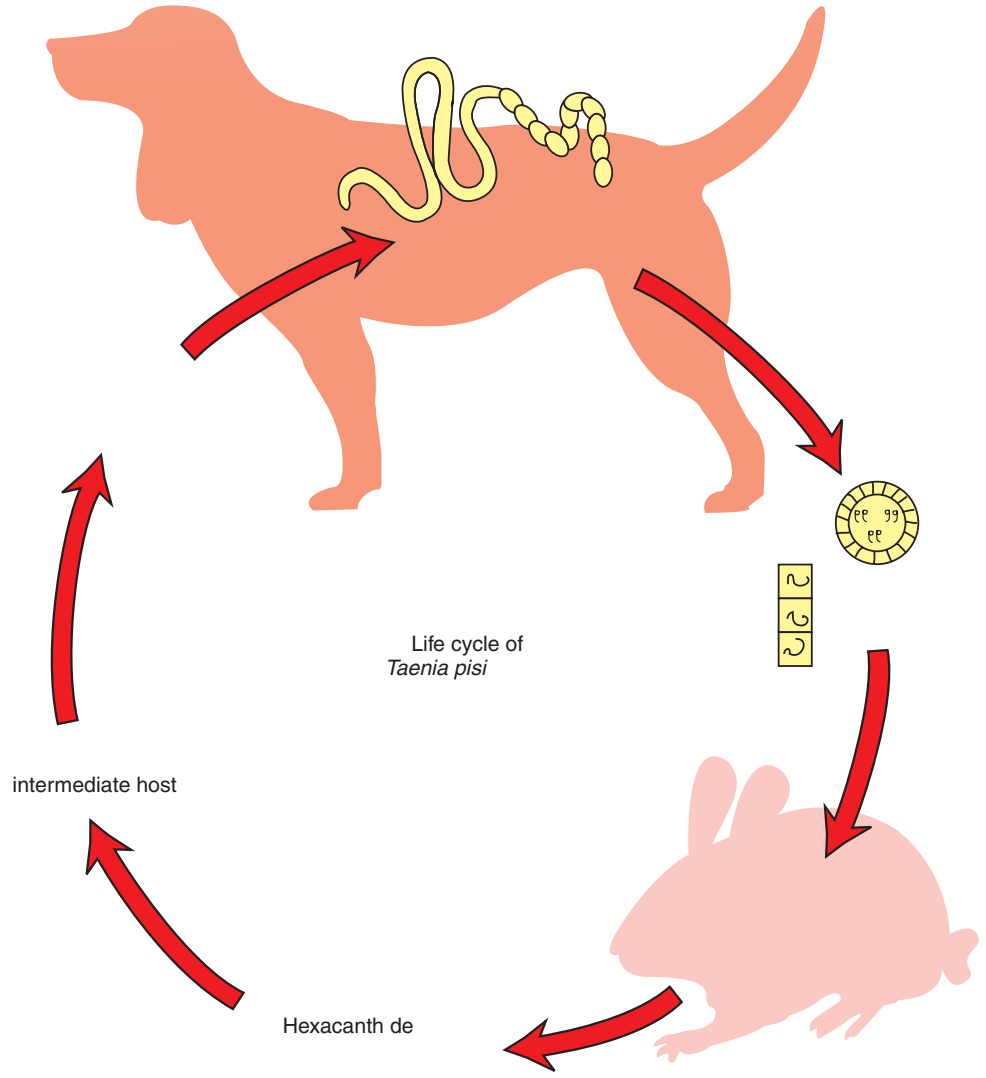
Multiceps multiceps *Multiceps serialis* e eworms of l estine ggs *M. multiceps* e o ter, hereas *M. serialis* e elliptic ure o y o oth contain ncosphere ee ggs f eline eniids, ggs *Multiceps* species e *Echinococcus* ecies.

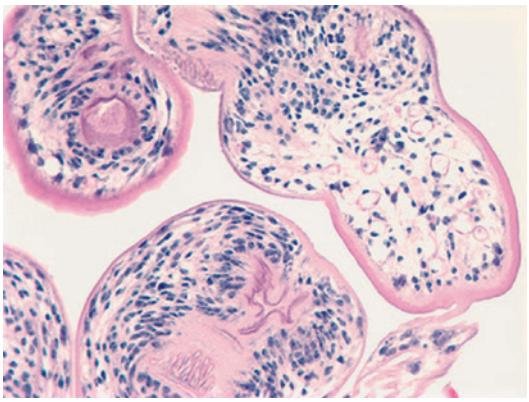
Echinococcus granulosus *Echinococcus multilocularis* e tapeworms ciated ultilocu ydatid *E. granulosus* ydatid eworm of ogs, hereas *E. multilocularis* ydatid eworm of hese ortant asites ecause xtreme zoonotic otential gg *E. granulosus* void ures o y o t ontains le ncosphere ee gg *E. multilocularis* ovoid measures to It contains le ncosphere ee ggs pearance *Taenia* *Multiceps* ecies.

species

The adult *Echinococcus* tiny tapeworm only to length. The entire tapeworm only three proglot tids: ne ure roglottid, ure roglottid, gravid proglottid Fig. hen passed, tiny gravid pro glottids are small they are often overlooked by client, eterinary echnician, eterinarian. efinitive diagnosis f *Echinococcus* ection chieved entify dult eworms en om 's estinal ract. are hich *Echinococcus* ection uspected, antemortem diagnosis accomplished by purging dog or with arecoline hydrobromide per collecting eces. his rocedure ually erformed nly hen infection rongly uspected. ire orms roglot tids y e ollected om ecause vere oonotic otential, vacuated erial handled ith tion. ubber loves orn. fter feces ve een xamined, rated.

Anoplocephala perfoliata, Anoplocephala magna, Paranop locephala mamillana e quine eworms. *A. perfoliata* found small large intestines cecum. *A. magna* ound estine ccasionally omach. *P. mamillana* ound estine ccasionally omach. ggs *A. perfoliata* ve k one r re ened ure o in iameter. he ggs *A. magna* re imilar ut lightly maller, measuring o he ggs *P. mamillana* e val ve ure o ggs f three ecies ve ee-layer ggshell. rmost led yriform paratus, hich ear-shaped. ggs of l quine eworms ecovered ecal





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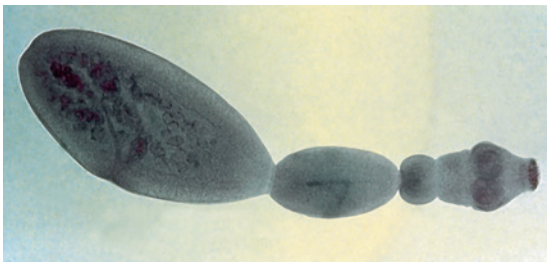
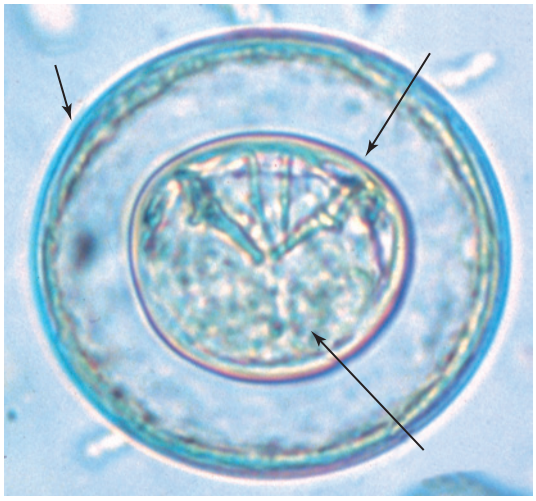


species is a tiny tapeworm that is only
(From Hendrix CM, Robinson E:
parasitology for veterinary technicians,

flotation. he repatent eriod ee ecies anges om o ys.

Moniezia ecies e eworms intestines f ep, oats. eworms roduce eggs ith haracteristic oidal yramidal hen viewed ith ompound oscope, ggs pear uare or riangular tte. wo ecies ommon: *Moniezia benedeni* *Moniezia expansa* ep, goats. he ggs ecies erentiated standard ecal flotation rocedures. g. 46.11 ows representa tive eggs of *Moniezia* species. The eggs of *M. expansa* appear triangular measure to diameter. The eggs of



(Hymenolepididae), a common

M. benedeni appear square, they are approximately diameter. The prepatent period for tapeworms approxi mately ys.

Thysanosoma actinoides fringed eworm" found ucts, eatic ucts, estine ruminants. ggs eworm ccur ckets eggs, ith vidual ggs uring y

Cysticercus tenuicollis bladderworm (larval or metacestode stage) of *Taenia hydatigena*—may be found attached to greater omentum within abdominal cavity of many ruminants. These cysticerci e ually nosed uring ostmortem tion. *Cysticercus bovis* bladderworm (larval or metacestode stage) of *Taenia saginata,* which beef tapeworm of human beings—may usculature ovine intermediate sticerci olloquially eferred beef ually nosed uring mortem ection. eings ecome ected dult eworm oorly ooked eef.

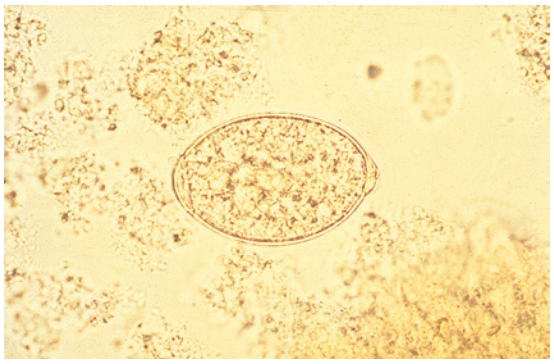
Cysticercus cellulosae bladderworm (larval or metacestode stage) f *Taenia solium,* hich ork eworm beings—may usculature orcine intermediate These cysticerci are colloquially referred to "pork ually nosed uring ostmortem inspection. Human beings become infected with adult tapeworm *T. solium* y ing oorly ooked ork ontaining cysticerci. Human beings may become infected with *C. cellulosae* in the uscles or ithin ervous issue .g., in rain ye) y esting ggs *T. solium*

Vampirolepis Hymenolepis or *Rodentolepis Hyme nolepis diminuta* asitize estine odents occasionally of dogs humans. The parasite unique it s ble o omplete ts ife ycle ithin ingle ndividual. he eggs e ound eces val cysticercoid evelops eetles, cts. Some of eggs of *V. nana* may hatch within intestine, xacanth mbryos urrow ucous mbrane

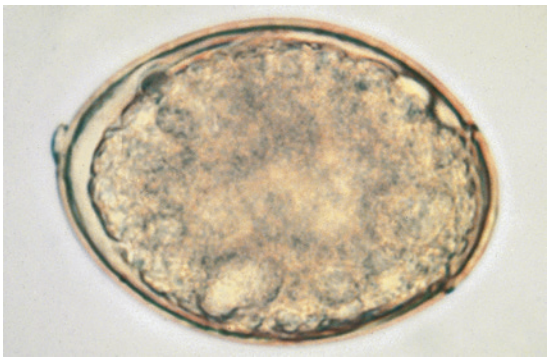
Spent proglottids of

form sticercoids. er eenter estinal umen to omplete evelopment. ggs with eces estion eetles which sticercoids evelop. *H. diminuta* ection equires ingestion of infected insect, human infection with tapeworm ely.

Spirometra ecies e ften eferred per" eworms or anosis eworms eworms often ound estines ogs live Florida along Gulf Coast of North merica. This tapeworm unusual produces operculated egg. Each proglottid of *Spirometra* species central spiral uterus associated terine ore ough hich ggs eleased. tapeworms characteristically release eggs until they exhaust their uterine ontents. ravid gments ually harged into et's eces.



(From Hendrix



Diagnostic parasitology for veterinary technicians,

This eworm ecause, ched 's junum, ure roglottids ften parate longitudinal for short distance. The tapeworm appears to unzip," which origin of common per tapeworm." ent ped" unzipped" roglottids ften appear eces et.

The gg f *Spirometra* ecies esembles digenetic rematode gg inct per culum ne ll. ggs val yellowish-brown. hey verage y y ve asymmetric appearance, they are rather pointed one end. When eggs rupture, distinct operculum visible. The eggs are mbryonated hen eces.

Diphyllobothrium species are often referred to broad fish" tapeworms. eworm wever, probably does grow large dogs Each proglottid f eworm entral osette-shaped terus associated uterine pore through which eggs are released. These tapeworms continually elease eggs il exhaust their uterine contents. The terminal proglottids become senile rather ravid, etach ather vidually.

The eggs of *Diphyllobothrium* species resemble of e enetic rematode). gg val, distinct perculum ll. ggs brown, verage o y o m. They tend to be rounded on one end. The operculum present on nd pposite ounded ggs mbryo nated hen eces

Pseudotapeworms of veterinary importance include

Trematodes re nsegmented nd eaflike. he rgans re mbed ded ose issue enchyma), muscular chment rgans uckers. erior ucker cated entral ucker cetabulum located n entral urface orm ody r here ee roups trematodes, ut nly enetic rematodes asites domestic Monogenetic trematodes are primarily external parasites f hibians, eptiles.

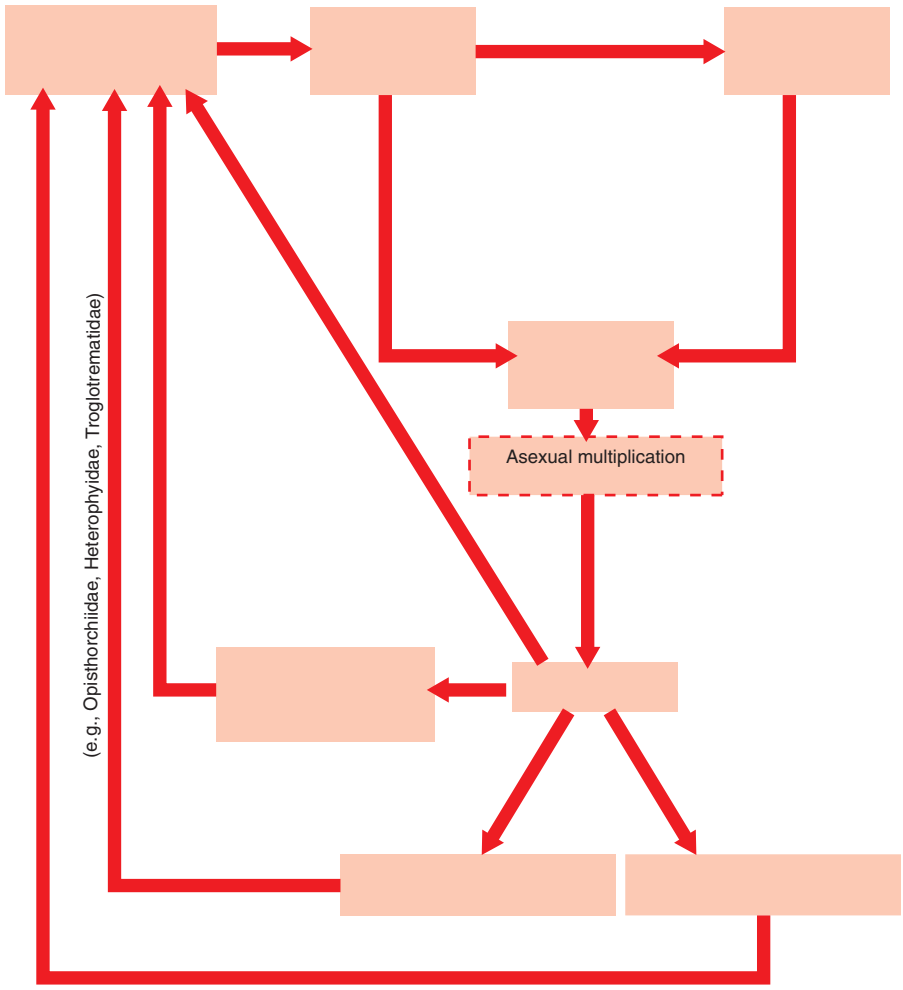
Digenetic rematodes ve ter ody ticle. They ve estive ract onsists pharynx, phagus, estine vides blind sacs (ceca). The organs are visible trematodes are eproductive rgans. rematodes ve emale eproductive rgans vidual, ut few ve parate xes *Schistosoma* rvous stem xcretory stem resent.

The e cle enetic rematodes omplicated hey ough veral erent val **(mira cidium sporocyst redia cercaria metacercaria)** they typically equire re ermediate hich nearly always mollusk (e.g., slug). Multiplication takes place oth efinitive ermediate

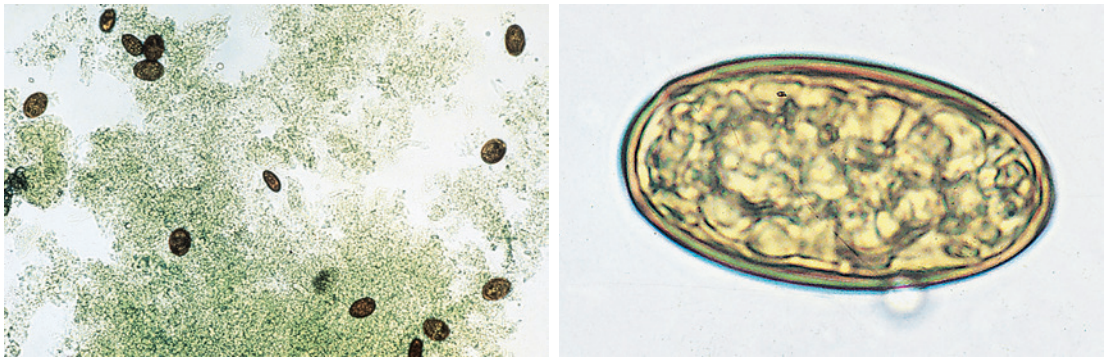
The eggs of digenetic trematodes are capped (operculated), they contain ciliated embryo called miracidium. Through penetration estion, acidium nters uitable evelops ough veral ventually ive to ile, eferred ercaria. ercariae released from swim actively. Sometimes, depending on ecies ercariae ncyst egetation. encysted tacercaria, ective or efinitive host. n her pecies, ercaria ay enetrate kin definitive or encyst another intermediate Table summarizes rematodes eterinary ortance.

Platynosomum fastosum lizard-poisoning e" Fig. dult ver, allbladder, ducts, ommonly, estine. rownish operculated ggs o y o

Nanophyetus salmincola salmon-poisoning e" dogs acific orthwest egion orth merica. adult e estine rves ector for ickettsial ents, hich roduce salmon oisoning" "Elokomin ever" ogs. ggs mbryonated when ure o y o Fig. hey ve inct perculum blunt oint pposite perculum.



Georgis' parasitology for veterinarians,



brownish, operculated eggs are 34 m to 50 m by 20 m to 35 m. (From Hendrix CM, Robinson E: *nostic parasitology for veterinary technicians,*

Alaria ecies e estinal ogs are found throughout northern of North merica. Their ova e e, olden rown, perculated they ure o y o *Heterobilharzia americanum,* histosome, blood e asitizes nteric eins e estines ortal eins og. enzootic ississippi elta oastal Louisiana. lthough fluke inhabits vasculature, pres ence s anifested loody iarrhea. nfected ogs lso xhibit emaciation anorexia. Diagnosis involves identification of thin-shelled gg, hich proximately y

Information About Some Trematodes of Veterinary Importance

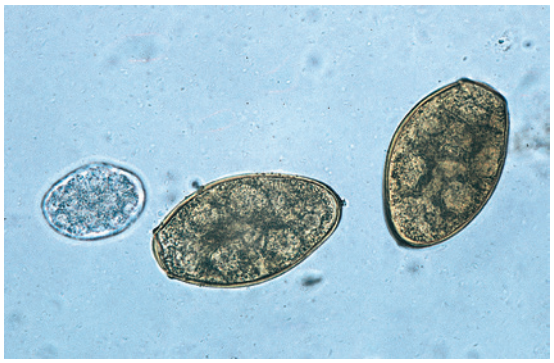
	Intermediate

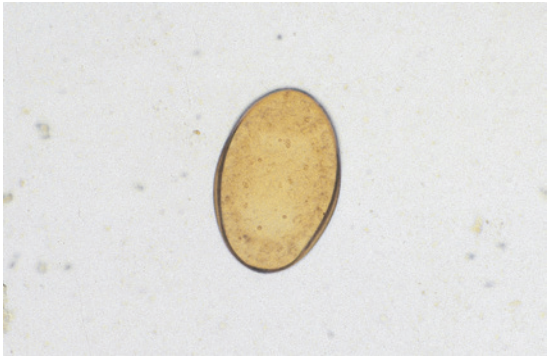
Continued

Information About Some Trematodes of Veterinary Importance—cont'd

	Intermediate

(From Hendrix

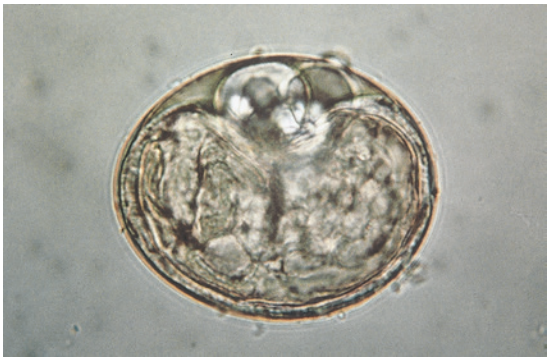




parasitology for veterinary technicians,

are broader in the anterior

lowed by prominent shoulders. (From Hendrix CM, Robinson E: *Diagnostic parasitology for veterinary technicians,*



a miracidium. (From Hendrix CM, Robinson E: *Diagnostic parasitology*

which contains miracidium. Fig. shows morphologic features of egg of *H. americanum.* The prepatent period for proximately ys.

Paragonimus kellicotti lung e" ogs. ermaph roditic adult flukes occur cystic spaces within lung paren chyma of both dogs These cystic spaces connect to terminal ronchioles. ggs tum eces. The egg yellowish-brown with operculum, measures to by to Fig. Fluke eggs are usually recovered with fecal sedimentation techniques; however, eggs of *P. kellicotti* may be recovered with standard fecal tation utions. ggs *P. kellicotti* y e ecovered tum ollected racheal dult within stic ces enchyma observed racic adiographs. e's repatent eriod o ys ng.

Fasciola hepatica liver e" ep, ruminants. rmaphroditic dult bile ducts of liver Fig. The eggs measure by they are yellowish-brown, oval, operculated Fig. 46.23 The prepatent eriod for *F. hepatica* is approximately 56 days ng. *F. hepatica* reatest conomic ortance all f asitize eterinary ecies. cle of ecies uite omplex

Dicrocoelium dendriticum lancet fluke" of sheep, goats, xen. hese iny eside ranches ile ucts. rown ggs ve inct perculum, y ure o y o ggs of orementioned rematodes ecovered from eces ecal dimentation ommercially vailable fluke gg ecovery

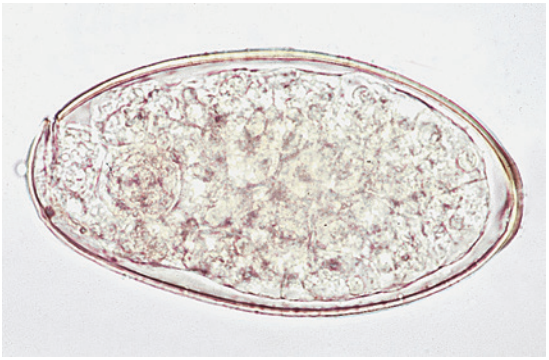
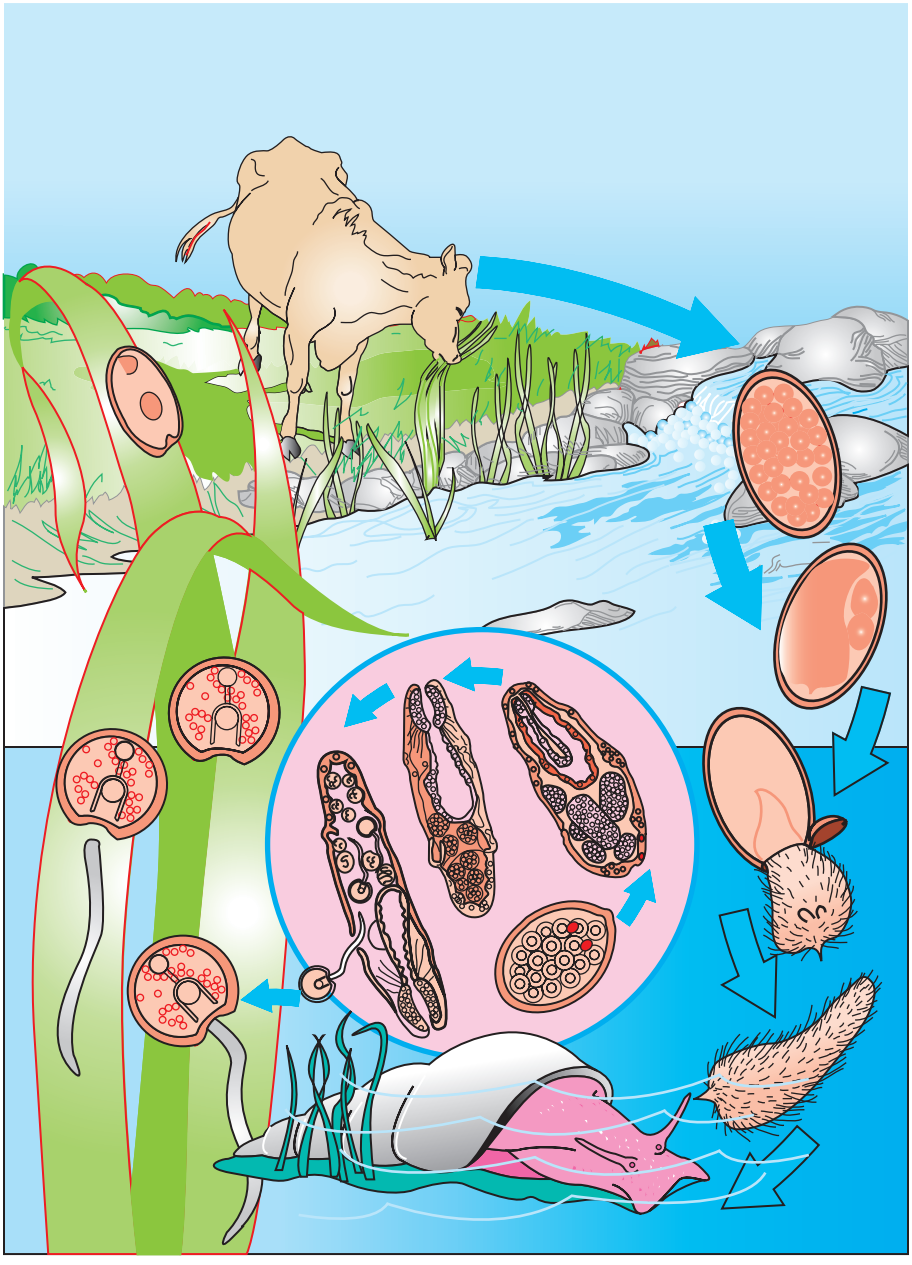


Fig. 46.23 Characteristic operculated ovum of *Fasciola hepatica,* the

"Rumen flukes" are composed of two genera: *Paramphistomum* *Cotylophoron* These adult flukes reside rumen reticulum of sheep, goats, many other ruminants. The eggs *Paramphistomum* species measure to by to whereas eggs of *Cotylophoron* species measure to by to The prepatent period *Paramphistomum* species to days.

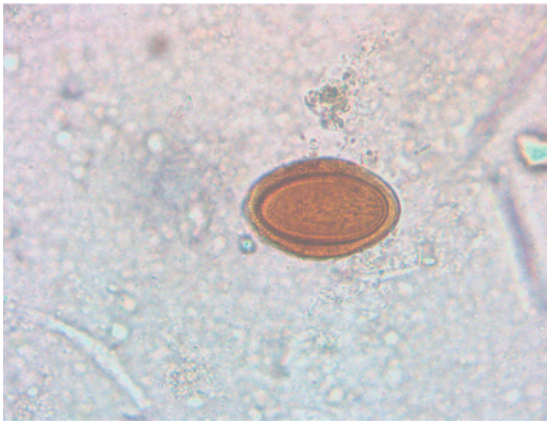
Schistosoma (Bilharzia) species are blood flukes of humans. Cercariae enter through direct penetration of These flukes are to *Heterobilharzia americanum,* they inhabit blood vasculature of mesenteric veins blood vas culature associated with major organs large small intestines urinary bladder) of abdominal cavities of humans.



the host by way of the common bile duct and the intestinal tract. If these eggs are carried to water, a ciliated miracidium develops within them over a period of several weeks or months, depending on the temperature of the water. After hatching, the miracidia seek certain species of lymnaeid snails,

generation of sporocysts and two generations of rediae. The second generation of rediae produces free-swimming cercariae that leave the snail and

objects, including aquatic vegetation. Ruminants



Acanthocephalans (thorny-head worms) are uncommon parasites with omplicated cles. matodes, ve separate xes.
proboscis ch estine wall. horny-head orms ve rue rb nutrients through their body wall. canthocephalans are usually recovered cropsy

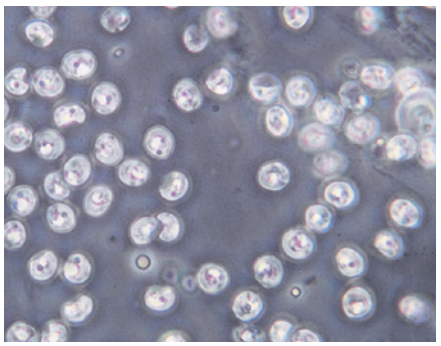
The canthocephalan *Macracanthorhynchus hirudinaceus,* hich asite asite dubious honor of possessing longest scientific among asites omestic *Oncicola canis* cantho cephalan estine ogs.

Chapter eview uestions ppendix

- Phylum Platyhelminthes includes flatworms are com monly eferred rematodes estodes.
- Members of subclass Eucestoda are referred to true tapeworms.
- Members ubclass otyloda eferred pseudotapeworms.
- Tapeworms ve ect cle volves more ermediate
- A rue apeworm as wo our uscular uckers cetabula rve o ch rganism estinal
- The rgans chment or udotapeworms of e rgans wn othria, hich cated lateral ect olex.
  Dipylidium caninum ommon eworm l estines ogs
- The ermediate *Dipylidium caninum*
- Dogs ecome ected eniid eworms ingesting sticercus-infected ermediate

Echinococcus granulosus *Echinococcus multilocularis* e tapeworms are associated with unilocular multilocular hydatid st
Anoplocephala perfoliata, Anoplocephala magna, *Paran oplocephala mamillana* e quine eworms.

- Trematodes are flatworms with complex life cycles include several different larval stages miracidium, sporocyst, redia, cercaria, tacercaria) ypically equire or re ermediate hich ly ways mollusk
- Trematode asites ogs lude *Paragonimus kellicotti, Platynosomum fastosum, Nanophyetus salmincola, Alaria* ecies, *Heterobilharzia americanum.*
- Trematodes lude *Fasciola hepatica, Dicro coelium dendriticum, Paramphistomum, Cotylophoron.*



After studying this chapter, you will be able to:

- List ommon rotozoal asites eterinary importance efinitive
- Describe onditions er hich rotozoal asites develop o
- Describe cle *Giardia*
- Describe eneral cle orozoans.
- Describe cle *Toxoplasma gondii* eline non-feline
- List ommon ickettsial asites eterinary importance.

Phylum Sarcomastigophora,
Giardia,
Trypanosomes,
Leishmania,
Trichomonads,
Histomonas,
Entamoeba,
Phylum Apicomplexa,
Cystoisospora,
Toxoplasma,
Cryptosporidium
Sarcocystis
Babesia
Cytauxzoon,
Hepatozoon,
Eimeria,
Plasmodium,
Phylum Ciliophora,
Rickettsial Parasites,
Key Points,

Amastigote
Bradyzoites
Cilia
Coccidiosis
Flagella
Hemoprotozoa
Infectious enterohepatitis
Merozoites
Oocyst
Promastigote
Protozoa
Pseudopodia
Rickettsia
Tachyzoites
Trophozoite
Trypomastigote
Undulatory ridges

There are about known protozoans be found wide variety of habitats. Only small percentage of protozoans are asitic. **Protozoa** e le-celled rganisms more mbrane-bound uclei ontain ecial ized toplasmic rganelles. arasitic rotozoa three rimary hyla: arcomastigophora, picomplexa, iliophora. rotozoans ect ariety tissue es efinitive ommon for ir etection lood hich led blood protozoa or **hemoprotozoa** or fecal which they are led estinal rotozoa. moprotozoa en United tates re ound rythrocytes red lood ells RBCs]) within stained blood smear. Ticks usually serve intermediate ransmit ontain moprotozoa from ne cles rotozoa or complex. Reproduction may be asexual (binary fission, schizogony, amy, onjugation). certain roups rotozoa, eproductive or identification. **trophozoite** wn egetative form) rotozoal cle feeding, vement, eproduction. able ummarizes some ommon rotozoal asites eterinary ecies.

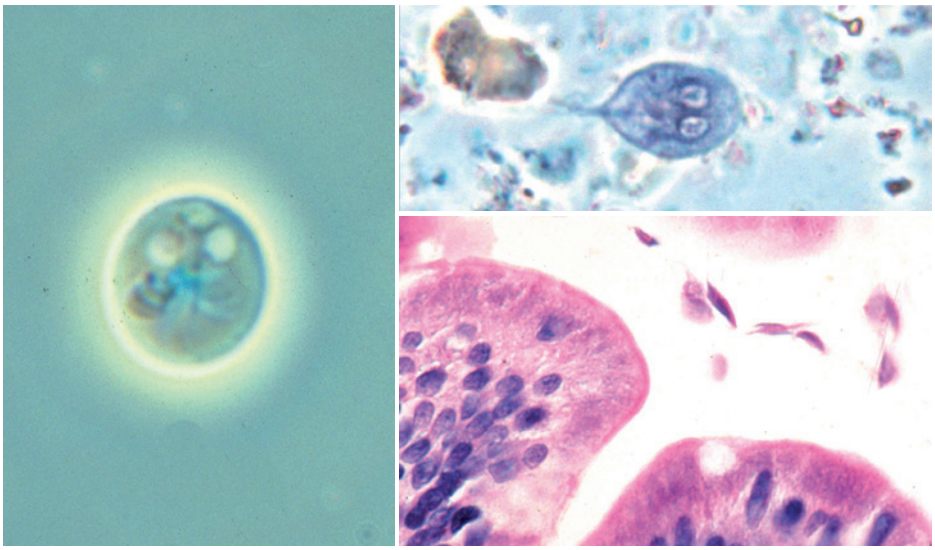
Select Protozoal Parasites of Veterinary Species

	Intermediate		Intermediate
Isospora ohioensis			

Organelles for locomotion consist of **flagella** (long, whiplike structures), (short flagella, usually arranged rows or tufts), **pseudopodia** (temporary extensions retractions of body wall), **undulatory ridges** (small, snakelike waves form cell membrane move posteriorly). Locomotor organelles modifications of them are frequently used to help identify the type of protozoa recovered from an animal. The trophozoite often too fragile to survive transfer to new generally infective. Transmission to often occurs when protozoan cyst stage. Most metabolic functions are suspended when parasite encysted. The cyst wall prevents desiccation. The cyst stage occurs under certain conditions include following:

- Lack of nutrients
- Low oxygen tension
- Lack of water
- Low
- Accumulation of waste
- Overcrowding

Protozoal parasites are usually transmitted to a new



trophozoite in a trichrome-stained fecal smear.

lumen. (From Bowman D: *Georgis' parasitology for veterinarians,*

The hylum arcomastigophora ludes ebas flagellates. There are about known species phylum, but nly out asitic. ellates or re ella uring rophozoite cle trophozoite asite. ebae move ia udopods, ve rophozoite form nera eterinary ortance phylum nclude *Trypanosoma, Leishmania, Giardia, Trichomonas, Histomonas, Entamoeba*

Giardia species are flagellated protozoans are often recovered from feces of dogs with diarrhea, but they may be ecovered om rmal ools. asite occurs rphologic orms: eeding known s he rophozoite tage nd 2) esistant yst tage Fig. orses, ows, ep, oats, usceptible to nfection. he otile tage ear-shaped nd orsoventrally flattened, nd ontains our airs agella. easures to by to Two nuclei prominent adhe sive e resent ortion ell, resemble yes ck bserver.

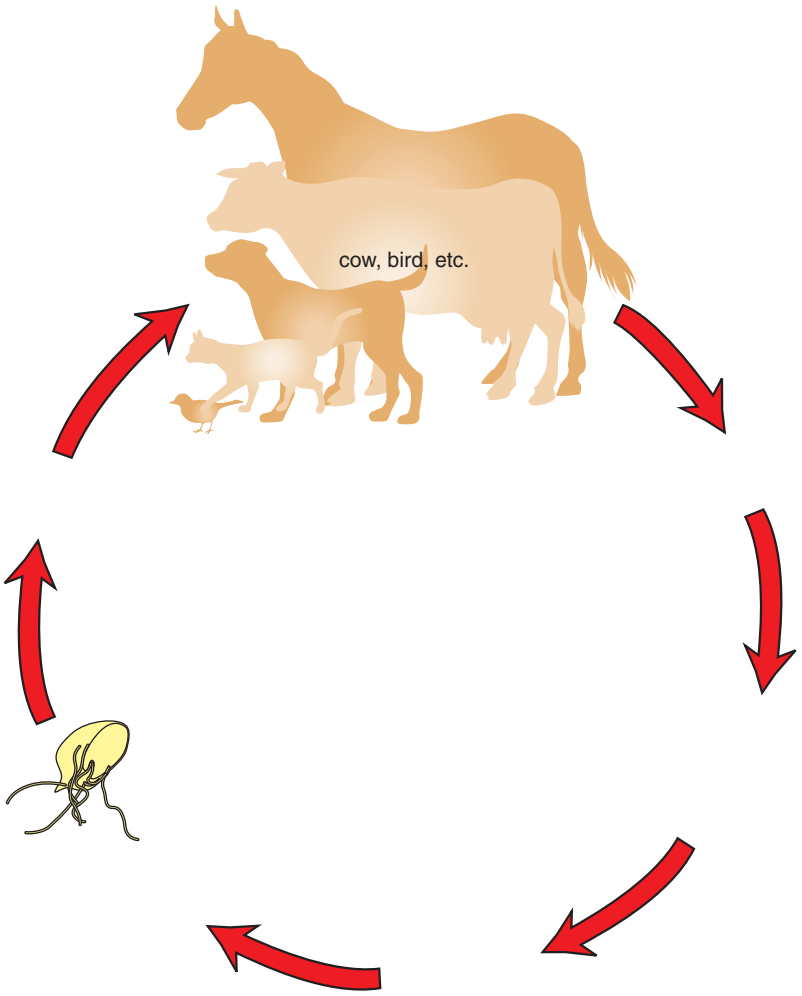
The ure vals ure o y o hey ve efractile uclei. Immature sts, which represent recently ncysted motile forms, contain nly uclei. ogs, rhea egin ly ays fter xposure *Giardia,* ith ysts rst ppearing feces eek. ummarizes cle *Giardia.*

seen in animals with diarrhea, whereas cysts are more commonly observed

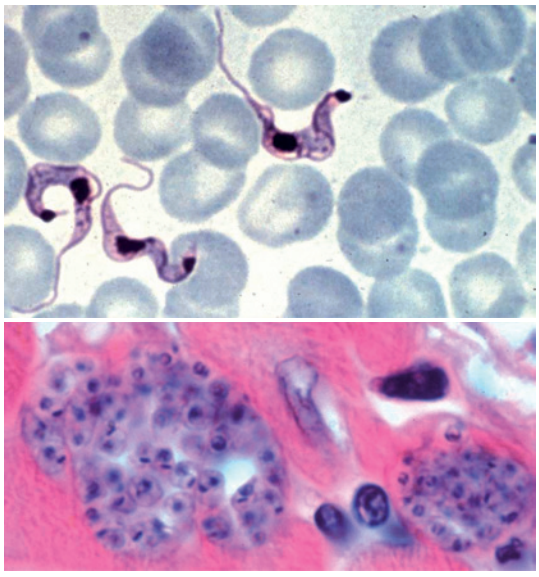
Diagnosis de ecal ion. ulfate (specific ravity, onsidered ion dium for ecovering ysts ften orted, milunar appearance. rophozoite ccasionally on ect eces onic ugol's iodine y isualize ernal ructures rophozoites. ecal unodiagnostic om monly d.

Trypanosomes are group of hemoprotozoans are occasion ally ound thern nited *Trypanosoma cruzi* asite f ccasionally ogs. irds may become infected. Rather being found within trypanosomes are extracellular they swim" within blood. They e o ide, banana-shaped. hey ve eral ulating mbrane hiplike ellum) or he led **trypomastigote** he st stage, hich wn **amastigote** xists, be found within cardiac muscle other tissues. These parasites are transmitted by blood-feeding arthropods (reduviid bugs).

Leishmania ecies e moprotozoans ect veral species, including dogs, humans. The parasite ransmitted enus *Phlebotomus Lutzomyia* ut xperimental ections ve ccurred certain ick reeds. *Leishmania* ecies ere be f oncern nly ets raveled tside orth America. However, parasites have been found several U.S. states, ly exas. wo orms have been recognized. The mucocutaneous form, which haracterized res, rimarily *Leishmania braziliensis.* he isceral orm, hich rimarily



Diagnostic parasitology for veterinary



. The top image shows a trypomastigote in a Wright-stained buffy coat preparation from a naturally infected dog. The bottom image shows the amastigote stages in heart muscle. (Courtesy

Leishmania donovani *Leishmania infantum* led *Leishmania chagasi* affects several internal organs (e.g., spleen, liver, one row).

The e cle rganisms asite transmitted uring ellated **promastigote** e ia ite from ct ector. romastigotes hagocytized by host's macrophages Fig. The promastigotes multi ply y inary evelop igote then eleased hen ell uptures. eleased igotes are then phagocytized by other macrophages. Large numbers of organisms an resent in iety issues. mastigotes are ked hen lood om hese evelop romastigotes to complete life cycle. Diagnosis generally requires fluorescent antibody esting nostics.

Trichomonads ng, nder rganisms el lum ched orsal urface, reby orming structure ipples rganism lides ough ebris.

Tritrichomonas foetus asite eproductive ract of he rganism esides repuce ulls

Fig. 47.4 *Leishmania infantum*. The top image shows a macrophage from the bone marrow of an infected dog that contains large numbers of amastigotes. The bottom image is of a dog from Brazil that is infected showing the typical cutaneous manifestation
Georgis' parasitology for

vagina, ervix, terus ows. nfection er tility, ontaneous ortion, yometra. *T. foetus* ear-shaped proximately o ng, three erior ella. iagnosis volves emonstration ile rophozoite upernatant entrifuged from agina repuce.

Trichomonas gallinae found crop crop from pigeons, doves, poultry. It transmitted only by direct contact ith ected ird er een on taminated y ected ird. *T. gallinae* crotic er ations esophagus, crop, proventriculus. Nonpathogenic species f richomonads ecum olon of many domestic Diagnosis involves demonstration of parasite direct of crop contents, characterized by four anterior gella. air-dried smear be stained with right's The parasite assumes oval shape, lue ed ostyle.

Histomonas meleagridis nfects urkeys, hickens, heasants, nd vian ecies. ransmitted hen ird va f matode ransport *Heterakis gallinarum*. Earthworms rve atenic or matode, birds ected rotozoa hen matode. ellated rophozoite eleased om nematode vae ecal umen. ella nters ecal pithelium ver eboid orm, where eproduces ion issue cro n urkeys, rganism ver led **infectious enterohepatitis** r blackhead." iagnosis equires histopathologic xamination ver.

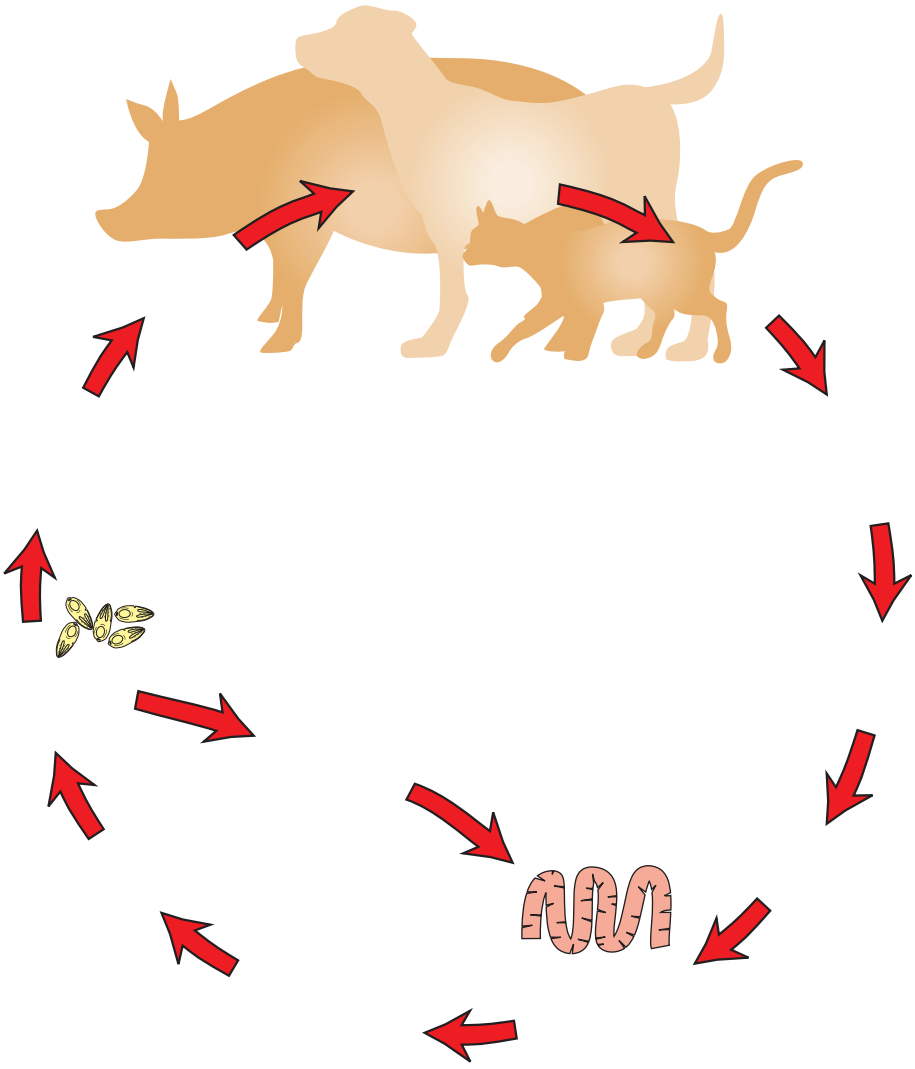
Entamoeba histolytica s primarily rasite umans in ropi egions. rophozoites arious eboid rgan e equently emonstrated ecal fecal ion ep, oats, rses, swine. These generally are of clinical importance. *E. histolytica* may roduce cute hronic rhea ogs. ther ecies of ebas ve een ed rimates tortoises.

Apicomplexans are orozoans. There are about ecies, all are parasitic. Sporozoans are unique all of their life cycle es xcept or gote. ontains orozoan cle. orozoal asites within ells, ommonly ccur esti ract ells lood ells. ***Oocyst*** iven cyst e f roup estinal rotozoa. enera greatest ortance eterinary ecies lude ollowing intracellular asites:

Cystoisospora (Isospora)
Toxoplasma
Cryptosporidium
Cytauxzoon
Sarcocystis
Plasmodium
Babesia
Eimeria

Cystoisospora species are protozoal parasites of small intestine of both dogs cats. They produce clinical syndrome wn **coccidiosis** hich ne ommonly nosed protozoal ens. occidiosis arely problem mature The oocyst diagnostic stage bserved ecal ion eces. oru lated esh eces, aries common ecies

The occidians ocyst urements ollows: *Cystoisospora canis,* o y o m; *Cystoisospora ohioensis,* o y o *Cystoisospora wallacei,* o y



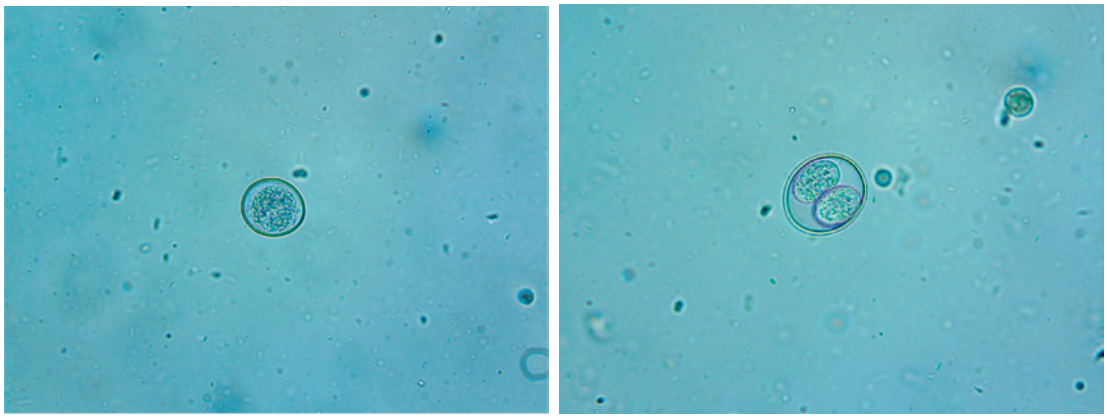


Fig. 47.6 *Cystoisospora felis* unsporulated oocyst and sporulated oocyst . (From Bowman D: *Georgis' parasitology for veterinarians,*

to he eline occidians urements follows: *Cystoisospora felis,* to by to *Cystoisospora rivolta,* o y o m. The repatent eriod aries ecies, ut ually to ys.

Coccidians are among the most commonly diagnosed

Isospora suis coccidian parasitizes small intestine of swine, especially young piglets. Oocysts are usually found with fecal ion eces. hey ubspherical, ck opyle, ure o ostmortem diagnosis lets xhibit ut shedding ocysts chieved opathology direct of jejunum been stained with Diff-Quik. Diagnosis bservation ed **merozoites** The repatent eriod ys ng.

Toxoplasma gondii r estinal occidian oocysts re sually iagnosed ia tandard ecal otation. ocysts *T. gondii* e orulated eces, ure y everal unodiagnostic volve f hole lood rum vailable or *T. gondii* infection. The prepatent period highly variable. It ranges om ys, epending oute ection. The e cle omplex volves veral erernt including **tachyzoites bradyzoites** rozoites, ogameto cytes, crogametocytes.

generally only

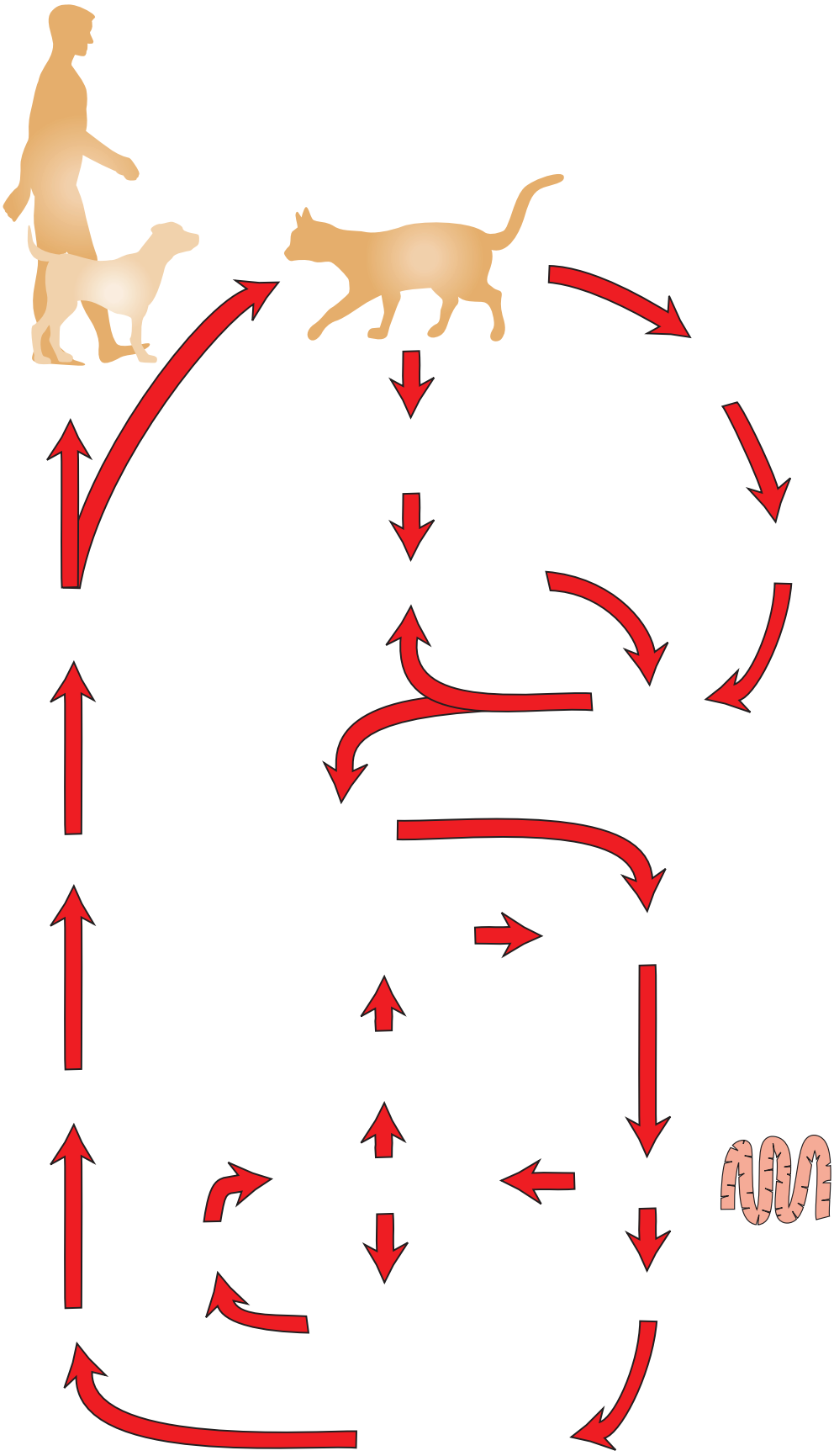
Although efinitive *T. gondii,* arious life cle asite ect ecies, lud umans lthough rganism ually harmful to healthy humans, serious problems fetuses f regnant omen. oxoplasmosis read om o eople estion ectious ocysts eces, ut ontract undercooked regnant omen void of er loves ardening.

Cryptosporidium r occidian asite asitizes l estine ide ariety luding ogs, ticularly ves. orulated ocysts eces e val herical ure nly o m. Diagnosis de ecal ion. ocysts extremely bserved er over slip, h ocus oocysts asite va xamination fecal s ecial dified helpful. Because people may become infected with *Cryptosporidium* ecies, eces uspected boring protozoan led reat

Sarcocystis occidian asite intestine. everal ecies ect ogs entifica tion f vidual ecies uite ocysts *Sarcocystis* ecies e orulated hen eces. ch ocyst ontains orocysts, ch sporozoites. vidual ocysts ure o by o y ecovered om fecal ion eces.

Babesia canis intracellular parasite found within erythrocytes ogs, eferred oplasm because f ear-shaped ody iagnosis volves bservation philic, ear-shaped rophozoites on stained blood smears. *Babesia bigemina* intracellular parasite asite e oplasm o ng y proximately ide. t haracteristically ear-shaped, ccurs s orm cute rythrocyte. intermediate for protozoan parasite tick *Boophilus annulatus. Babesia equi Babesia caballi* e racellular parasites rses. hey eferred quine oplasms. iagnosis volves bservation philic, ear-shaped rophozoites n lood rophozoites *B. equi* y e round, eboid, yriform. rganisms ined, which ives ffect altese ndividual rganisms are o ng. rophozoites *B. caballi* e yriform, round, or oval to long. They occur characteristically s cute ch r.

Cytauxzoon felis r racellular asite een sporadically reported of various locales (e.g., Missouri, rkansas, Georgia, Texas) throughout nited States. It ransmitted ough ite *Dermacentor variabilis* other ick ecies, luding *Amblyomma americanum.* s range f ick ectors revalence expanded, now been identified mid-Atlantic. The parasite ects omestic elids. ector icks ect the ost ith chizont orm, hich hen nter acrophages. The schizonts undergo asexual reproduction within macrophages, cells become large enough to occlude venous flow. The macrophages subsequently rupture, releasing merozoites infect rythrocytes. rythrocyte orm led oplasm een escribed eing ed bejeweled ing" which are referred to ring form stained blood smears Fig. Piroplasms may undergo asexual reproduction d o estruction rythrocyte, Acute cytauxzoonosis occurs during schizont phase lead to multiple-organ failure death. Piroplasms may be seen during cute ection urvived cute oplasm orm ccurs er ourse Fine-needle aspirates of lymph nodes, liver, or spleen may demonstrate evidence of schizont-filled macrophages earlier



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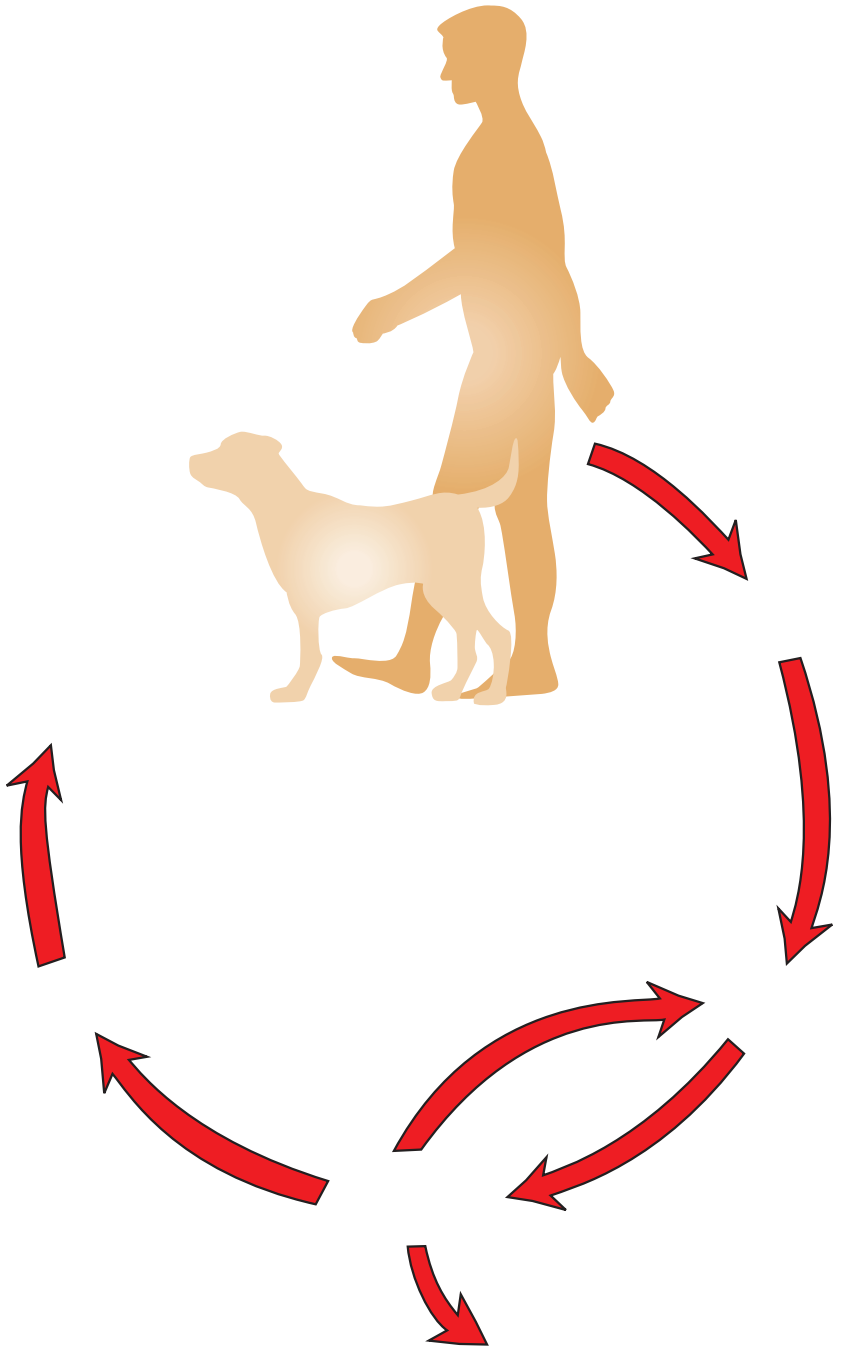
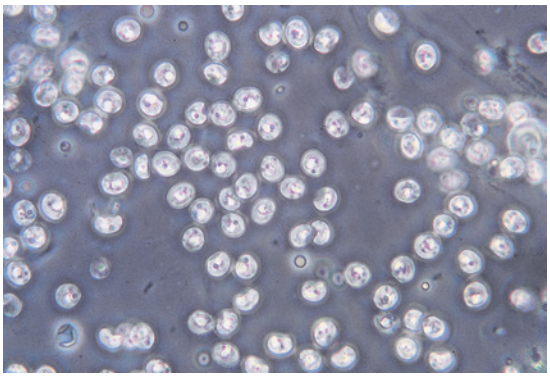


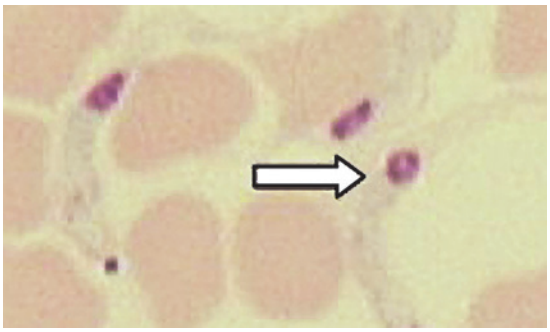
Diagnostic parasitology for veterinary technicians,

ourse olymerase eaction assays e vailable or onfirmation

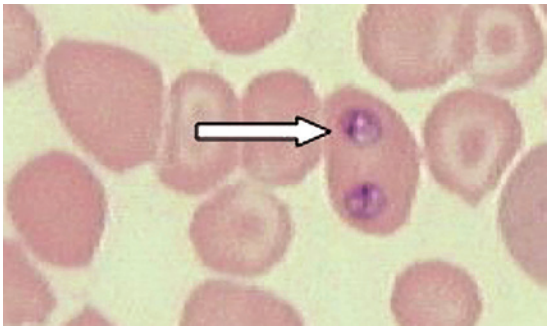
Hepatozoon canis nd *Hepatozoon americanum* re ntracellular, malaria-like asites ect ogs. lood orms parasites amonts) rotozoan asites ukocytes. eukocytes ontain amonts *H. canis* are ommon eripheral lood hereas amonts *H. americanum* e are. hizonts ndothelial cells of spleen, bone marrow, liver. The gamonts are urrounded elicate lue with k, eddish-purple ucleus. umerous ranules are ound toplasm ukocyte. onion tissue sts *H. americanum* e ound eletal uscle of ogs usual asite dog ecomes ected er estion ected ick,



species. (From Hendrix CM,

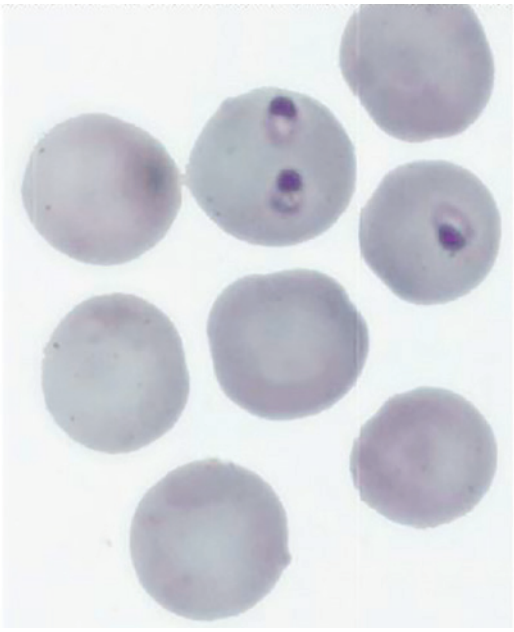


Diagnostic parasitology for veterinary technicians, ed 3, St Louis,



Amblyomma americanum *H. canis* ell dapted varies from producing subclinical to *H. americanum* produces violent frequently course of orized ve ossed ecies rier om ild omestic og.

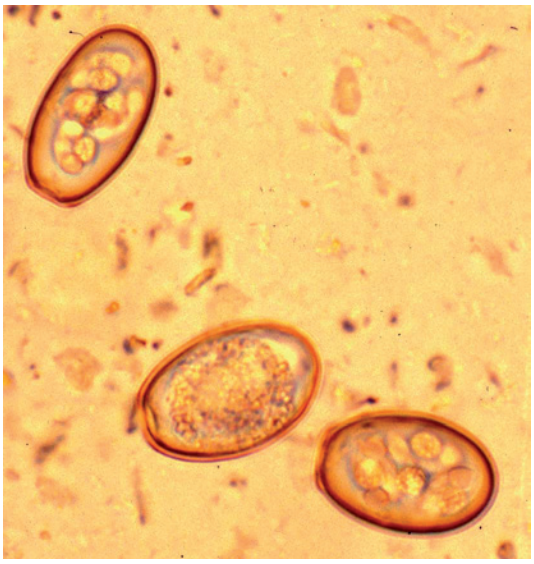
Eimeria leuckarti occidian tine f rse. rotozoan emonstrates oocysts o y o ith k distinct opyles, rown olor. ocysts be ecovered ecal ion, oc oocysts. They are frequently observed on histopathologic examination. repatent eriod anges om ys. Ruminants rve ecies *Eimeria* he identification vidual ecies occidia ften because ir ocysts ommon ecies occidia *Eimeria bovis* *Eimeria zuernii,* e erentiated ecal tion. cysts *E. bovis* e val, ve opyle, they ure y hereas *E. zuernii* e spherical, ck opyle, ure o y o hen ocysts ecovered fecal ion, bservation ually ed coccidia." Several ecies *Eimeria* e le ecting abbits ut *Eimeria stiedai,* f ticular ortance. Heavy ections uct lockage ver ure. Mortality abbits.



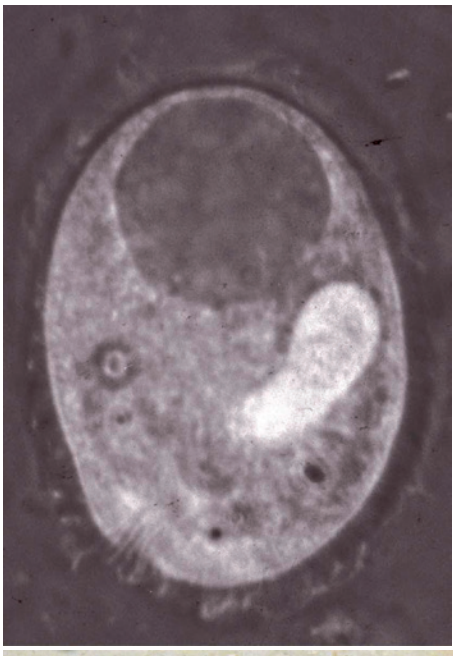
ring–shaped *Cytauxzoon* piroplasms (From Little S: *The cat*, Philadelphia,

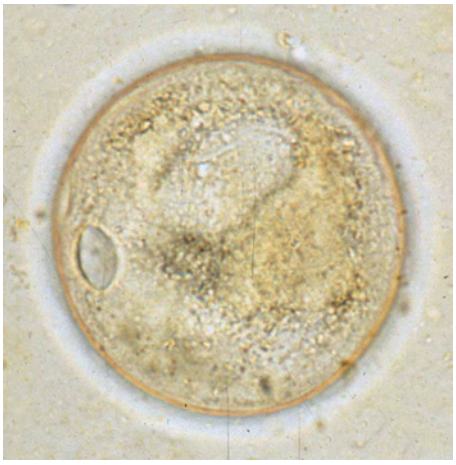
TECHNICIAN NOTE are often fatal,

Various ecies *Plasmodium* birds, eptiles. rganism ransmitted toes, evelops rozoites patocytes. hepatocytes upture, eleased rozoites vade erythrocytes reticulocytes. The diagnosis of infections with *Plasmodium* ecies ccomplished emonstrating organisms lood rgan ression



histopathologic examination of liver the spleen. Molecular nostic vailable. *Leucocytozoon* *Haemoproteus* e asites ect lood ells of irds.





Trophozoite (electronic flash photograph)
Cyst. Trophozoites abound in the large intestine of normal swine, and cysts are passed in their feces. (From Bowman D:

There are about species phylum Ciliophora, of which about asitic. enus, *Balantidium,* f veterinary nificance.

Balantidium coli ed rotozoan e estine lthough ommonly bserved during oscopic xamination rheic eces, generally considered nonpathogenic. Two morphologic stages may e ound eces: yst tage tile rophozoite stage . ary protozoan parasite. The trophozoites may be by m, with y-shaped cronucleus. rganism overed ith umerous ows ves out microscopic vely ility. herical ovoid o ter, reenish-yellow olor. ecognized microscopic xamination estinal ontents diarrheic eces.

The **rickettsia** are group of obligate intracellular gram-negative bacteria. he onomic ickettsiaceae Table hich lude enera *Rickettsia, Orientia, Coxiella,* naplasmataceae Table which include genera *Anaplasma* Fig. *Ehrlichia* Fig. *Wolbachia, Neorickettsia.* The organisms are transmitted via arthropod lminth ectors.

Chapter eview uestions ppendix

Pathogenic Rickettsiaceae That Affect Animals

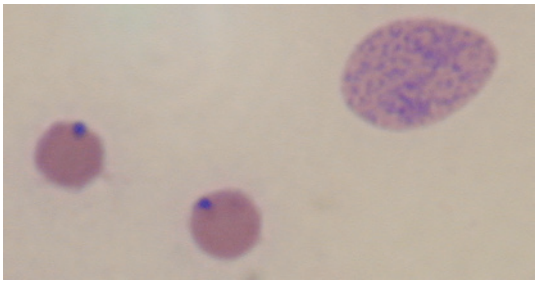
From Songer JG, Post KW: *Veterinary microbiology: bacterial and fungal agents of animal disease,*

Anaplasmataceae of Veterinary Importance

Continued

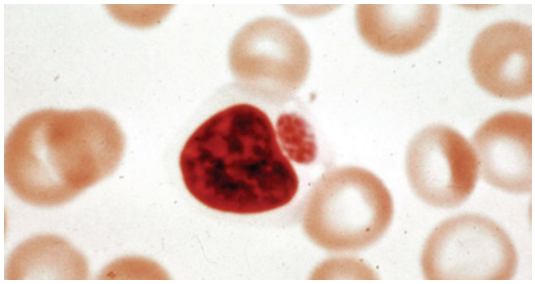
Anaplasmataceae of Veterinary Importance—cont'd

Human granulocytic ehrlichiosis;
Veterinary microbiology: bacterial and fungal agents of animal disease,



cally parasitized erythrocytes and an immature form. (Courtesy Raymond

fungal agents of animal disease,



fungal agents of animal disease,

- Parasitic protozoa be found three primary phyla: Sarcomastigophora, Apicomplexa, Ciliophora.
- Protozoal parasites are usually transmitted to new during cyst stage.
  Giardia species common flagellate infect variety of including humans.
- Trypanosomes *Leishmania* species are zoonotic parasites are primarily found southern United States.
  Tritrichomonas foetus parasite of reproductive tract of infertility, spontaneous abortion, pyometra.
- Apicomplexans (sporozoans) are found within cells, they commonly occur intestinal tract cells blood cells.
- Cats are only definitive for *Toxoplasma gondii,* various life-cycle stages of parasite infect other species, including humans.
  Cytauxzoon felis intracellular parasite sporadically found of usually
- A variety of *Eimeria* species are capable of infecting ruminants small
- Rickettsia are obligate intracellular parasites with two primary taxonomic Rickettsiaceae Anaplasmataceae.

After studying this chapter, you will be able to:

- Describe eneral haracteristics rganisms phylum rthropoda.
- Differentiate etween cts achnids.
- Describe eneral cle cts.
- Describe eneral cle achnids.
- Describe eneral haracteristics
- List ecies ommonly ncountered veterinary ecies.
- Differentiate etween allophaga noplura ecies.
- Describe cle
- List escribe asitize eterinary species.
- Describe cle icks.
- Differentiate etween icks icks.
- List ommonly ncountered ecies icks parasitize eterinary ecies.
- Describe eneral haracteristic coptiform
- Discuss eneral cle coptiform
- List ommonly ncountered ecies parasitize eterinary ecies.

Order: Siphonaptera (Fleas),
Orders: Mallophaga and Anoplura (Lice),
Order: Diptera (Flies),
Blackflies idges,
Sandflies
Deer orseflies,
Sheep eds,
hat roduce yiasis,
Anopheles, Aedes, Culex ecies osquitoes),
Class: Acarina (Mites and Ticks),
Ticks,
Mites,
Pentastomids (Tongue Worms),
Phylum: Annelida (Segmented Worms),
Hirudo Medicinalis edicinal eech),
Key Points,

Acariasis
Arachnids
Ectoparasite
Flea-bite dermatitis
Hirudiniasis
Hypostome
Instar
Mange
Myiasis
Nits
Nymphs
Pediculosis
Periodic parasite
Pupa
Tick paralysis
Warbles

Organisms hylum rthropoda haracterized presence f inted gs. hey ve hitinous xoskeleton omposed gments. re dvanced roups, segments ve ogether orm ody uch head, thorax, abdomen. rthropods have true body cavity (coelom), culatory stem, estive stem, espiratory system, excretory system, nervous system, reproductive system. The sexes are separate, reproduction by of eggs. y ertain roups thropods asitic. embers of r roups ct ermediate or revi ously ndoparasites. hen asite esides surface of called **ectoparasite** Most ectoparasites are ither cts **arachnids** ., icks, mites). ew val matodes ctoparasites. eeches

(bloodsucking annelids) also considered to be ectoparasites. Infestation eches eferred **hirudiniasis**

The ollowing eneral haracteristics erentiate major lasses thropods eterinary ortance:

- Insects ve ee gs, ee inct ody egions (head, rax, omen), ennae.
- Arachnids (adults) have four pairs of legs, body divided into two egions cephalothorax nd bdomen), nd ntennae.

Pentastomids ongue orms) are another group rasitic arthropods arely ncountered espiratory sages f ertebrates. rganisms esemble orms ather thropods uring dult dults ve of ved, etractile oklets mmature are elike, ee gs.

The thparts cts ary ructure, ependingfeeding its, daptations or hewing/biting, onging (lapping rcing/sucking. rax ve two s f tional ings ddition ee jointed gs. xes parate, eproduction esults roduction ggs vae. evelopment ften volves three r re arval tages alled **instars** hich re ollowed formation of **pupa** change form or transformation (complete tamorphosis) dult cts, development ccurs om gg ough veral ure stages **nymphs** hich esemble dult orm ut smaller omplete tamorphosis). emon strate complete metamorphosis, lice demonstrate incomplete metamorphosis. nsects roduce efinitive dults, vae,

The achnids lude icks, ers, orpions. Ticks re ortant roups achnids veterinary ers orpions harm omestic oxic enoms. rachnids generally small may even be microscopic. Their mouthparts are borne on structure called capituli, they consist of f bile dapted or tting helicerae) of sensory structures The **hypostome** s tructure with ecurved eeth chment ears roove ermit thropod va lood ymph. cle onsist gg, va, nymph, adult. There be more one nymphal instar. Nymphs resemble adult form, but they are smaller. There ually nly val hich ers om ymphs dults nly ee gs.

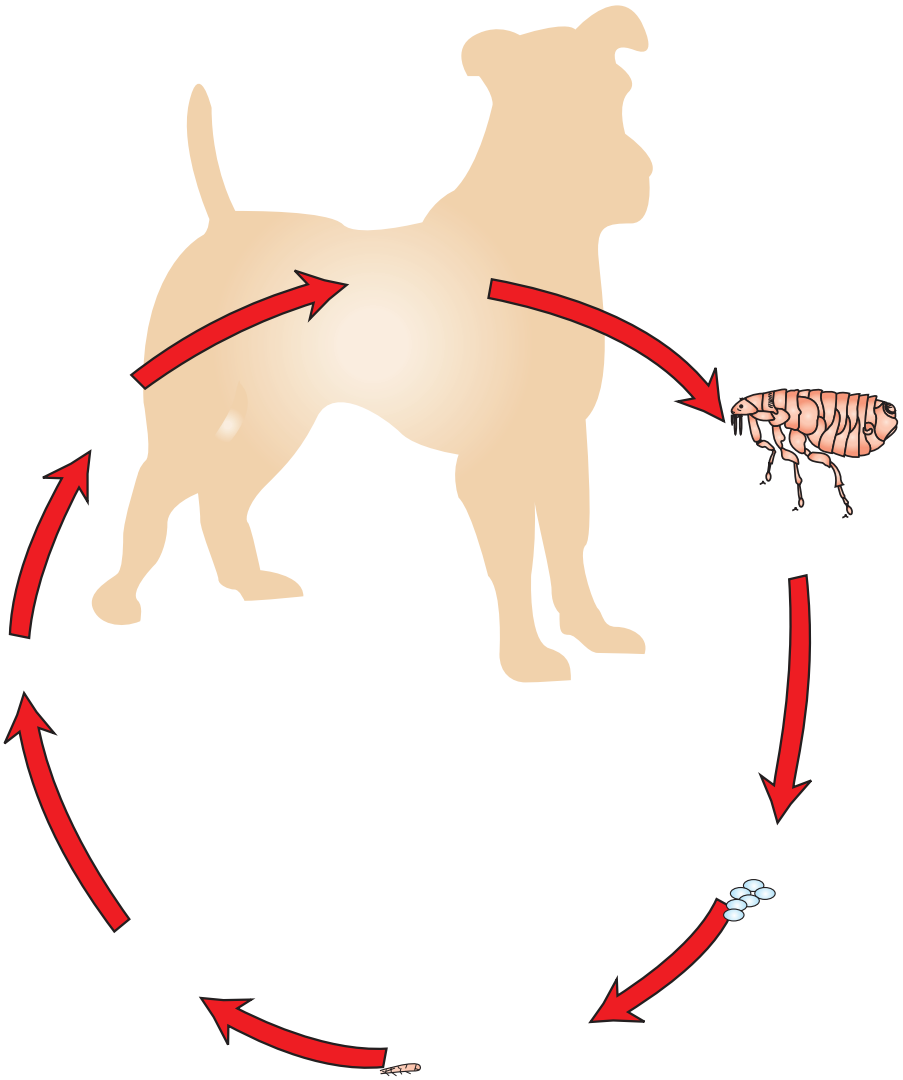
are blood-sucking parasites of dogs, rodents, birds, people. They are vectors of several such bubonic tularemia. More species of have been identified throughout world. dult are always parasitic, feeding on both mammals and birds. Dogs are host comparatively few species of Cat dog *Ctenocephalides felis*

Ctenocephalides canis, respectively—can act intermediate for common tapeworm, *Dipylidium caninum.* Heavy infesta tions ith ecially roduce va igenic ritating, ense ru ritus ching) ypersensitivity. ondition wn **flea-bite dermatitis** r y ermatitis.

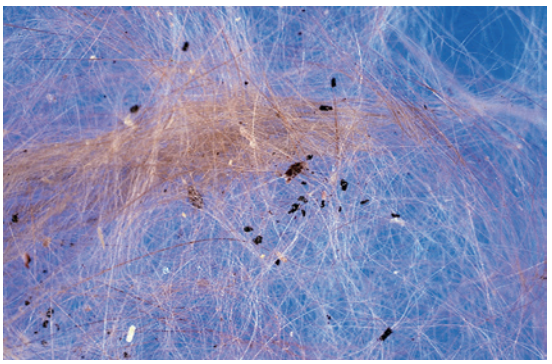
are laterally compressed, wingless insects with legs that are dapted or dult ve rcing/sucking siphonlike) outhparts hat re sed uck he lood of ir hey ve apidly om infestations are encountered frequently on dogs hey etected entral omen, er demonstrate complete metamorphosis Fig. Eggs deposited n evelop vae environment. vae ccasionally animal's edding, niture, acks evices nvironment. vae gotlike, head capsule bristles Fig. larvae feed on organic debris, luding xcrement dult oppings are eddish-brown, omma-shaped ehydrated lood Fig. oppings oat estation.

The ecific entification equires xpertise entomologist. Other of veterinary importance are *Pulex irritans, Xenopsylla cheopis, Pulex irritans, Xenopsylla cheopis, Echidnophaga gallinacea.* ve referred ut attack y ce lood referred vailable. Adult survive for extended periods off of y vily remises.

Echidnophaga gallinacea known stick-tight flea" of oultry. ommon hickens owl, feeds n ogs eeding female rts thparts remains attached site. These specimens resemble attached ticks; wever, e ommonly rses barns here eral here xcessive edding used, ve een ves umbers



Ctenocephalides felis, the cat flea. Flea larvae resemble tiny fly maggots; they are 2 mm to 5 mm long, white (after feeding, they become brown), and sparsely covered with hairs. (From Hendrix



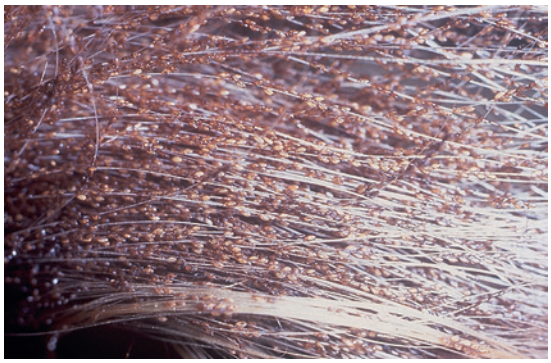
the cat flea. Flea dirt can be used to diagnose current or recent infestations

produce nificant *Pulex irritans,* uman
been recovered from dogs especially southeastern
United es.

Lice e orsoventrally ened, ingless cts lawed
appendages or hey ve
three body divisions: head, with mouthparts antennae;
rax, ee gs ck ings;
omen, hich ortion ears eproductive
organs. Lice are separated into two orders on of whether
their mouthparts are modified for biting/chewing (Mallophaga)
or ucking noplura). ucking eed lood ve
slowly n hey er hewing
they e ed ray; olor ually epends
of blood ingested from They have long, narrow head.
Biting ice eed pithelial ebris nd an ove apidly ver he
hey ve road, ounded ider idest
portion f rax), ler ucking
they are generally yellow color. Lice are host-specific, they
remain close association with they have preferred
locations n Table lue ggs
Fig. o eathers iny:
they e proximately val,
white. its ch proximately ys er eing
dult emale ymphal pear
ance o dult. owever, ler cks tioning
reproductive rgans enital penings. ee ymphal
stages e ch rogressively er redecessors.
nymphal om eeks.

Anoplurans are larger than Mallophagans and have

The adult stage appearance to nymphal stage,
but er. tional eproductive rgans.

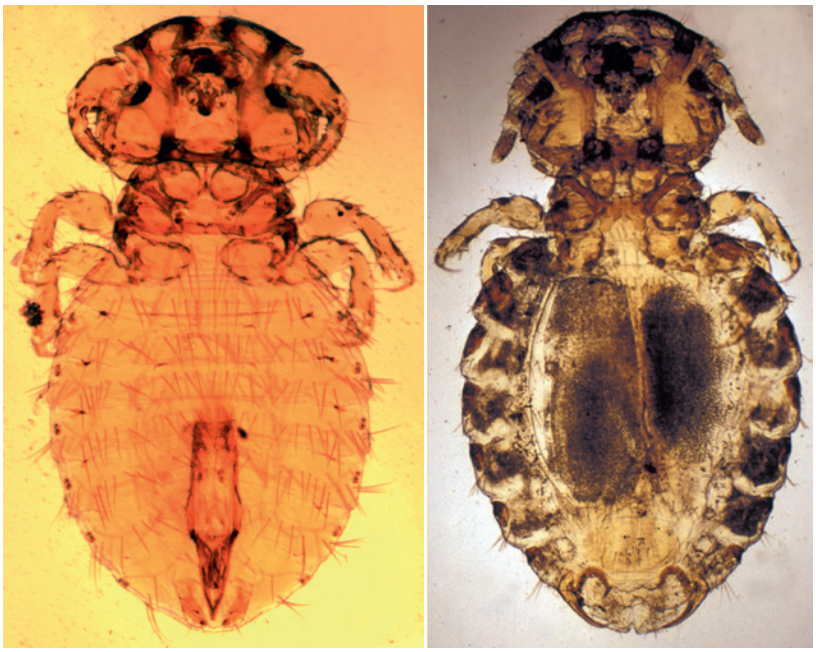


Thousands of nits can be cemented by female lice to the hair coat of a domesticated animal. This calf's tail contains thousands of nits. (From Hendrix CM, Robinson E: *Diagnostic parasitology for veterinary*

female e opulate, emale ys ggs ements
r eather, cle egins
weeks o omplete cle. ymphal dult ve
more ys emoved om ggs ch
within to weeks during warm weather, but they seldom hatch
off of Transmission usually by direct contact, but
occur via equipment contaminated with eggs, nymphs, or adults.

Louse estations **pediculosis** end o e re vere
young, , r oorly urished imals, ecially vercrowded
conditions uring older ucking roduce
anemia, hereas iting ritating urbing

Common biting lice of domestic include *Tricho dectes canis* og) . *Damalinia equi* rse), *Dama linia bovis* ow), *Damalinia ovis* ep), *Damalinia caprae* (goat), *Felicola subrostratus* ommon ucking
domestic lude *Linognathus setosus* og) . *Haematopinus asini* (horse), *Haematopinus vituli, Haematopinus eurysternus, Solenopotes capillatus, Haematopinus quadripertu sus* ow), *Linognathus ovillus, Linognathus pedalis* ep), *Haematopinus suis*



is on the right. (From Bowman D: *Georgis' parasitology for veterinarians,*

Fig. 48.7 Sucking louse *Linognathus setosus* of dogs. (From Hendrix

Diagnosis involves careful examination of coat or feathers f ested hich eveals accompanying lippings rve ood ce for e. he estation oats easily verlooked. nifying inocular headband magnifier may help with observation of adult nymphal lice crawling through or clinging to or feathers or tiny emented vidual ny bserved may e ollected orceps ced op mineral il oscope entification lice eyond rder ually essary.

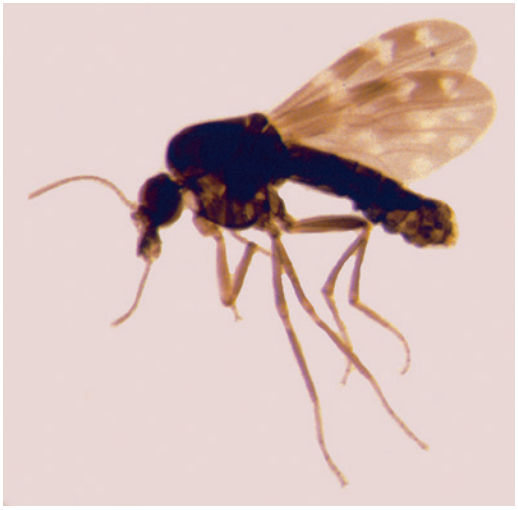
Its mbers ary egard ood ce reference, developmental stage parasitizes or produces lesions. are diverse group of insects undergo complete metamorphosis. ings mbranous, cts ructures led ers. The thparts dapted or onging or rcing/ sucking. roduce ting ites, ucking blood, roducing ypersensitive eactions, epositing ggs sores, rating uring val ough issues ough **warbles** annoyance, acting vectors intermediate to other pathogenic ents. dult ipterans equent ertebrate ermittently eed lood eferred to **periodic parasites** hen ipteran vae evelop tissues r rgans ertebrate roduce ondition known **myiasis** s periodic parasites, blood-feeding Dipterans may e lassified egard hich eeds ertebrate blood well food preference. In certain Dipteran groups only emales eed ertebrate lood; emale equire vertebrate lood or ying ggs. cond roup blood-feeding ipterans, emale equire vertebrate lood

Flies produce harm by inflicting painful bites, sucking

migration through tissues of the host, and acting as vectors and intermediate

Diptera e omplex rder cts. dults, members ve ings, hich rigin ordinal two," *-ptera,* ing."

Biting es no-see-ums") *Culicoides* ecies y e emales are loodsuckers ite. ome ecies



allergic dermatitis, whereas others transmit helminths, protozoa, viruses. Horses often become allergic to bites of *Culicoides* gnats, they will scratch rub bitten areas, thereby alopecia, xcoriations, kening ondi tion as everal ames, ncluding Queensland tch," sweat tch," "sweet ch," summer ermatitis" er ecause ften en uring mer rve ermediate or *Onchocerca cervica* matode ofilariae horses. hese ransmit luetongue irus ep.

Members of genus *Simulium* (blackflies, buffalo gnats) are small ange om ve characteristic umped ck. hey ve road, otted ings with rominent eins ings. These tiny have serrated, scissor-like mouthparts inflict ites. hey roduce reat umbers, xsanguinate

Because females lay their eggs well-aerated water, e ften icinity iftly wing reams. They e ift rs, ve reat ting ites ucking 's lood. eep from grazing or them to stampede. animal's ears, neck, d, omen vorite eeding eed n oultry, rve ermediate for rotozoan asite *Leukocytozoon.*

Sandflies *Phlebotomus* *Lutzomyia* species) are mothlike e wn rimarily or ransmission leishmaniasis iral emales uck lood.

Muscid lude ce rn le ce uck lood, but they are annoyances, because they are attracted to excrement secretions. Both act intermediate for spirurid para sites *Habronema* pp., *Thelazia* pp.) nd hey an echanically transmit cteria. rn *(Haematobia irritans)* stable ites uck lood. orn end

mately the same size as the housefly,

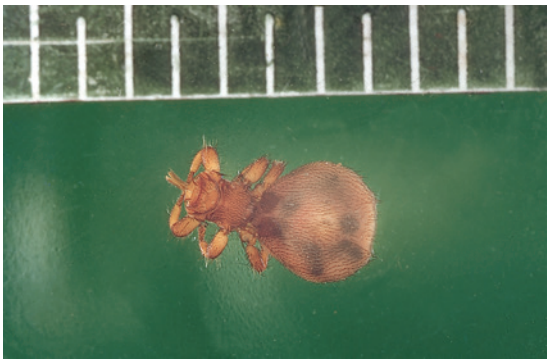
f ir ves ys or rt eriods, uring hich btains lood his tdoor wever, uring during ainy eather, nter

The le read cterial iral and orses, nd ntermediate ost or he tomach orm of rses *(Habronema)* he le *Stomoxys calcitrans,* often led biting ." proximately *Musca domestica,* hich ommon ather possessing sponging mouthparts, stable bayonet-like proboscis protrudes forward from head Fig. The usually on with head pointed upward. It sedentary does move on The inflicts painful ites hat uncture he kin nd leed reely. hen arge numbers f ck roduction fall. eef efuse raze ytime hen are cked umbers esult, do ain eight. worldwide. nited entral theastern here aised. female vid lood eeders, eeding omestic hey ually ck gs entral omen, they y ite eed ears of dogs with pointed ears, especially German Shepherds. The s f ogs ve een itten ften emonstrate of resence ied, usty lood

Chrysops species (deer *Tabanus* species (horseflies) are large, uring ng. vy-bodied, obust Dipterans ith owerful ings yes. orseflies deer are largest Diptera group, which only

emales eed ertebrate lood. ws *Tabanus* species, largest blood-feeding Dipterans. Horse are larger eer eer ve om cranial o ings.

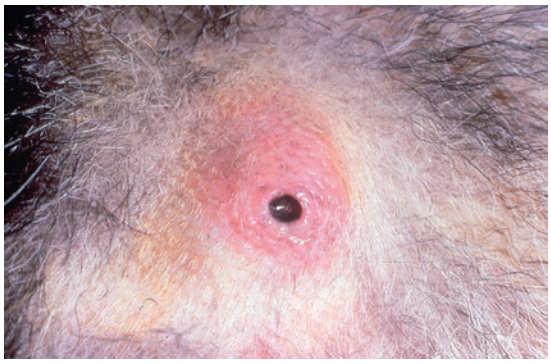
Adult lay eggs vicinity of open water. Larval stages of are found aquatic to semi-aquatic environments, often uried eep ottom onds. Adults e en ummer, ond unlight. Female eed icinity pen er ve ecip rocating, scissorlike mouthparts. They sharp, bladelike mouthparts cerate issues ozing ertebrate blood. These feed primarily on large such rses. referred eeding lude erside abdomen ound vel, gs, ck ithers. These generally feed number of times multiple feeding sites efore op eeding. hen urbed swatting eflex itching), ve ut lood ontinues oze om pen ound. ites ected horses become restless. Because they often feed on multiple ct chanical ransmitters irus quine ectious hey are rious vestock, ransmit todes, ct chanical ectors cterial, iral, rickettsial ents.



species is often found within the feces of an equine host. Note the presence of anterior hooks, with larva attached to the gastric mucosa. (From Hendrix CM, Robinson *Diagnostic parasitology for veterinary technicians,* ed 4, St Louis,

Hippoboscids ep eds *(Melophagus ovinus)* e orso ventrally ened, ingless esemble icks. hey uck blood, they spend their entire lives on (sheep). They ruritus ool. eds y, ry, o rt road, thorax rown, omen road rayish rown. The gs e rong med out laws Some y eds ve louselike" pearance, ut elated o

Blowflies, ewworm er bright coloration. The adults do suck blood, but they deposit their ggs ecaying rganic er, ptic ving The larvae of *Callitroga hominivorax* *Wohlfahrtia opaca* are only primary invaders of living tissue North merica. Other mbers racted ptic wn condary vaders. *(Gasterophilus* p., *Hypoderma* p., *Cuterebra* p., *Oestrus ovis)* e eelike adults of which do feed. The adult glue their eggs to s f eposit ntrance burrows. he vae ch enetrate Some rate xtensively ough 's ody, rs develop cally. val resent passages f ep *(Oestrus),* omach rse *(Gas terophilus),* orsal ubcutis ow *(Hypoderma) Cuterebra* are parasites of rabbits rodents, but they may infest dogs, humans. They produce large pockets subcutaneous issues ell e wn bles



like, subcutaneous sites, with a fistula (pore or hole) that communicates

(From Hendrix CM, Robinson E: *Diagnostic parasitology for veterinary*

species. This is one genus from among several

Although iny, agile ipterans, uitoes of oracious lood eeders omestic human eings emales uck lood, are or ransmission umerous protozoal, iral, matode people. osquitoes vestock, have een wn eep om razing ertain or o ede. eeding umbers swarming mosquitoes may significant anemia domestic umbers uitoes roduced om eggs were relatively small bodies of water. Mosquitoes spread malaria *Plasmodium* species), yellow fever, elephan tiasis ng eople rve ermediate or heartworm, *Dirofilaria immitis.*

Infestation by mites or ticks referred to **acariasis** Ticks are blood-sucking arachnids. They are dorsoventrally flattened unengorged state. The tick's head, which known capitu lum, serves organ of cutting attachment. It made of enetrating, hor-like ucking rgan led ypostome our ccessory pendages tting helicerae pedipalps) hat ct ensors nd upports hen he ick astens to host's body. The mouthparts may be concealed under tick's ody, xtend om order. ticks re ornate; that they re eddish or hogany, ithout markings. ome ecies rnate ve inctive hite patterns n he ark cutum ackground. dult icks ave ight legs, ith laws gs. hey ch feed n ne ee erent uring cle therefore eferred ee-host icks Fig.

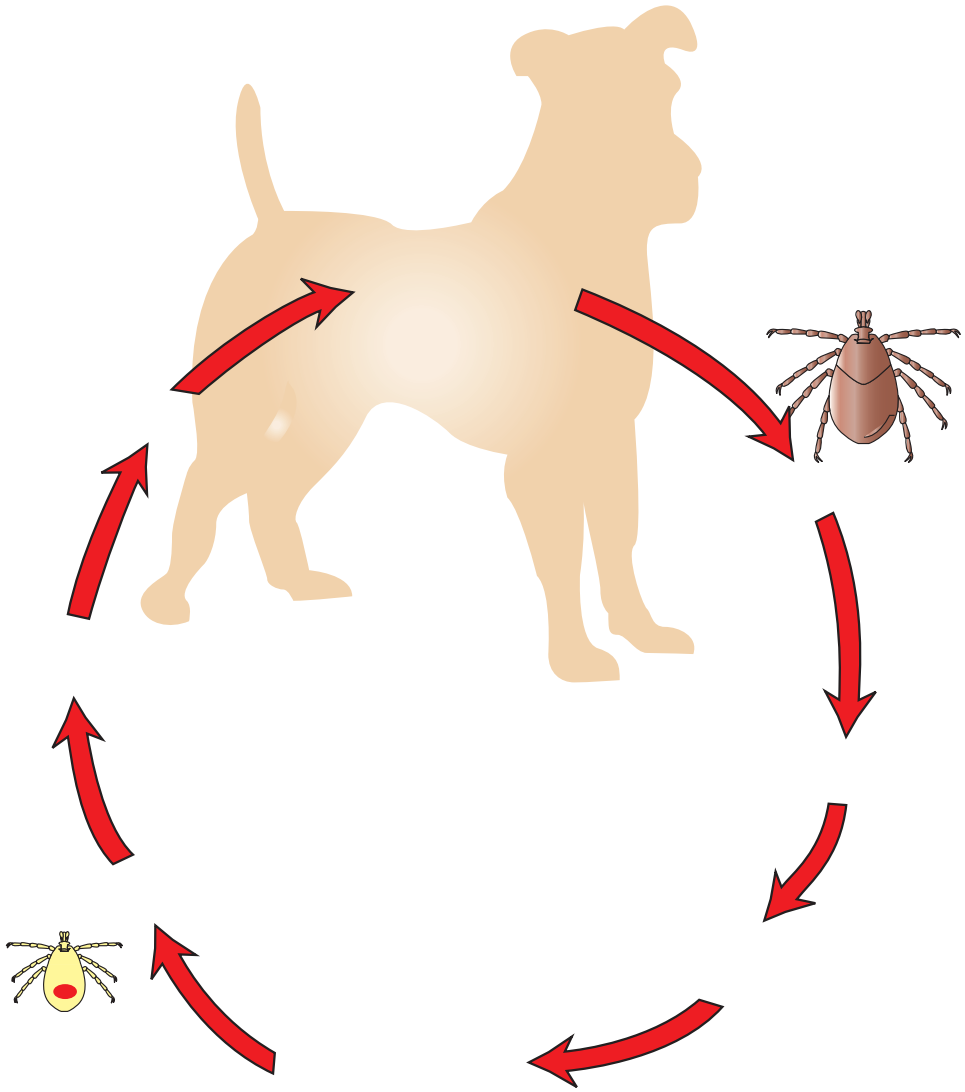
There e ypes icks: icks xodidae) ticks (Argasidae). Hard ticks are important vectors of protozoal, bacterial, viral, rickettsial The saliva of female ticks of some species toxic, produces flaccid, ascending paralysis people **tick paralysis** Tick species are com monly ciated ick alysis *Dermacentor andersoni* Rocky Mountain spotted fever tick), *Dermacentor occidentalis* Pacific Coast tick), *Ixodes holocyclus* ustralian paralysis tick), *Dermacentor variabilis* ood ick).

The dults, vae, ymphs ch eed on blood. Eggs are deposited environment. Hard ticks are dorsoventrally flattened, with well-defined lateral margins unengorged state. They have hard, chitinous covering *(scutum)* on orsal urface ody. ard icks ve rooves, margins, ches estoons) or entifica tion poses. mportant icks orth merica lude *Rhipicephalus sanguineus* . *Dermacentor variabilis* Fig. *Dermacentor andersoni, Dermacentor occidentalis, Dermacentor albipictus, Ixodes scapularis, Ixodes cookei, Ixodes pacificus, Amblyomma americanum* . *Amblyomma maculatum, Haemaphysalis leporispalustris Rhipicephalus annulatus. Rhipicephalus sanguineus* usual become lished oor wellings ennels.

There are two families of ticks: hard ticks (Ixodidae)

Soft icks ack cutum, nd heir outhparts re ot isible from he orsal urface. he ateral dges he ody re ounded. The emales eed ften, ggs Soft icks e re esiccation icks, they an ive or everal ears rid onditions. here re hree genera of veterinary importance: *Argas* species, *Otobius megnini,* *Ornithodoros* ecies.

Argas ecies e ctoparasites irds. vae, ymphs, dults ve acks evices oultry feed ht out nce hey of roductivity, vere hey rve ector for cterial ickettsial irds. *O. megnini,* tick, occurs on housed stock, dogs, even people. Only larval and two of the nymphal stages are parasitic Fig. They live external suck blood, thereby ion roduction xudate. *Ornithodoros* ecies ve rimitive or dy rees. enus robably re



important to people rodents to domestic but *Ornithodoros coriaceus* known to transmit agent of foothill abortion California.

Mites are arachnids occur parasitic free-living forms, some of which act intermediate for cestodes. Most

. Unfed adults mm
long and bluish gray in color. (From Hendrix CM, Robinson E: *Diagnostic parasitology for veterinary technicians*

parasitic mites are obligate parasites, which spend their entire life cycle on produce dermatologic condition referred to **mange** few species found on birds rodents live off of visit only to obtain blood (e.g., *Dermanyssus gallinae, Ornithonyssus bacoti* Most mite infestations are transmitted through direct contact with infested Burrowing mite infestations are diagnosed with deep scrapings periphery of lesions.

The first group of parasitic mites be classified together sarcoptiform mites. Sarcoptiform mites have several common key characteristics or features. These mites may produce severe dermatologic problems variety of domestic The dermatitis produced by mites usually accompanied by severe pruritus. Sarcoptiform mites are barely visible to naked eye, they are approximately size of grain of Their bodies are round to oval. Sarcoptiform mites have legs with pedicels or tip. The pedicels may be long or short. If pedicel long, may be straight (unjointed) or jointed. At tip of each pedicel may be tiny sucker. The description of pedicel (e.g., long or short, jointed or unjointed) may be used to identify sarcoptiform mites. Another group of mites parasitic only larvae trombiculid mites or "chiggers."

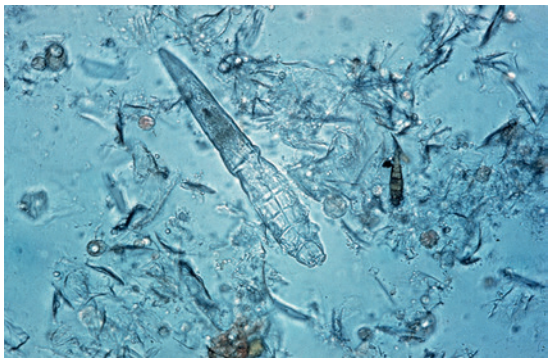
Georgis'

parasitology for veterinarians,




Sarcoptiform vided coptidae, hich urrow unnel pidermis, Psoroptidae, hich eside urface external The burrowing mites (Sarcoptidae) include ollowing: *Sarcoptes scabiei* . *Notoedres cati* . *Knemidokoptes* ecies. hese unnel superficial layers of epidermis feed on tissue Infes tations egin calized ion but y read apidly ecome eneralized. ver 15-day period, the female deposits 40 to ggs within the tunnel. After gg eposition, emale vae merge om eggs ithin ys, unnel er urface. vae ymphal minute pockets of epidermis. Nymphs become sexually active adults ithin ys, cle egins

Sarcoptic *S. scabiei* ect species, including people, but commonly seen on dogs pigs. It characterized by of intense pruritus. Each ecies wn ariety *S. scabiei,* oss- transmission oes ccur. owever, emporary estation may e ce ithout olonization otodectic mange *Notoedres* re estricted egard ange, ccurs ccasionally abbits. midokoptic mange (caused by *Knemidokoptes* fects ird s. This mite tunnels superficial layers of epidermis of pads eet. vere ere



mites resemble eight-legged

(From Hendrix CM, Robinson E: *Diagnostic parasitology for veterinary*

. This species is commonly recovered from infested rabbit ears. (From Bowman D: *Georgis' parasitology for*

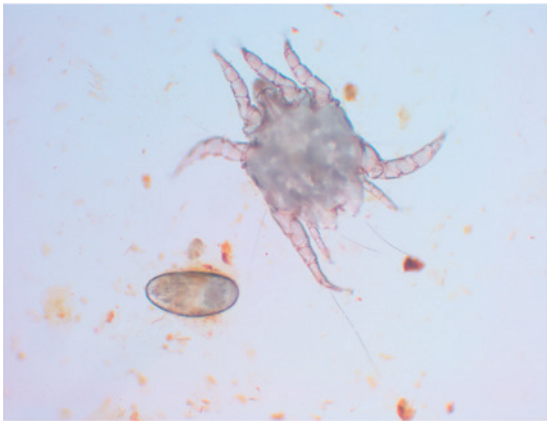
may e ected. haracteristically roduces ellow to gray-white resembles honeycomb. This condition may e ing. asites rce erlies reby ion xudate hardens on surface displaces superficially. This rocess kened, ure *Demodex* species are burrowing mites live follicles baceous hey onsidered part f rmal emodectic mange ommon ogs, calized generalized. mmunodeficiency—both enetic uced es—is cessary or estation ecome linically apparent. The characterized by of hair, thickening of pustule formation. Pruritus manifestation of ype eep apings ecover ar-shaped or

Nonburrowing mites (Psoroptidae) include *Psoroptes, Chorioptes, Otodectes.* These mites live on surface of feed on keratinized hair, tissue *Psoroptes* species Fig. *Chorioptes* species, *Otodectes cynotis, Psorergates ovis, Cheyletiella* ecies e nburrowing Psoroptic ortant ep. ctive uperficial eratinized yer ut rce ith thparts. esicles evelop, usting intense pruritus. Chorioptic mange severe tends to remain localized. *Chorioptes bovis* more important species, ommon asite *Cheyletiella Otodectes* ecies e asites ogs Members of genus *Cheyletiella* produce condition referred o uff." *Otodectes cynotis* . live external of dogs brownish, exudate accumulates, with crust formation, ulceration, sec ondary cterial ections. nfested atch equently at he ars nd hake heir eads. ead haking an esult he rupture of blood vessels hematomas of The mites be found exudate crust within

Pentastomids ongue orms) esemble ut are ctually elated thropods. *Linguatula serrata* "canine pentastome" or tongue worm." Pentastomes are usually parasites of snakes reptiles, but tongue worm parasitizes respiratory passages of dogs. It resembles lminth, ut lassified ype thropod, ecause has itelike arval tage. he entastome ggs easure by inside of egg, mitelike larval stage with inted laws ften isible.

Leeches e onsidered rue but they are often described parasitic worms. ectoparasites of uman eings, omestic eches are mbers hylum nnelida irudinea. Leeches y ve gic eneficial eterinary medicine.

The erm *hirudiniasis* erived om nomenclature, defined invasion of mouth, pharynx, r eches chment eches Leeches are voracious blood feeders; depending on number ch ecome om lood eeches ve ecently ained vor



Adult and ova of the ear mite,

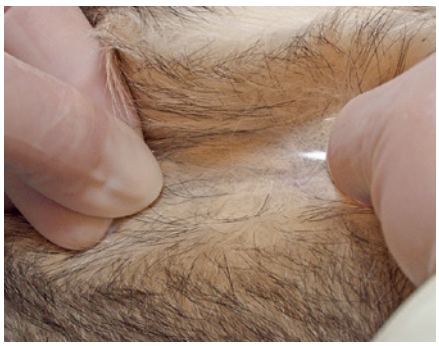
postsurgical ools econstructive ovascular urgery. *Hirudo medicinalis,* medicinal leech, been used recon structive ovascular urgery eings; uch eterinary orthcoming.

Leeches are segmented worms with slender, leaf-shaped bodies e evoid ristles. ypical ech uckers: large dhesive ucker ler surrounds eches er, ew e er; errestrial arieties.

Chapter eview uestions ppendix

- Ectoparasites of domestic include insects (e.g., lice, iting achnids icks).
- Immature or larval stages of nematodes and some dult stages of nematodes may parasitize animal's or subcutaneous tissues.
- Insects parasitize domestic are primarily members of rders emiptera rue ugs), allophaga hewing lice), noplura (sucking lice), Diptera (two-winged Siphonaptera
- Insects ve ee gs, ee inct ody egions (head, rax, omen), ennae. Arachnids dults) ve gs, ody divided into two regions (cephalothorax abdomen), ennae.
- The e-cycle icks onsist gg, va, nymph, dult. here re ymphal instar.
- Infestation hewing ucking eferred pediculosis.
- Infestation val terans eferred yiasis.
- Infestation eferred honapterosis.
- Infestation icks eferred cariasis. *Ctenocephalides felis* *Ctenocephalides canis* are dog
- Heavy estations ecially produce mia.
- Flea saliva antigenic irritating. It intense pruritus (itching) ypersensitivity, hich wn ite dermatitis r ermatitis.
- Lice are separated into two orders on of whether their mouthparts re odified or iting/chewing Mallophaga) sucking noplura).
- Flies roduce arm nflicting ainful ites, ucking lood, producing ypersensitive eactions, epositing ggs res, larval ration ough issues cting vectors ermediate genic ents.
- Adult ticks have eight legs, with claws on ends of legs, y eed ee erent uring cycle.
- Hard icks xodidae) ortant ectors rotozoal, bacterial, iral, ickettsial
- The saliva of female icks some species toxic and produces flaccid, ending alysis eople tick alysis).
- Most asitic bligate asites end entire e cles roduce ermatologic condition eferred
- Sarcoptiform vided coptidae, hich urrow unnel pidermis, Psoroptidae, which reside on surface of or within xternal *Demodex* ecies e urrowing ve ollicles baceous
- Nonburrowing soroptidae) lude *Psoroptes, Cho rioptes, Otodectes.*

Sample Collection and Handling



After studying this chapter, you will be able to:

- Describe ollection ecal om
- Describe ollection ecal om
- Explain rocedure or ollection through aping.
- Explain rocedure or ollection f ellophane reparation thod.
- Describe ollection or blood asites.
- Describe ollection vacuum leaner echnique.

Cellophane tape preparations
Fecal loop
Pooled sample
Skin scraping
Vacuum collection

Parasites ect ral vity, phagus, omach, l estines, ernal rgans of ually etected oscopic xamination feces. iagnosis ually volves entifying ecific e-cycle stages f asites eces. lude eggs, ocysts, vae, gments eworms), dult rgan xternal asites etected ough **skin scraping** **cellophane tape preparations** **vacuum collection** brushing or ombing oat.

Fecal amples hat re ollected or outine xamination hould be fresh possible. Specimens be examined within ew s xcretion efrigerated ed equal part of formalin. In older appearance of ggs, ocysts, cle ered result f asite evelopment.

efecated. eces ored ype ontainer, such pered ar. eterinary may dispense containers to their clients for purpose. In ither nly eces equired or proper xamination. deally, be ollected om erent efecated erial. ll specimens be properly identified with owner's s ecies

Fecal ollected ectly om eterinary loved er **fecal loop** . love eces emain love, love urned ied, eled. To ollect ecal op, ply of ubricant op, ently rt into ectum ollect ecal material. amples ollected ecal op for ect xamination nly, ecause ollected relatively

Several methods are used to collect feces from companion An wner ay ollect ecal ample mmediately fter nimal

Fecal specimens are collected from livestock may be obtained either ectly om vidual ectum om

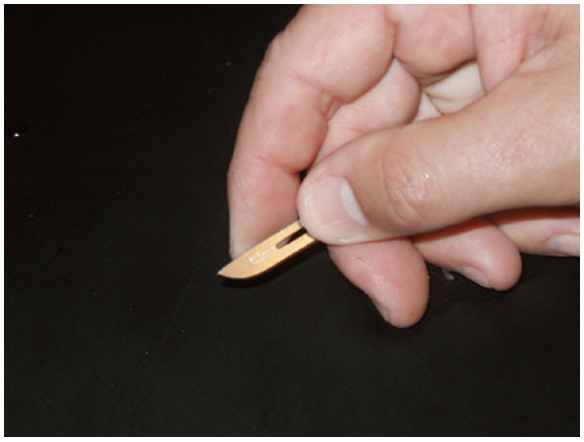
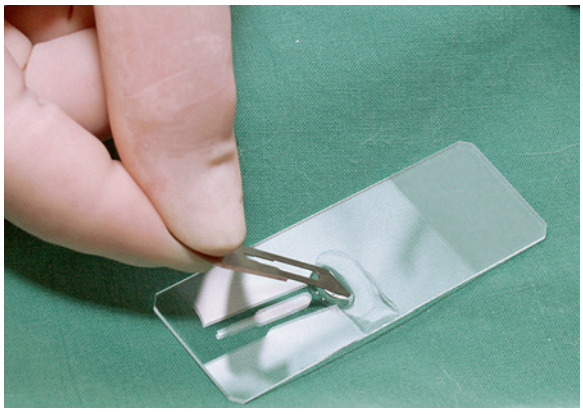
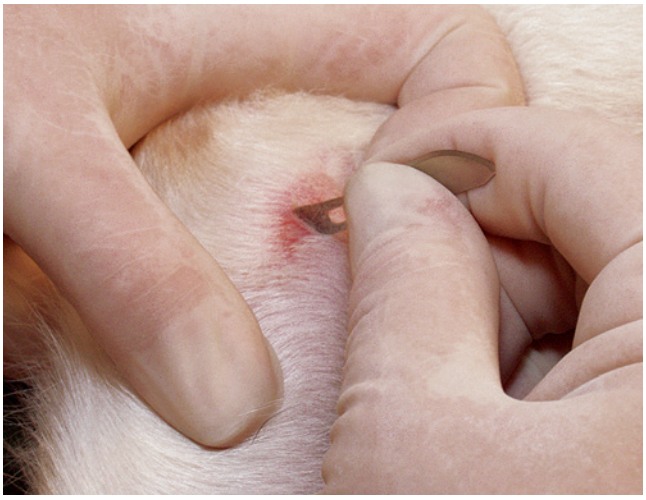


Fig. 49.2 A scalpel blade may be held safely between the thumb and



Dip the scalpel in a small amount of mineral oil before scraping



number f **pooled sample** amples ol lected ectly om vidual loved emain love, love urned ied, eled.

Pooled ollected om umber are housed ogether. The samples are then commingled in gle container. hese valuated etermine egree of ection roup. ooled ollected y ype ontainer ightly sealed. hese eled ecies en roup umber.

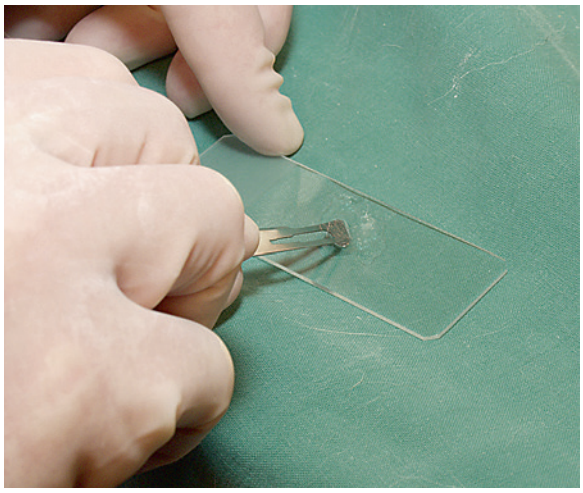
Skin aping ommon nostic rocedure to valuate uspected xternal asites. quip ment eded to collect skin scrape sample includes an lectric clipper ith lade, el ral l opper ypical likely o bor ticular asite aped ins or *Sarcoptes scabiei* ar.

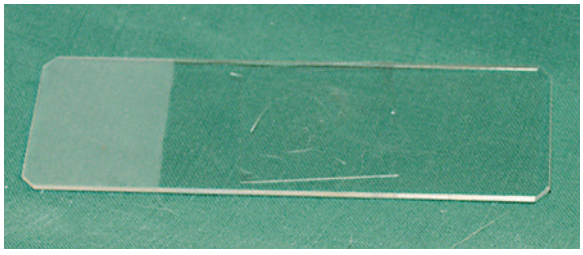
The aping erformed el lade, or without handle. steel spatula (Sargent-Welsh Scientific, Detroit, may be used. The scalpel blade be held between thumb forefinger Fig. Before aped, lade ped op mineral oil on slide Fig. or drop of mineral oil may be ced n

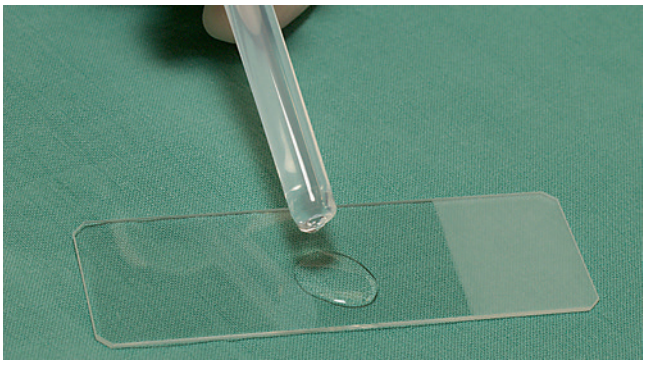
During rocess, lade er pendicular o esult ccidental verage aped approximately o ultiple aped to ease hances ollecting asite.

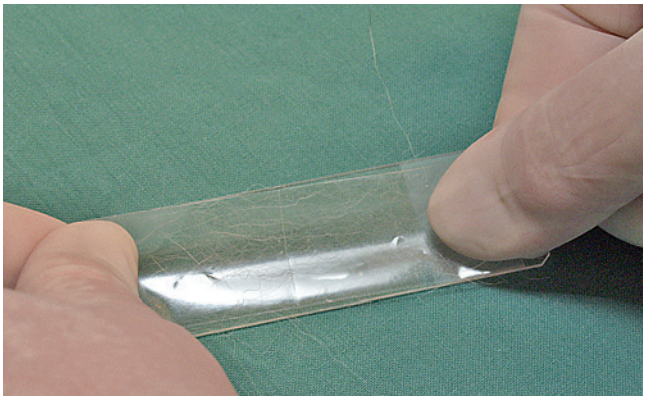
The epth f aries ypical cation asite uestion. hen or ve tunnels (e.g., *Sarcoptes* species) or follicles (e.g., *Demodex* species), scrape the until all amount of capillary blood oozes from scraped area Fig. Clipping area with no. lade efore nables etter isualization lesion removes excess impedes proper scraping interferes with collection of epidermal debris. Scraping interface between affected unaffected sites important. For surface-dwelling mites (e.g., *Cheyletiella, Psoroptes,* or *Chorioptes* species), aped uperficially ollect r usts. lipping efore cessary hen infestation urface-dwelling uspected.

All scraped debris on the forward urface the blade then spread op ral glass overslip ced erial, eady for oscopic xamination objective ns.



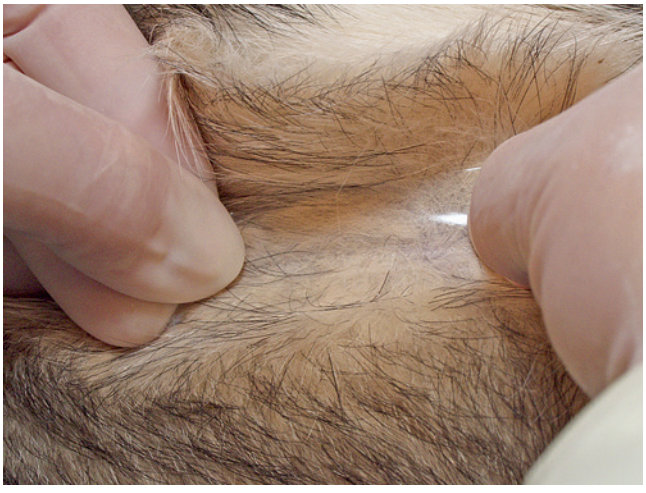






Spread the scraped material onto the glass slide in the drop of

clinical techniques,



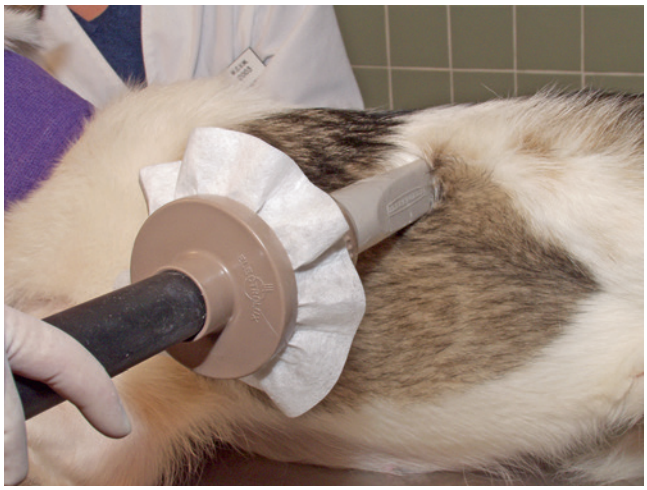
but necessary. The slide then examined microscopi cally or asites.

collection facilitated by spraying pet with insec ticide. fter ew utes, ead op Alternatively, ollected ooth omb available eterinary upply ore et ore.

Parasites rimarily resent urface ollected acuum leaner. Gentle estraint ient enerally equired, ecause ise f acuum leaner ress ients. perform procedure, place piece of filter paper over end of acuum ube chment pply acuum to ultiple ecially here isible debris resent.

When attempting to demonstrate lice or mites live primar ily n urface *Cheyletiella, Psoroptes, Chorioptes* species), cellophane tape preparation may be used. Clear cellophane tape applied to to pick epidermal debris . ibbon ral ced slide, dhesive urface ced mineral il dditional ral overslip may e ced revent om rinkling,

Necropsy ostmortem xamination) ortant thod of nosing luding asitism. ypes of ns roduced ure asites, dult asites found ody vity issues, opathologic examination ected issues or eteri nary echnicians esponsible or ollected for ure roperly ontained, reserved, labeled, ped.



parasites that are found are gently removed with thumb forceps and

contents of the digestive tract, including scrapings from the interior

parasites that are found are gently removed with thumb forceps and

Two thods re ed to recover rasites om gestive tract at necropsy: the decanting method the sieving method Boxes either method, veterinary techni ust parate erent estive ract work ith ontents ch ction vidually.

Parasites recovered from digestive tract may be preserved alcohol or neutral buffered formalin for later identi fication. Occasionally bladder worms or cysticerci may be found attached o iscera omestic handled ith ecause ladder allergenic oonotic.

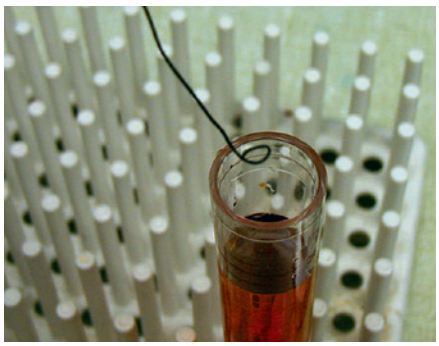
Franklin Lakes, ll be labeled with owner's animal's date of collection. (See Chapter nit or re etails egarding lood ollection.)

Sterile quipment equired or ollection blood from Blood may be collected with f ringe edle acutainer ecton ickinson,

Chapter eview uestions ppendix

- The nosis ntary asitism equires nation f ecal ecimen or resence ggs va), oocysts, vae, eworm roglottids, dult asites.
- Fecal om ollected er evacuation ecal op thermometer.
- Fecal amples rom roups arge nimals re ften ollected ooled
- Parasites eside rimarily urface be ollected ellophane thod.
- Deep scraping required to collect parasites reside ollicles.

Diagnostic Techniques



After studying this chapter, you will be able to:

- Describe ross xamination ecal
- Describe rocedure or erforming ecal ect
- Describe rocedure or erforming ecal ion.
- Discuss dvantages dvantages arious ecal flotation utions.
- Describe rocedure or erforming entrifugal flotation.
- Explain aermann paratus.
- Describe rocedure or erforming oat
- Describe dified nott's

Baermann technique
Centrifugal flotation
Direct smear
Fecal sedimentation
McMaster technique
Modified Knott's test
Simple fecal flotation
Zinc sulfate

Parasites cated ral vity, phagus, stomach, small large intestines, internal organs, f iagnostic tum, feces, lood, rine, ecretions he eproductive rgans, nd he epidermal yers ollected or xamination should e s esh ossible xamined oon ossible, preferably ithin er ollection. take roper recautions hen orking revent contamination ork nvironment nsure ersonal hen ents ransmissible eople. Wear loves, equently er soap, lean ect ork er xaminations. addition, ortant quipment equently.

TECHNICIAN NOTE

The maintenance of good records important. Label with lient's ollection, ient's ecies. ecords lude entification or mation, procedures performed, results. adequate history ludes uration any dications iven, nvironment, accina tions eceived, ocking ensity, umber ected or rd ck xaminations ccompany

The microscopic examination of reliable method or etection asitic ections. inocular microscope 0 bjective nses eded. stereo microscope helpful for identification of gross parasites. rated ometer cessary determine sizes specific differentiations of some parasitic stages, uch ofilariae hapter of ook). enerally ed dium overslip op.

be roughly stematically iewed bjective lens, eginning orner overslip opposite nd. arasite ually focus ubbles dge overslip. ny erials or bjects bserved iewed erified re ow erful bjectives. ood orking wledge binocular microscope of adjustments needed to produce Kohler illumination are essential for parasitology examinations.

Depending ient's ory, ely ecific asite estations uspected. or mation lps hoice erformed.

The asitologic xamination eces egins ross nation of noting consistency color well presence f lood, ucus, dor, dult arasites, oreign odies (e.g., string). Normal feces be formed yet soft. Diarrhea or constipation an ccur ith arasitic nfections. ost ecretions are clear moderately cellular. yellowish discoloration with excessive mucus could signal infection. Blood be fresh right ed tially ested molyzed), pear k eddish rown lack ry. xcessive enerally ritation ucosal mbrane, with roliferation ucus-producing ells. ommon asitic ections espiratory stem wer digestive tract. dult parasites such roundworms tapeworm proglottids entified omitus eces.

the consistency and color of the sample as well as the presence of blood,

Fecal direct smears are of evaluation procedures. Feces, tum, gma, lood bserved echnique escribed rocedure ype

and 400 magnification for eggs, cysts, tropho

requires quipment erials, apid or asite ecal or **direct smear** reparation btained om f ecal op ectal rmometer er uring s emperature). rocedure volves small eces microscopically for presence of eggs larvae. This method will low or isualization rophozoite protozoal asites uch *Giardia*

Unfortunately, direct alone adequate examination or asites. isadvantages lude of eces xamined, hich ufficient etect parasite burden, of extraneous fecal debris on slide, which could be confused with parasitic material. However, e orporated outine asitology examination.

Flotation methods are based on differences specific gravity of e-cycle asites eces fecal ebris. **Simple fecal flotation** xample ion method rocedure ecific ravity efers eight of bject ompared eight qual olume distilled er, tion solved material solution. Most parasite eggs have specific gravity that etween Table Flotation

and then cover the top with the cheesecloth squares while pouring the suspension into the shell vial. If using a metal strainer, pour the suspension

l the shell vial to form a convex dome (meniscus) at the rim. Do not overfill

solutions e ormulated ecific ravity her f ommon asite va. herefore, va urface ution. aturated utions ugar various ion utions ve ecific gravity ranges between Fecal debris eggs with specific gravity greater of flotation solution do at. uke ggs enerally vier ecific gravity f outinely ion utions, ew exceptions *Paragonimus, Nanophyetus* y usually ecovered echnique. ematode vae recovered, but they are frequently distorted result of crenation, thereby entification ecific ravity flotation solution too high, plug of fecal debris floats traps parasite stages thereby obscuring them from view.

Commonly ion utions lude ugar, dium chloride, dium rate, nesium ulfate, **zinc sulfate** Each solution advantages disadvantages, including cost, availability, efficiency, shelf life, crystallization, corrosion of equip ment, of Table Selection often determined by ype ractice ommon asites ncountered ea. ecific ravity ion utions checked ith ydrometer djusted more r re er ution. eaving xtra ystals of n ottom ution nsures ution urated.

Several ompanies ve ckaged ion lude prepared utions dium rate ulfate, osable plastic ials, rainers hey onvenient, ut they are more expensive. Supplies to conduct flotation procedures an cquired hrough uppliers cientific quip ment hemicals. roducts vailable otential or ersonnel oming ontact ecal material

This procedure principle to flotation procedure except er ution ed, eci men rained emove xcess ebris. overslip dded, specimen centrifuged to for minutes. Centrifugal orce overslips ce uring provided tubes are balanced. bacteriology loop then used o emove op om urface ube, drop examined microscopically Fig. **Centrifu gal flotation** re nsitive ion. ecovers more eggs cysts from time Procedure However, equires ccess letop entrifuge ccommodate otation uckets. ed-angle work well for procedure described, but they be adapted for procedure by filling tubes omitting overslip uring entrifugation.

The **fecal sedimentation** procedure used when suspected para sites produce va that are too large to be recovered with standard flotation va). ecal ed volume of water strained into centrifuge tube. The e entrifuged or utes lowed emain undisturbed for to minutes. The supernatant poured off, and ipette sed emove rop he ediment. ne rop from per, wer ortions diment

*Check the specific gravity with a hydrometer. Store solutions at room temperature.

. These kits are based on the principles of the simple flotation procedure. (From Hendrix CM, Robinson *Diagnostic parasitology for veterinary technicians,*

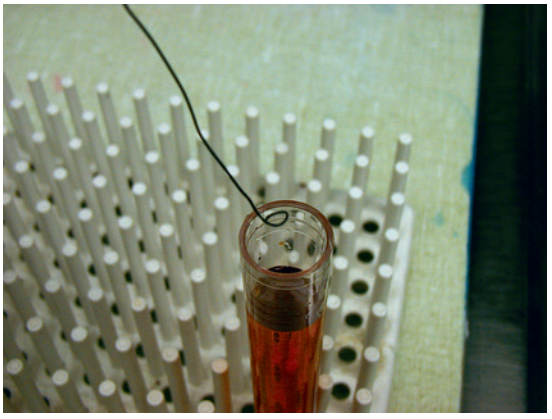


Fig. 50.3 The use of a bacteriologic loop to transfer a drop from the top of a fecal flotation emulsion after the centrifugation procedure. Note that loop is bent at a 90-degree angle to the wire handle. (From Hendrix

removed, ee ops xamined oscopi cally. edimentation oncentrates asite ell ecal debris rocedure ecause ebris, asite may e bscured rom iew. his echnique lso ore abori edimentation rimarily hen ections suspected. ggs orted flotation solutions with higher specific gravity, thereby o ecognize ew ops etergent e dded er urfactant emove xcess ebris om

This thod ften ecover va *Oxyuris* worms). t an lso elp ith he dentification apeworms. piece f ellophane rapped ongue epressor with dhesive aised, tongue epressor ressed mly emoved, plied water on then examined microscopically Procedure

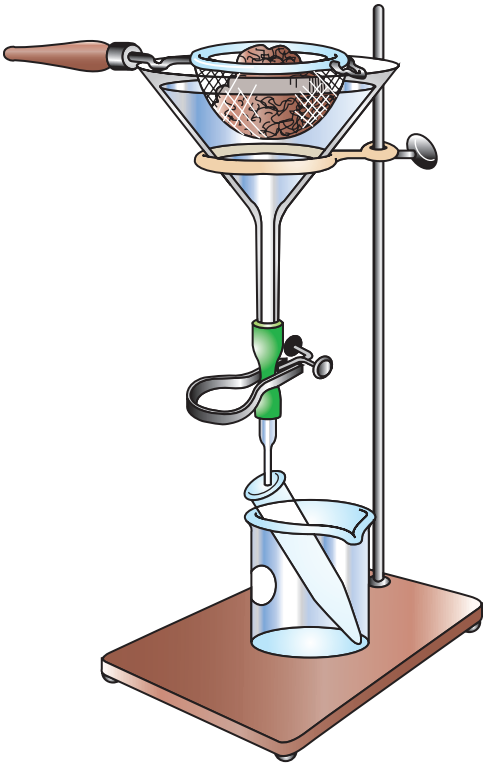
The **Baermann technique** metimes ecover vae from fecal The procedure requires construction of Baermann apparatus, which consists of large funnel supported ing ce ubber ubing ched of funnel placed collection tube. The fecal placed funnel on top of piece of metal screen Fig. Warm ater armed hysiologic aline assed hrough he

a balance tube of equal weight that contains another sample or

from the tubes by lifting straight up, and then place

into a 50-mL conical centrifuge tube. (Suspending a funnel over the tube

*For centrifuges with fixed-angle heads, do not fill the tubes, and



Georgis' parasitology for veterinarians,

he vae imulated ve er, they then to bottom of apparatus. drop of material n he ollection ontainer xamined icroscopically for resence vae. aermann echnique recover nematode larvae from feces, fecal cultures, soil, herbage, tissues Procedure The warm water stimulates vae o rate elax. hey sink o he ottom he pparatus, here hey an ollected relatively free of debris. Free-living larvae must be distinguished from asitic ecially ollected round, om om rbage. equire expertise f xperienced lminthologist. reserve

which is more practical in a practice setting, is to use long-stemmed plastic

the slide for larvae, and identify them. The slide can be passed

bottle of water can be used to moisten the fecal mixture if it becomes too

recover developing stages. Some larvae migrate up the wall of the jar and

by dding ormalin ellet or ubmission expert. Kill free-living larvae by adding hydrochloric acid to pellet, examine preparation without fixation. Unfortunately, identification of motile larvae more

The aermann echnique erformed eces omestic when lungworm infections *Dictyocaulus, Aelurostrongylus, Filaroides, Crenosoma, Muellerius, Protostrongylus* are suspected. Ideally, be fresh collected rectally. For dogs Baermann technique be used when infection with *Strongyloides* species are suspected. If fresh, fecal culture may be needed to distinguish first-stage hookworm vae om st-stage vae *Strongyloides.* The d-stage iform va *Strongyloides* nostic haracterized phagus larva orked ipartite) are en hen handling *Strongyloides* ecal ures ecause oonotic potential f rganism.

Some parasites produce intestinal bleeding. This bleeding may be evident ank lood ecal kened eces. Some estinal leeding nly entified hemical testing. his eferred ecal ccult lood esting. everal types of kits are vailable for this procedure, and ey rimarily act o entify resence moglobin

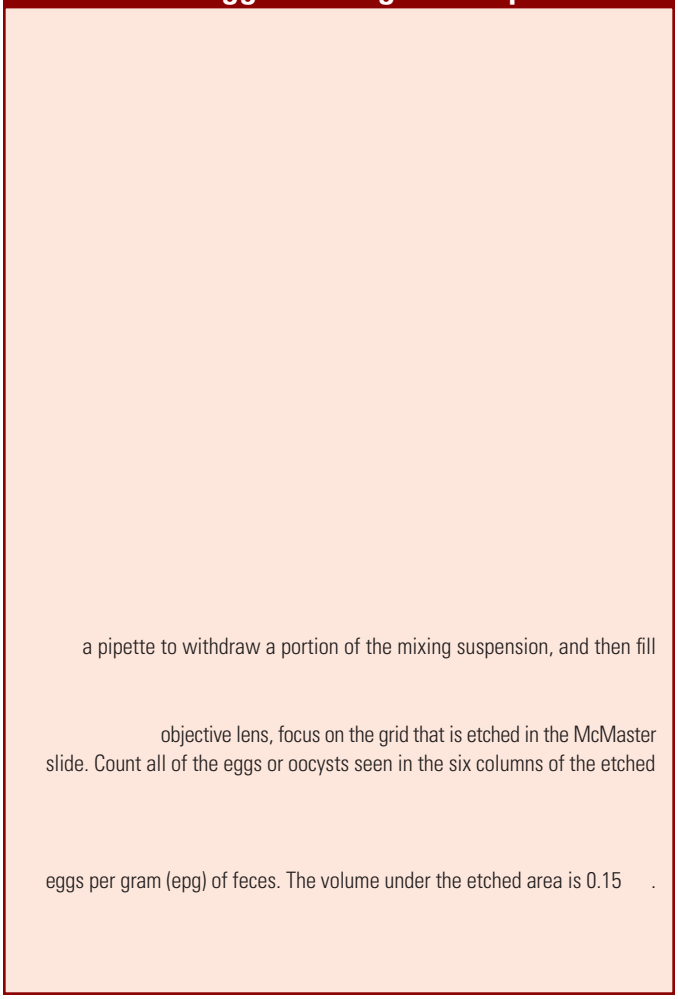
The examination of vomitus may diagnosis of parasitism. Some parasites (e.g., *Toxocara canis* are often present omitus ected ients.

Fecal ure erentiate asites ve ggs or vae inguished xamination of fresh fecal Procedure Trichostrongyle eggs ruminant eces re ndistinguishable om trongyle ggs. Small strongyle ggs rse ecal guished om rongyle ggs. st-stage okworm vae og r ee-living matode vae soil r n rass inguished om st-stage *Strongyloides* vae. fter ecal ure, d-stage vae of y f asites entified enus vel. Because life cycles, pathogenicity, epidemiology of some species y er, entification cessary or roper treatment ontrol. dentification equire xperienced lminthologist.

An dditional ype ecal ion dified **McMaster technique** his echnique rovides imate number of eggs or oocysts per gram of feces, primarily used ith vestock ecies rses rocedure igi nally dapted om echnique eople ected with okworms imate orm opulation However, impossible to calculate actual worm population especially livestock horses, because many factors influence gg roduction, umber ggs roduced varies ith ecies umber orms resent.

Typically, livestock horses are infected with several species of orms ecies re rolific pathogenic rs. ddition, ften esult om damage roduced ure asites.

Quantitative Egg-Counting Technique



a pipette to withdraw a portion of the mixing suspension, and then fill

objective lens, focus on the grid that is etched in the McMaster slide. Count all of the eggs or oocysts seen in the six columns of the etched

eggs per gram (epg) of feces. The volume under the etched area is 0.15 .

ruminants, the parasites of interest coccidia trichostron gyles. n rses, asites erest strongyles. Both trichostrongyles strongyles infect ruminants rses. ggs richostrongyles rongyles be readily distinguished from one another, they are referred to ollectively rongyle ggs. evertheless, xcess of e onsidered ive ections, hereas f re derate ections. gg count indicate low level of infection or severe infection which asites ecoming ure. gg always e erpreted iew bserved; age, x, utritional vel ocking density f rd ck.

Egg ounts ve een pidemiologic vestigations rd ement rograms redictors pasture ontamination ransmission otential or erent geographic egions vidual ormation applied toward prevention programs involve strategic of broad-spectrum anthelmintics and pasture rotation schemes aimed educing ective vels ures xposure rates. hen rd onducted, vidual taken om rd. gg to nitor evelopment esistance lmintics. gg counts e one efore reatment ee eeks er treatment to determine effectiveness of anthelmintic used evelopment esistance iven orm opulation.

---

y ecognize ertain ructural acteristics f rophozoites ugol's methylene blue are common are used with direct smear rocedure. hese tains ot reserve he lide, ut hey do cilitate xamination ecimen, reby identification r.

If protozoal parasite be identified with direct smear, fecal contains protozoal trophozoites be dried; stained ith right's, diagnostic oratory.

The cid-fast echnique entify *Crypto sporidium* ecies eces. ryptosporidium asite gastrointestinal tract of many including human beings. The ocysts o ter, undetectable flotation solution to inexperienced eye. etect ocysts ecal

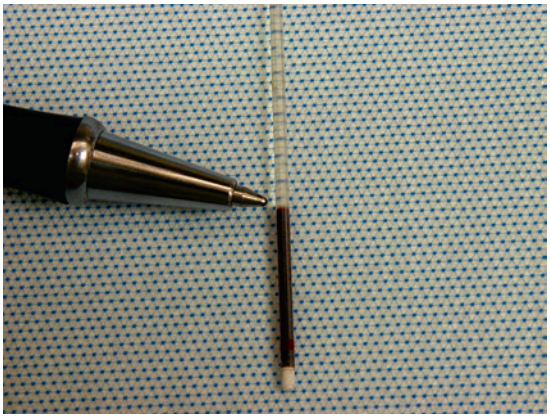
Diff-Quik tain an sed elp ith he dentification *Isospora* ecies. n estinal ucosal examined or nostic hizonts, rozoites) asite. rocedure volves ucosa the ejunum nd mearing he crapings nto icroscope lides. After the slides are dried, they are stained ith Diff-Quik and examined ith il-immersion bjective

---

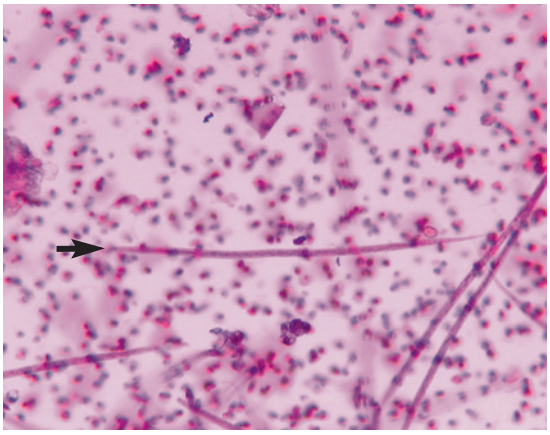
The xamination lood eveal dult asites ir arious e-cycle ither ee lood intracellularly. ariety thods or eter mination. hin lood repared way or hite lood ell erential prepared. The reparation or ype escribed hapter asites ried with flow to feathered edge of slide. Parasites may e cated etween ells, urface ells, cytoplasm of cells. Thin blood are effectively used to study rphology rotozoan ickettsial asites. parasitemia w, ections lood or uffy oat re ffective ecause oncentrates er olume ells rocedure

The uffy oat oncentration echnique or detection f rotozoa ickettsiae hite lood ells. microhematocrit tube centrifuged done for packed cell volume etermination. icrofilariae rotozoa e ound op olumn technique uick, ut erentiate *Diro filaria immitis* om *Dipetalonema reconditum.*

se the file to etch the glass below the buffy coat. Snap the tube by applying



syringe, and fill it with tap water. Allow a few milliliters of air



(From Hendrix CM, Robinson E: *Diagnostic parasitology for veterinary*

The direct drop of blood evaluations, although ccurate esult used. drop of anticoagulated whole blood examined micro scopically. he vement asites xtracellular be etected thod.

The er echnique thod esigned oncentrate microfilariae blood Procedure The principles applied are o dified nott's xcept blood ough illipore er, hich ollects microfilariae. ommercial etergent ysing solution erential rocedure quicker dified nott's ut ferential haracteristics ofilariae bvious. Identification volving haracteristics ed able is ot ossible ith ommercial its, ecause he haracteristics of ofilariae ion ormalin.

The **modified Knott's test** used to concentrate microfilaria, lp erentiation *Dirofilaria* om *Acantho cheilonema (Dipetalonema)*. The procedure requires mixture of blood formalin centrifuge tube. The mixture incubated room temperature for to minutes then centrifuged for minutes. The supernatant poured off, drop of methylene blue s dded he ediment he ube. rop his ixture n ransferred or oscopic valuation

Using the Modified Knott's Technique

*Artifact of formalin fixation.

pply a coverslip, and examine the sediment microscopically for microfilariae

Procedure echnique oncentrates ofilariae while yses ed lood ells. hen reparing formalin solution, important to remember formaldehyde equivalent to formalin. It important to er hysiologic repare ution, because hysiologic oes yse ed lood ells. or accurate differentiation of microfilariae, microscope must ve rated ometer. ccurate differentiating haracteristics ody ody e f haracteristics consistent. The modified Knott's technique detect occult heartworm ections.

The modified Knott's test, the buffy coat smear, and

ariety f vailable entify igens ibod o ecific asites. rity

enzyme-linked immunosorbent assay principle. The tests are highly accurate precise, they detect occult infec tions. anine tworm ections *Toxoplasma* ections are routinely diagnosed with methods. The method involves noclonal ibodies etect igens adult heartworms serum or of dogs. These proce dures are rapid to perform. They are more sensitive ecific ofilariae etection thods. meri eartworm ociety rently ecommends igen-detection thods or outine eening. ntigen-detection methods e referred ofilariae oncentration thods ecause uch errant culate ofilariae or only ery rt owever, igen vels lood of ected oo etect. ther thods (e.g., adiography) mployed

Approximately of heartworm-infected dogs have occult infections. Occult infections are characterized by lack of circu lating ofilaria. hey ccur ection et ent, opulation dult tworms onsists nly immune reactions of to microfilariae eliminate stage from bloodstream. Occult infections occur infected with adult heartworms are given heartworm-prevention dications vermectin roup, ecause interfere ith ogenesis erilize orms.

The identification of specific protozoal parasites (e.g., *Crypto sporidium, Giardia* are performed with of molecular diagnostics, uch olymerase eaction.

Cellophane tape preparations, described previously, be used to recover external parasites live primarily on surface of mites, lice, Parasites live fol licles or burrows are usually diagnosed with of standard skin-scraping rocedures.

Samples y ollected om espiratory genital tracts with cotton These be examined micro scopically ith reparations or tol ogy ranstracheal ronchial used o ecover asites espiratory stem. arasites inary stem ually ecovered sediment xamination echniques. mpression used or racellular asites. hey be ful or asitic, oplastic, emortem ostmortem. rocedures described n ore etail nit requently, rotozoal rgan produce systemic These organisms may be located eticuloendothelial ells ymph des, ver, ung, bone row, en, rain, ys, uscles. ddition, ver, ungs, ymph des, one row, en er out ed normal ells lood, reby ollect asitized ells. oxoplasmosis, hrlichiosis, esiosis asitic diagnosed tology echniques.

Skin apings nostic ool or ermato logic conditions, especially mange domestic Because

some e well urrows ollicles eep pidermis, uperficial apings roductive. ther mites ve re uperficial yers roduce crusty r eep apings equired estations. ometimes erfere with isualization oaking potassium ydroxide ution ve eratinized eleases ite estations ually calize specific cations ially, epend n ecies volved. er, ecome generalized re *Sarcoptes* *Cheyletiella* es ransmitted eople roduce pruritic reactions require attention. The specific identification f es ccomplished onomic keys.

Cheyletiella infestation be diagnosed by combing coat of infested over piece of black paper observing paper for moving ndruff." *Otodectes cynotis* infestations of the external be diagnosed with otoscopic examination or y ebris oscopically ral

Tritrichomonas foetus flagellated protozoan parasite of reproductive tract of early-term abortions repeat reeding. rganism om abomasum orted etuses, terine harges, aginal and reputial ashes. owever, umbers resent re sually low, culture of materials facilitates diagnosis. Occasionally, estinal ellates ontaminate be erentiated om *T. foetus,* hich ee ella one flagellum attached to undulating membrane. Isolates f *T. foetus* e ropagated ough veral of culture medium, whereas intestinal flagellates usually Materials may be collected shipped to diagnostic facilities for ulture nd dentification. nPouch Biomed iagnostics, White ity, xcellent ransport dium.

Tables nostic haracteristics internal asites omestic ppendix verview oonotic ernal asites.

Chapter eview uestions ppendix

Continued

Diagnostic Characteristics of Internal Parasites of Domestic Animals—cont'd

Diagnostic Characteristics of Internal Parasites of Domestic Animals—cont'd

From Sirois M: *Principles and practice of veterinary technology,*

Diagnostic Characteristics of Blood Parasites of Domestic Animals

From Sirois M: *Principles and practice of veterinary technology,*

- Methods for examination of fecal specimens include both gross microscopic examination of feces.
- The microscopic examination of fecal samples may involve the direct examination of or concentration of material with fecal flotation or fecal sedimentation techniques.
- Hemoparasites (blood parasites) be identified with micro scopic examination of peripheral blood smears or by variety of concentration techniques (e.g., modified Knott's procedure).
- Fecal concentration methods are preferred for identifica tion of parasite ova, larvae, oocysts feces. Larger volumes of feces are used compared with direct smear, thereby more likely developmental stages will be seen, present, feces.
- Fecal flotation solutions with specific gravity of to are used to float parasite ova, cysts, larvae while fecal material to bottom of container.
- The fecal solutions of choice are Sheather's sugar solution, sodium nitrate solution, sulfate solution.
- Fecal centrifugation method of choice for fecal flotation testing, because floats higher concentration of ova, cysts, larvae compared with fecal flotation.
- Fecal sedimentation used to test for trematode eggs, which are heavier other parasite eggs thus do float well.
- •A blood will reveal such blood parasites *Babesia* *Theileria* within red blood cells. However, be used for accurate differentiation of *D. immitis* *A. reconditum*
- The buffy coat technique and the modified Knott's technique be used to properly differentiate between *D. immitis* *A. reconditum*

Unit Outline
Chapter 51: Sample Collection and Handling,
Chapter 52: Preparation of Cytology Smears,
Chapter 53: Microscopic Evaluation,
Chapter 54: Cytology of Specific Sites,

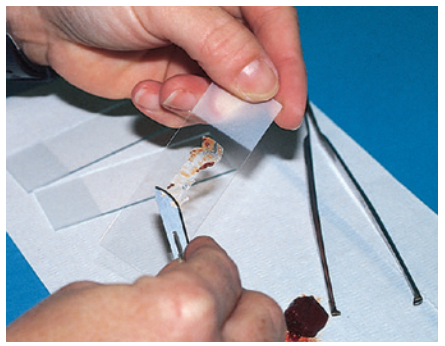
The objectives for this unit are:
Describe the collection and handling of cytology samples.
Describe the preparation techniques that are used with cytology samples.
Discuss the general procedure for the evaluation of cytology samples.
Describe the general characteristics of samples taken from inflammatory lesions.
Describe the general characteristics of samples taken from neoplastic lesions.
Discuss the microscopic appearance of cells in cytology samples taken from a variety of sites.

Exfoliative cytology is the study of cells that have been shed from body surfaces. It refers to the examination of cells that are present in body fluids (e.g., cerebrospinal, peritoneal, pleural, and synovial fluids), on mucosal surfaces (e.g., in the trachea or vagina), or in secretions (e.g., semen, prostatic fluid, milk). The primary purpose of the cytology evaluation is to differentiate inflammation from neoplasia. The types and numbers of cells that are present in a properly collected and prepared cytology specimen can provide rapid diagnostic information to the clinician. Samples for cytology evaluation can be collected quickly and do not generally require special ized materials or equipment for proper evaluation. With careful attention to quality control—including the use of appropriate collection, preparation, and staining techniques—a high-quality cytology sample can be obtained. Such samples yield valuable results for the clinician and often preclude the need for more invasive procedures to determine a patient's diagnosis, treatment, and prognosis.

Cytology provides somewhat different information than a histopathologic evaluation. Histopathology observes cells in relation to their neighboring cells. The histopathologist evaluates the cellular architecture. The prepara tion of a sample for histopathology involves several complex steps and some specialized equipment. To prepare a sample for histopathology, the tissue is first immersed in fixative. Several steps are involved in dehydrating the tissue before it is imbedded in paraffin. The paraffin block is then sliced, and the slice is mounted on a glass slide before it is stained. Cytologic evaluations observe the cells individually or in small groups. The cells in a cytologic preparation are randomly distributed, with no evidence of their in vitro relationship to each other.

For additional sources for this unit see the Resources Appendix at the end of this textbook.

Sample Collection and Handling



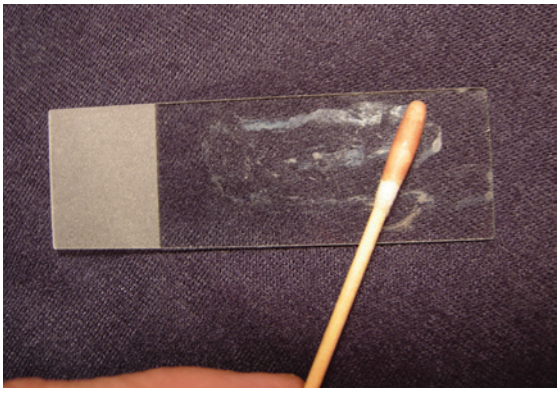
After studying this chapter, you will be able to:

- List echniques or ollection cytology
- Describe rocedure or ollecting bing.
- Describe rocedure or ollecting imprinting.
- Describe echniques or edle iopsy collection.
- Describe echniques or ranstracheal collection.
- Describe eneral rocedure or ollecting centesis.
- List thods oncentrate tology

Abdominocentesis
Arthrocentesis
Centesis
Fine-needle biopsy
Paracentesis
Punch biopsy
Thoracocentesis
Transtracheal wash
Tzanck preparation
Wedge biopsy

Cytology from on animal's body or are btained om urgical rocedure ollected swab, scrape, or imprint technique. **Fine-needle biopsy** be used for some well **Centesis** refers o ollected om ody vities.

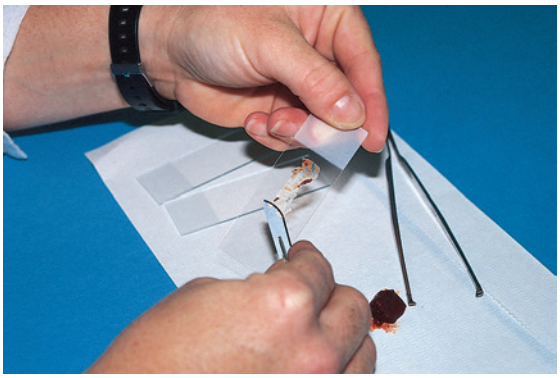
Swabs e enerally ollected nly hen rints, apings, ates de, uch racts vaginal ollections. bed erile cotton or rayon Fig. Sterile isotonic (e.g., be used to moisten swab. Moistening helps o ell uring ollection reparation. or ollection aginal estrain osition levated. inse ulva, rt ubricated eculum smooth plastic tube to point just cranial to urethral orifice agina. ells ollected ve xfoliated, or d, om aginal pithelial ells utrophils)



through vagina from uterus, especially erythrocytes during proestrus and rus the bitch. If collecting om ed istened. After ollection, ently olled surface f oscope be rubbed across slide surface, because excessive ell

ontain xcess may erfere valuation ffect, entle cessary. e riefly ough entle om yer ve xcess be voided, ecause ill estroy ellular omponents side om ram rocedure, only cumstance er hich tology equire plication.

Smears f apings repared om issues lected uring cropsy urgery om xternal living Scraping advantage of collecting many cells om issue, refore dvantageous hen



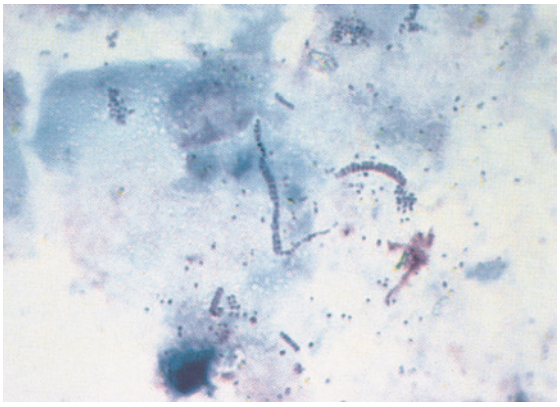
A scalpel blade can be used to collect cells from solid masses.

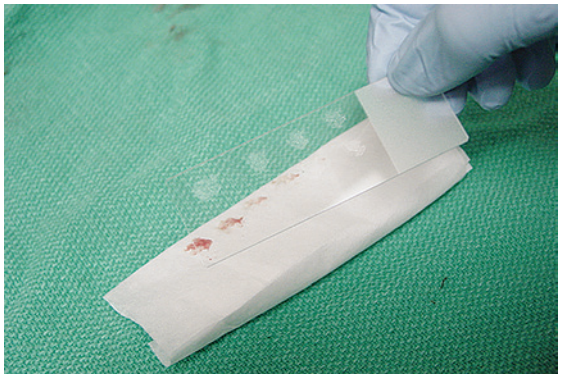
lesion m ields ew ells. dvantages scrapings e re ollect they collect only superficial result, scrapings from superficial ften eflect nly condary cterial ec tion or inflammation-induced tissue dysplasia, which markedly hinders ir or oplasia.

To btain aping, calize el blade perpendicular to lesion's cleaned blotted surface. Pull lade cross veral erial ollected on lade ransferred oscope slide . read re echniques described er hapter or reparation from ates

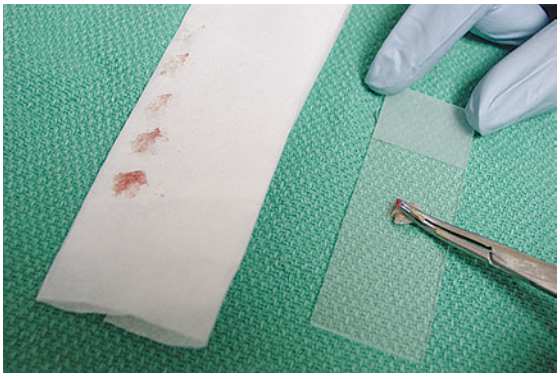
Imprints, which are referred to impression smears, may be prepared from external lesions on living or from tissues removed uring urgery cropsy. ollect equire estraint, ut ollect ewer ells scrapings usually contain greater of contamination (bacterial ellular) ompared edle iopsies. As esult, rints om uperficial ften eflect nly cal condary cterial ection ion-induced tissue ysplasia. cteria issue ys kedly er ccurate neoplasia.

The **Tzanck preparation** type of imprint collection be d n xternal erform rocedure, repare lean rinted efore cleaned, esignated umber should hen leaned ith aline-moistened urgical onge reimprinted with slide marked imprint Number The lesion n ebrided eimprinted hich ked rint umber resent, er side f rinted ked imprint Number Fig. Imprints from tissue exposed by removal of scrapings or from exposed tissue oth ollected.





(Dermatophilus congolensis)



tissue fluid before the imprint slide is prepared.

To ollect rints om issues ollected uring urgery necropsy, lood issue emoved om urface eing rinted lotting clean, absorbent material Fig. Excessive blood tissue inhibit tissue cells from adhering to glass slide, thereby producing oorly ellular reparation. ddition, xcessive its ells om reading shape they usually have on air-dried smears. If delay occurs from ime ollection rint en, el lade xpose urface efore blotting urface. microscope ouched lotted urface tissue to be imprinted. Multiple imprints are generally made on each slide Fig. Several slides may be imprinted slides e vailable or ecial cessary.

If obiologic erformed ortion ollected ody vity enetrated peritoneal thoracic cavities joints), then area of aspiration urgically repared. therwise, reparation essentially equired or accination enipuncture. alcohol

For ation thod edle iopsy, 25-gauge edle ringe. fter rated issue ler edle ringe The se f eedle arger han 1-gauge or spiration eldom advantageous, even for firm tissues such fibromas. hen larger needles are used, tissue cores tend to be aspirated, thereby result oor ield ee ells uitable or tologic preparation. ddition, er edles end reater blood ontamination.

The e f ringe nced onsistency issue eing ated. ofter issues, uch ymph des, often y e ated uccessfully ringe. tissues, such fibromas squamous cell carcinomas, require er ringe dequate uction or ufficient collection f ells. ecause ringe known or efore ation, ringe ful e.

Fine-needle iopsies ollected om including ymph des, dular ernal rgans. For taneous rovide dvantage ver methods y voiding uperficial ontamination cterial ellular). owever, ewer ells ually ollected with r thods, uch apings. edle iopsy e erformed ither ation naspiration method.

The o ated mly enetra tion f ontrol ection needle. The needle, with syringe attached, introduced into enter f rong gative ressure plied by ithdrawing unger proximately ee ourths olume ringe everal e ut ation rel of syringe contamination of by aspiration of

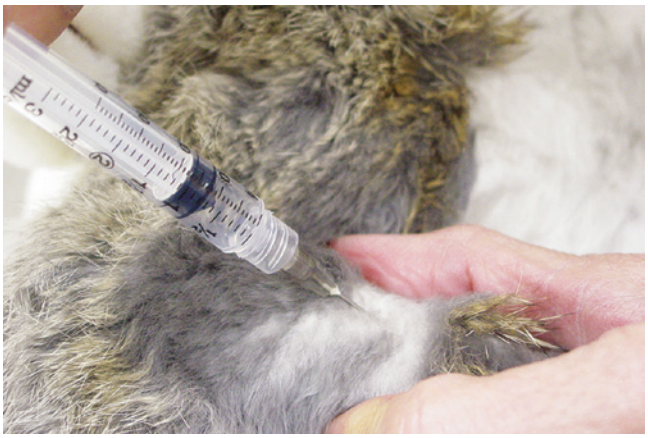


Fig. 51.8 Collection of a sample by the fine-needle biopsy nonaspira

issue urrounds voided. ccom hen nough low edle be edirected ved veral ithout danger f edle's ving gative ressure maintained during redirection movement of needle. However, hen nough or edle be edirected ved ithout er edle ving the ass, egative ressure elieved uring he edirection nd movement edle. uation, gative ressure applied nly hen edle igh-quality ollections often o ve ate erial isible ringe sometimes ven edle.

When erial bserved edle er several eas gative ressure elieved om ringe, edle ithdrawn om ext, edle emoved om ringe, awn o ringe. edle eplaced nto syringe, issue rel needle expelled onto middle of glass microscope slide by rapidly epressing unger. hen ossible, veral repara tions de, escribed ollowing ctions hapter.

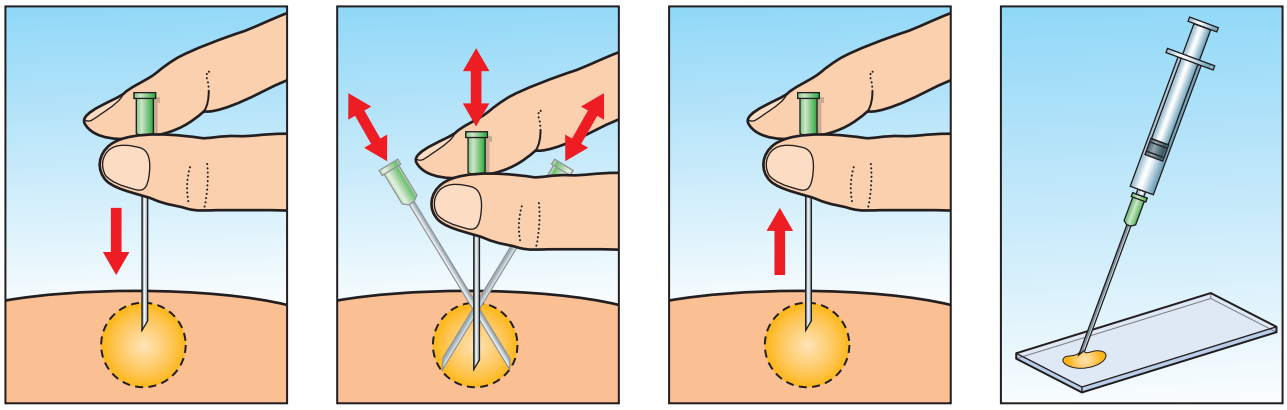
This echnique erform ation, ecause does volve ecting ringe edle unger held mly enetration o lp ect edle. auge edle roduced into ringe unger emoved attached o edle cilitate needle moved rapidly back forth through five to imes ract. ells ollected lary ction. edle emoved om attached to syringe been prefilled with air. The erial xpelled nto oscope rapidly depressing plunger Fig. The expelled material e ed echniques escribed or reparation

Generally, enough material collected to make only one smear. Therefore, rocedure epeated ee erent nsure dequate umbers eas f valuate.

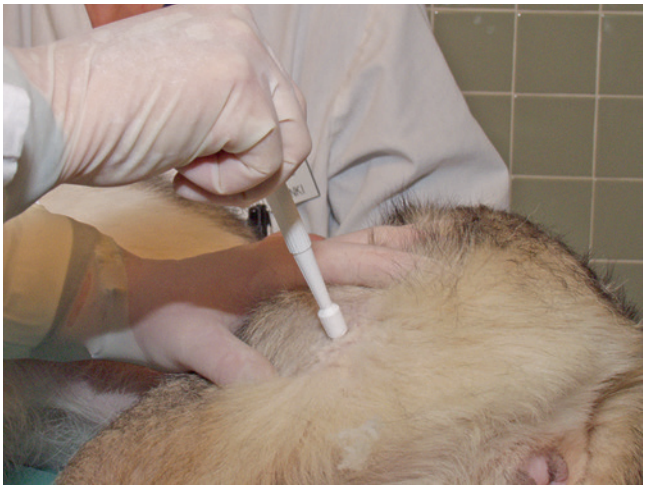
Tissue biopsy of piece of tissue for cytologic or histopathologic xamination oth. any rgans issues—including idney, iver, ung, ymph ode, rostate, kin, pleen, thyroid—and (tumors) may be biopsied. Biopsy tech niques lude entle rasion lade, edle ation, xcision, luding **punch biopsy** ndoscope-guided biopsy. The technique used varies with tissue to be biopsied. Considerations lude cation, ccessibility, ure issue. rospective iopsy lipped care en o void ritation ucement inflammatory tifact. ither ecom mended r cessary. ubbed, any urface ebris urbed, because y ffer aluable nostic lues.

Elliptic **wedge biopsy** specimens are commonly obtained with scalpel. The wedge biopsy offers advantages of large, variably sized ecimen riented gy echni Solitary lesions are often best removed with technique. When edge iopsy ecimen en, el lade d o xcise ntire edge en om area f ough ransition one, rmal issue. The pathology technician then trim specimen on long axis o rovide he athologist ith lide hat hows bnormal tissue, ransition one, rmal issue.

The h iopsy echnique umber dvantages ver wedge iopsy, ticularly eed roce dure. eyes taneous iopsy disposable iopsy ommonly Fig.



Disposable biopsy punch.



With he iopsy unch, -mm pecimens equire utures, or biopsy specimens require only one or two sutures. Ideally, o ee iopsy ecimens arious should e ollected. ocal nesthetic hould nfiltrated round e. he ently otated ection punch lade ctioned issue otated nly ection, ecause ck-and-forth tion eases elihood ecimen esult shearing orces.

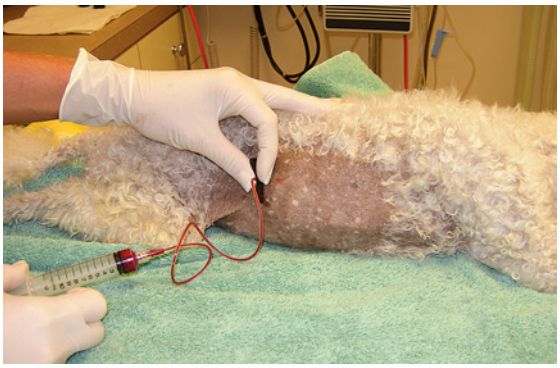
Tissue amples or istopathologic xamination ften re xed neutral phosphate-buffered formalin. To ensure adequate fixation, of tissue more wide be placed ight ars ontain ormalin proximately times specimen's volume. large tissues, be emoved ler ormalin ontent er een ed or

After ecimen een ollected ither edge or h thod, ecimen ently emoved grasping issue orceps. Fresh, unfixed tissue extremely fragile. Specimens collected by endoscopy ently om ndoscope with erile ecimen lotted gently n er owel emove xcess lood ced l ce ooden ongue epressor dboard). Skin ecimens placed with subcutaneous side own. ntly ressing iopsy ecimen cilitates adherence lows or roper omic rientation ecimen oratory. llow issue nto splint."

Specimens ched splint" rsed floated specimen side down fixative. The timely placement of ecimen ive itical, ecause tifactual changes y egin ccur ute er iopsy specimen btained. dequate ecimen ion equires hours before processing. Formalin freezes

Specimens collected by endoscopy should be gently flushed from the tip of the endoscope.

freezing may substantial artifactual damage unfixed specimens. Therefore, to ensure proper fixation without freezing artifact, specimens remain room temperature for efore xposure ossible xtreme



Collection of peritoneal fluid by abdominocentesis.

edle rted venth ighth ercostal ce along anial ect ib. bdominocentesis performed ient eral recumbency Fig. The needle introduced into ventral abdomen o ight proximately o rocedure htly erent or exotic f ollection eflect olume resent or ressure ody vity. nced by echnical roficiency perator of onscious ooperation volume ollected ecorded ollection. Certain ross haracteristics ody ecorded collection, including color turbidity. Subsequently, total nucleated cell counts, cell types, morphologic features are etermined.

Color turbidity are influenced by protein concentration cell umbers. ross oloration eased urbidity be d y rogenic ontamination eripheral lood, recent r morrhage, ion, ombination onditions.

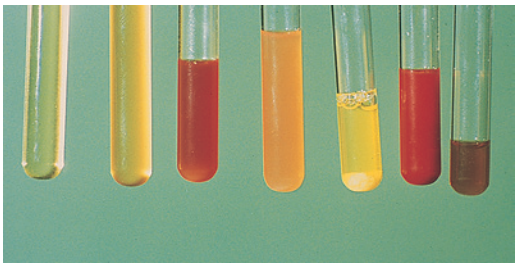
The erforation uperficial essels uring ollection result ontamination eripheral lood. uch dmixture of blood with may be obvious streak of blood otherwise clear some stage during collection. Blood-tinged may be result of recent or hemorrhage into body cavity being sampled. Both peripheral blood contamination ecent morrhage esult upernatant ed (erythrocyte-rich) diment er entrifugation. ecent lysis imparts reddish discoloration to supernatant. Hemor rhage ccurred ys reviously enerally ellowish upernatant esult moglobin reakdown products), usually with erythrocytic sediment Fig.

Cytologic xamination determining morrhage. lumps elets be bserved ecent ften rogenic perator-induced) hemorrhage. These clumps are obvious after approximately . lood resent vity or veral before crophage estion rythrocytes ecomes vident. If he emorrhage ccurred ay efore ollection, emo globin breakdown products such hemosiderin may be seen

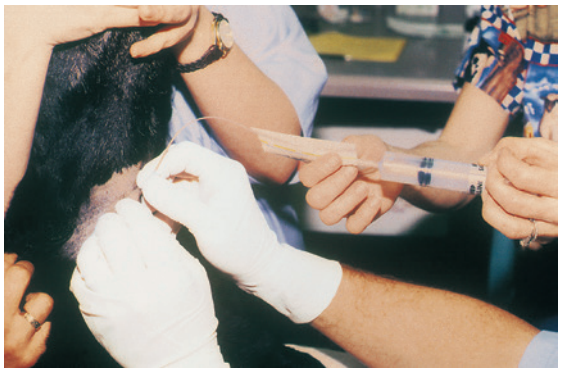
---

Centesis efers roduction edle ody cavity r rgan or pose emoving lection f om eritoneal vity **abdominocentesis** **paracentesis** racic vity **thoracocentesis** e commonly erformed mall nimal ractice. ystocentesis the percutaneous ation om inary ladder) discussed or tologic valuation e ollected om olumn, om joints **arthrocentesis** om ye. neral required for collection of cerebrospinal synovial ueous itreous umor.

Before ollection eritoneal ural e ptically repared quipment upplies e athered. reparing veral om soon ollected lpful, ntiful upply slides eady. ortion lected thylenediaminetetraacetic ube. 21-gauge needle commonly used be attached to ringe. or ients, racocentesis usually performed with standing position



Gross appearance of various effusions *(left to right):*



crophages. nflammation olor ody with he egree urbidity eflecting eukocyte umbers. olor may ary om ff-white ed-cream ty brown, epending umber rythrocytes volved egrity ells resent.

Consistent erminology hen escribing ell types. ecific etails rphologic eatures ch ell type eutro phils crophages valuated or resence vacuoles r hagocytized erial. eoplastic ells evaluated or hanges, uch otic basophilic toplasm.

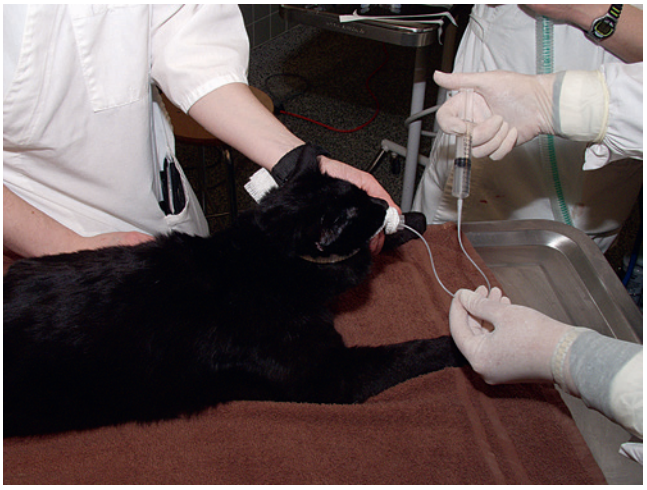


Fig. 51.17 Collection of transtracheal wash sample by the orotracheal

The tologic valuation btained om rachea, bronchi, r ronchioles monary

Transtracheal washes y e er formed y ter ough ndotracheal ube tized rotracheal proach), ough passages (nasotracheal approach), or through trachea (percutaneous approach) conscious sedated The transtracheal oute minimizes pharyngeal ontamination of ecimen, ut vasive rocedure onsequently requires aseptic technique. These procedures are commonly used oth

The ercutaneous ethod equires he 0-gauge through-the-needle ter. yngeal lipped of aseptically prepared. small (usually to f ocaine cted icothyroid brane urrounding edle rted trachea ough icothyroid mbrane, ter dvanced umen rachea erile physiologic ution ough ter rate of to per kilogram of body weight. hen oughs, ringe unger etracted veral times collected be placed into sterile tube. amples rocessed diately.

This echnique ay referred ery mall ractious nimals. The ient htly tized, propriately sized endotracheal tube be placed. polypropylene urinary or jugular catheter then placed through endotracheal tube, infused described for percutaneous method. ed ubber ter ut en ensure hat he atheter oes ot ollapse uring spiration, hich may ccur hly iscous epending on level of will often cough, be withdrawn within few seconds evaluated. Bronchoalveolar vage rotracheal echnique is sed o ollect amples pecifically rom he ower espiratory tract. ronchoscopy referred thod or erforming BAL, but specialized equipment (e.g., bronchoscope) required.

With either method, only small of infused ill vested ollection. ubsequent coughing f ontain ells erest, eleased uring oughing ubsequent lection ollected er een eturned to e. ced erile ube,

ion de ollection. uch ften contaminated, ut metimes or valuation when ollection ields ufficient ormation.

Samples with mucus generally correspond to small numbers of cells) be centrifuged low speed, smears e repared om diment. ontain much ucus ually umerous ells) ed centrifuge concentrated before made. Total nucleated cell ounts ually erformed racheal Cell umbers ubjectively ecorded om valuation smear. tracheal from normal contains few ells, ually often pears oscopically osinophilic rands y nmesh ells. pithelial ells rincipal ell type resent.

The cytologic evaluation of samples btained om the cavity y uring vestigation affect per way. rmal into cavity through with syringe tubing then aspirated. This procedure referred to Such ecimens rocessed or racheal Various abnormalities may be demonstrated with procedure, such inflammation secondary to sepsis, fungi yeasts, neoplasia. hese onfused love owder, which y resent ecimens.

When tologic de ell of oncentration ells ory. (Such concentration may be helpful even higher cell counts.) Four thods escribed.

To oncentrate entrifugation, entrifuged for utes o ith entrifuge adial arm ngth centrifuges) pm pm. fter entrifugation, supernatant separated from sediment analyzed for total protein oncentration. diment esuspended ew drops f upernatant ently ube.

op f esuspended diment ced de lood ompression reparation technique. hen possible, several smears be made each technique. The addition of may help cells to adhere to microscope slide. fter drying, slide may be stained with omanowsky

Gravitational dimentation thod concentrate ells. ommonly or erebrospinal evaluations. method glass cylinder (which e de tting ube) ched oscope The ube dipped lted ced ells proximately lowed out utes settle. The supernatant then carefully removed with pipette, ube etached. xcess ently emoved with rbent er.) ied, esidual efully aped Romanowsky

The mbrane ration ohol-diluted used o oncentrate ells. mbrane ore ually satisfactory. er ers ch ringe vailable. The ermitted ravity eed om ringe rel, gently injected through filter more drop/sec. The er er ept rizontal ribute ells evenly. ncreased esistance ration uggests ores are ecoming bstructed ells rotein, re e orced ough er. ration r, smaller olume ough er er esults less-crowded reparation.

After emoval om ringe er, er ed thanol or utes. olders vailable or ling er er uring ion richrome-type omanowsky unsuitable ecause er er oo ensely. satisfactory procedure be performed by immersing er er or utes ch ecific ubstance following rder: distilled er. ollowed utes matoxylin, utes er, utes ollak's minute acetic acid, minute ethanol, minutes -propyl alcohol (propanol), minutes mixture of propanol xylene. The finally undergoes three rinses of minutes each xylene. all stages, filter must be treated gently o void dging ells. epending filter, y ed uitable efore cement on oscope ell er oded with mounting medium with refractive index to of er er proximately overslip plied.

Cytologically, ells rapped mbrane er rounder seen after sedimentation therefore may be harder to distinguish), they are slightly different of focus. Furthermore, filter produces patterned background may be distracting. This distraction minimized by ensuring verstained propri ate ing dium. ore enerally oo e rap ee cteria. itatively, re ells collected y ration dimentation thods.

As ith ny uid ow ellularity, ytocentrifuge an sed for reparation tologic uch quipment enerally oo xpensive or eterinary ractice ustify purchasing. owever, ften eferral oratory. This echnique lows ells oncentrated circular ea

---

Chapter eview uestions ppendix

- Cytology from on animal's body or are obtained from surgical procedure be collected by swab, scrape, or imprint technique.
- Fine-needle biopsy be used for some well
- Fine-needle biopsy be performed by either aspiration or nonaspiration method.
- Centesis refers to collection of from body cavities.
- The collection of for evaluation of trachea, bronchi, and bronchioles can be performed with the transtracheal wash technique.
- Transtracheal be performed with either percuta neous or endotracheal technique.
- Concentration techniques may be needed for with low cellularity.

Preparation of Cytology Smears

After studying this chapter, you will be able to:

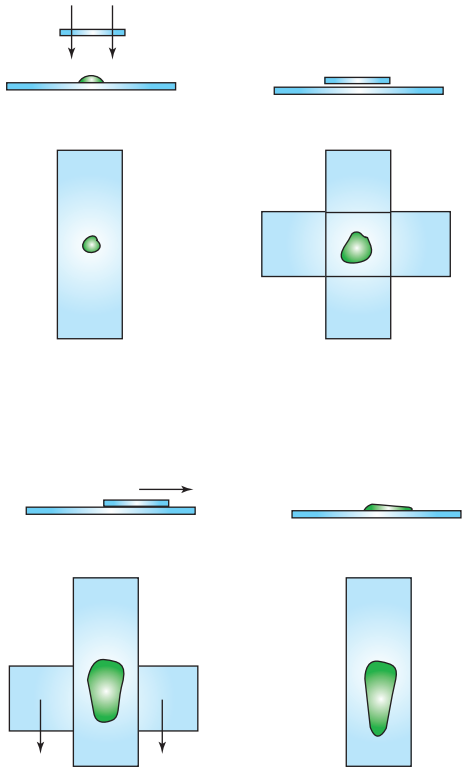
- List thods repare tology or valuation.
- Describe echnique or erforming ompression smear.
- Describe echnique or erforming
- Describe echnique or erforming
- Describe echnique or erforming dified compression
- Describe rocedure or tology
- List otential roblems encountered, escribe ossible utions.

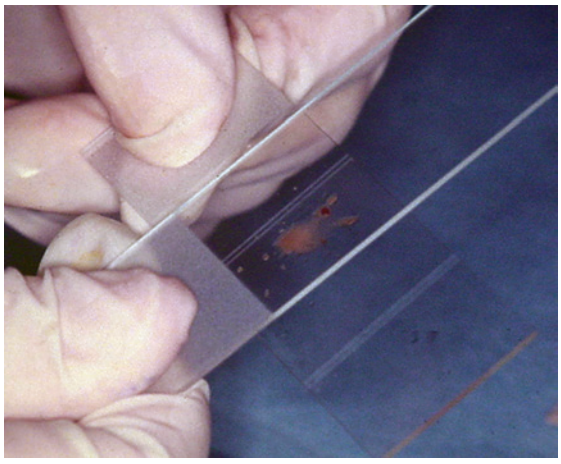
Compression smears
Fixative
Impression smears
Line smears
Modified compression preparations
New methylene blue (NMB)
Romanowsky stains
Starfish smears

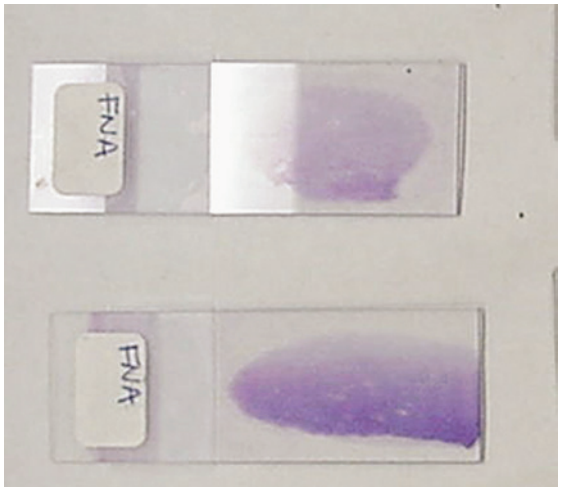
Cytology rocessed ariety echniques, including **impression smears** **compression smears** **modified compression preparations** **line smears** **starfish smears** or wedge smears. The exact type of preparation depends on characteris tics f he ample. ome amples ay lso equire oncentration by entrifugation. equire icoagulants preservatives. everal erent reparations ually de from ch lows or dditional nostic esting without dditional ollection. ariety echniques are available for cytology specimens. Some require processing re rocedure.

Several methods may be used to prepare smears for cytologic evaluation luding ymph des er rgans. xperience erson reparing haracteristics nce hoice preparation technique. combination of slide preparation techniques refore uggested. ome tologic reparation techniques escribed ollowing ctions.

The ompression echnique, hich metimes eferred squash prep," yield excellent cytologic smears. However, xperienced ften ields eadable tologic smears, ecause oo ells uptured ufficiently read. ompression reparation de expelling ate nto gently placing second slide spreader slide) over aspirate horizontal ight rep slide) Figs. The spreader slide then quickly smoothly cross rep ownward ressure be placed on spreader slide, because may exces sive cell rupturing, unable to be interpreted.







A portion of the aspirate is expelled
Another slide is placed over the sample,
thereby spreading the sample. If the sample does not spread well, gentle

to place excessive pressure on the slide, which could cause the cells to

but this may also result in excessive cell rupture.

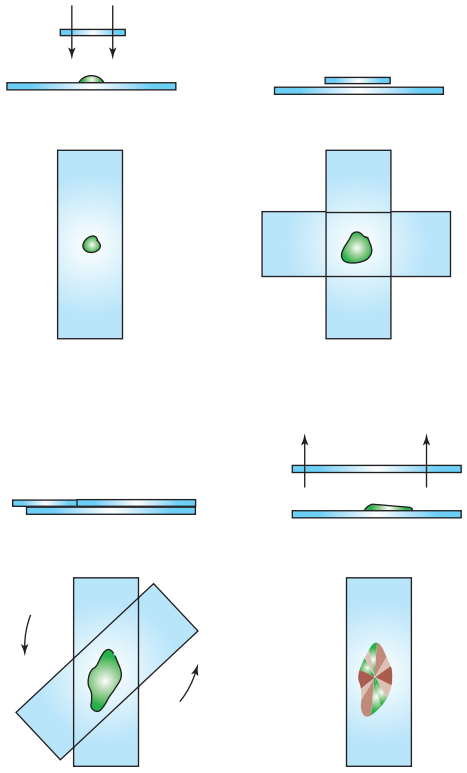
Preparation of a compression smear. Completed com

dification ompression reparation
tendency o upture ells ay econd lide ver spi
rate, otate cond egrees, ward
Fig.

combination procedure involves spraying aspirate onto
le oscope rep ce
rep rizontal urface,
spreader slide backward 45-degree angle the first slide until
es ontact proximately ate.
The spreader slide be smoothly rapidly forward,
lood ext, reader ced
horizontally ver ck ate ight
to he rep lide. he eight he preader lide sually uf
ficient to spread material. void temptation to compress
es ually. eep reader rizontal,
and se uick, ooth otion slide reader ide cross
rep

This procedure makes compression preparation of back
third f ate. ate
untouched. This procedure leaves front third of aspirate
gently read. ate agile issue,
contain ufficient ct ells or valuation. ck
aspirate been spread with forces of compres
sion preparation. If aspirate contains clumps of cells are
o read, lumps ufficiently read
ck reparation. ate
cellularity, emains re oncentrated
fficient udy.

Another echnique or preading spirates rag spirate
peripherally veral ections oint ringe
needle, roducing echnique
tends o agile ells, ut oes low yer
of issue emain ells. ometimes
layer f revents ells om reading ell er
feres ith valuation ell etail. sually, wever,
acceptable resent.



gentle digital pressure can be applied to the top slide to spread the

a squash preparation with subtle ridges and valleys of cells

Cytologic smears be prepared immediately after col lection. hen possible, for cytologic examination e ollected thylenediaminetetraacetic tubes. mears repared ectly om ell-mixed r om diment entrifuged edge (blood) smear, smear, or compression preparation techniques. The cellularity, viscosity, homogeneity of influence lection echnique.

When be concentrated by centrifugation or centrifuged ample ow ellularity, he ine mear echnique may be used to concentrate cells Fig. drop of ced lood technique xcept reading aised ectly upward proximately ee-fourths ough smear, ielding ontains uch her oncentra tion of cells rest of slide. Unfortunately, excessive f emain revent cells om reading ell.

The compression preparation technique often spreads viscous cks ticulate erial etter lood echniques. lood technique usually produces well-spread smears of sufficient cellularity om mogeneous ontain cells/ ut ften roduces ufficient ellularity from ontaining ells/ he technique oncentrate ellularity, but ften oes ufficiently read ells om hly el lular In general, translucent are of low to moderate cellularity, hereas paque ually ve ellularity. Therefore, translucent often require concentration, either by centrifugation or by technique. hen possible, concentration entrifugation referred.

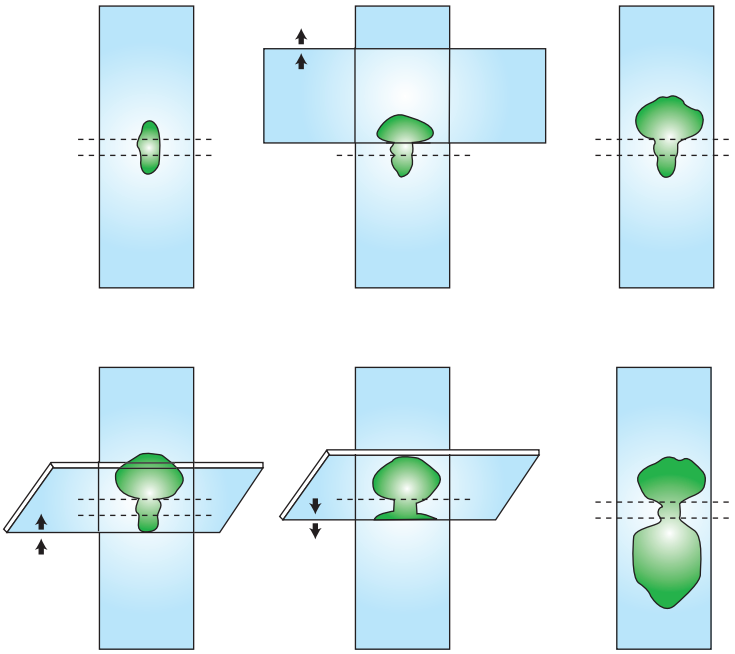
TECHNICIAN NOTE Samples with low cellularity and small volume should

To repare edge lood echnique, small op ced proximately o om nother led ckward o egree ontact op. When ws eways uncture etween slides, cond uickly othly orward until ained way om cond procedure eathered dge.

Although many incorporate cellular **fixative** accomplish parate ep rocedure dvantageous ensure hest-quality reparation. referred ive for tology ecimens be esh ontaminated ellular ebris. Methanol ontainers rotected om vaporation dilution esults om nvironmental umidity, hich ill introduce humidity artifacts onto slide. The prepared cytology slides emain ive or utes. onger fixative times will improve quality of procedure m

Several ypes ve een or tologic repara tions. he eneral ypes ommonly Romanowsky-type (e.g., right's, Giemsa, Diff-Quik, DipStat) Papanicolaou derivatives (e.g., Sano's trichrome). dvantages dvantages ypes e owever, ecause omanowsky-type e re ewarding, ractical, eadily vailable practice uations, emainder re dominantly omanowsky-stained reparations.

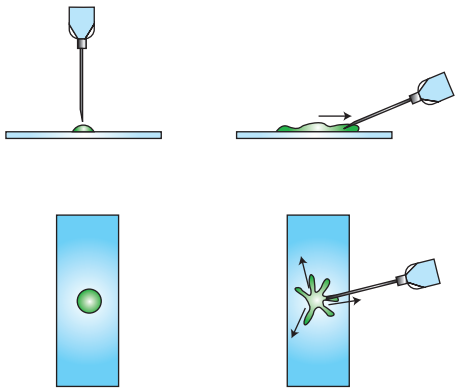
Romanowsky stains are inexpensive, readily available, to repare, hey rganisms cytoplasm f ells xcellently. lthough uclear ucleolar



A portion of the aspirate is expelled onto a glass microscope
Another glass microscope slide (the spreader slide) is placed over approximately one
third of the preparation. If additional spreading of the aspirate is needed, gentle digital pressure can be used.
The spreader slide is slid smoothly forward. This procedure makes

squash preparation (not depicted). Next, the edge of a tilted glass microscope slide (a second spreader slide)
is slid backward from the end opposite the compression preparation until it makes contact with approximately

preparation. The middle area is left untouched, and it contains a high concentration of cells.



The tip of a needle

results in a preparation with multiple projections.

detail erceived ell omanowsky
with Papanicolaou nuclear nucleolar detail usually
sufficient for differentiating neoplasia from inflammation for
evaluating oplastic ells or tologic vidence
potential iteria

Smears o e omanowsky
dried. ir ying tially reserves ells
them o dhere uring
rocedure.

Many omanowsky ommercially vailable, luding
Diff-Quik de ehring, eerfield, ll), edichem,
Inc., anta onica, alif), uick right's
ost—if l—Romanowsky cceptable or
tologic reparations. oes
undergo tachromatic eaction. esult, ranules
some ells hen ell ranules
cells may be misclassified macrophages, which
may lead to confusion during examination of some cell
tumors. Increasing fixative time to approximately minutes
may alleviate problem. In addition, during evaluation of
blood smears or bone marrow aspirates, Diff-Quik does
polychromatophilic ed lood ells ell ccasionally oes
basophils. The variations among different Romanowsky
problem after evaluators become
with outinely

Each ually ecommended ro
cedure. hese rocedures ollowed eneral, ut
they dapted ype
being d valuator's reference.
lower total protein concentration of

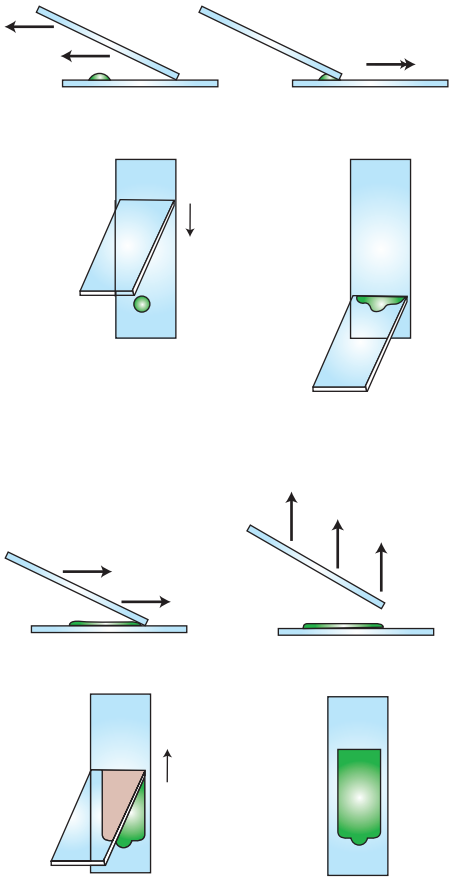


Fig. 52.6 Line smear concentration technique. **A,** A drop of fluid sample

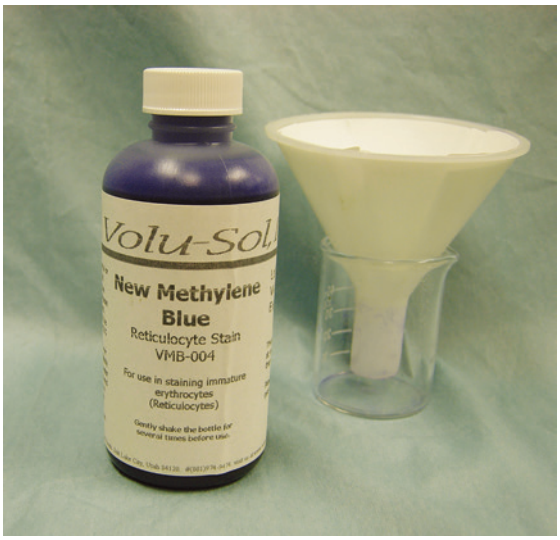
slide is slid backward to make contact with the front of the drop. When

After the spreader slide has been advanced approximately

with a feathered edge, the spreader slide is raised directly upward. This

rather than a feathered edge.

A three-step Romanowsky stain suitable for cytology samples.



New methylene blue stain is used when critical nuclear detail

ime eded ker
greater total protein concentration of more time
needed esult, rotein
w ellularity ominal etter
r ecommended hick
of neoplastic lymph nodes) may need to be stained for twice
recommended nger. ch echnician ends ve
different preferred technique. By trying variations
recommended ime ntervals or tains, he valuator an stab
hich imes roduce referred haracteristics.

New methylene blue (NMB)

djunct
Romanowsky toplasm eakly,
all, but provides excellent nuclear nucleolar detail. Because
toplasm eakly, uclear etail ells ell
clumps y etter isualized. nerally, ed lood ells
ith ut evelop lue

esult, ked ed lood ell ontamination oes
bscure ucleated ells.

New methylene blue stain provides excellent nuclear

The elicate apanicolaou ive xcellent uclear etail
elicate toplasmic etail. hey low iewer
through layers of cells cell clumps to evaluate nuclear
nucleolar changes well. They do cytoplasm strongly
omanowsky refore emonstrate to
hanges ell. hey emonstrate cteria
r rganisms ell omanowsky o.
Papanicolaou equires ultiple eps onsider
able ime. n ddition, cessary eagents ften
to locate, prepare, practice. Papanicolaou

ir erivatives equire ecimen et ed ust ed efore ells ve ied). et requires raying tologic ive thanol diately er reparation. hen to e ced de rotein-coated slide, hich revents ells om hen e rsed.

Poor uality ften erplexes vice xperienced cytologists. roblems voided lowing recautions en:

- Always new, clean slides. Even precleaned" slides be iped ith efore emove esidue.
- Fresh, ell-filtered eriodic ration equired) esh uffer ution uffer equired) used.
- Cytologic reparations ed diately er drying eing tside oratory. The tside oratory onsulted efore are ed.
- The urface ouched any ime y

Occasionally ontaminated oreign substance (e.g., lubrication jelly) alters specimen's ing. Table shows some of problems occur with Romanowsky roposed utions problems.

When valuation tologic reparation oes nish ufficient eliable ormation or ement of preparation may be submitted to veterinary clini pathologist or cytologist for interpretation, or alternative procedure iopsy opathologic valuation) performed. If possible, person to whom cytologic prepara tion sent be contacted, specifics concerning handling be discussed (e.g., number of smears to send, whether o efore

When ossible, ee -dried two r ee -dried omanowsky-stained be ubmitted. athologists -dried smears with Romanowsky or of their choice. The Romanowsky-stained smears fety factor. Some issues stain poorly hen ied ut or veral ys. In ddition, ccasionally ered uring ransport eceipt. ometimes oscopic examination of shards from broken prestained smears allows

Unfixed slides must not be in proximity to formalin containers.

for nosis. nly ew repared om ne ubmitted ied other submitted dried, fixed, stained. Smears be well labeled with alcohol-resistant ink or another permanent labeling thod. apanicolaou veral wet-fixed smears be submitted. hen biopsies or aspirates are btainable ranted, or collection, ecially ucosal urfaces om deep ithin ft-tissue

Fluid have smears prepared from them diately. irect oncentrated mitted. n ddition, ube vender op) erile serum ube ed op) ubmitted. nucleated cell count total protein concentration be per formed n ube cessary, hemical analyses erformed rum ube

Slides must be protected when they are mailed. Simple card board mailers do provide sufficient protection to prevent slide breakage they are mailed unpadded envelopes. Marking envelope with phrases such ragile," Glass," reakable," cancel" effect. Placing pad of bubble wrap or olystyrene ch er ually revents slide breakage. Slides may be mailed plastic slide holders or vative ers, uch

Unfixed slides be mailed with contain formalin, they be protected against moisture. Forma er haracteristics er ell ysis

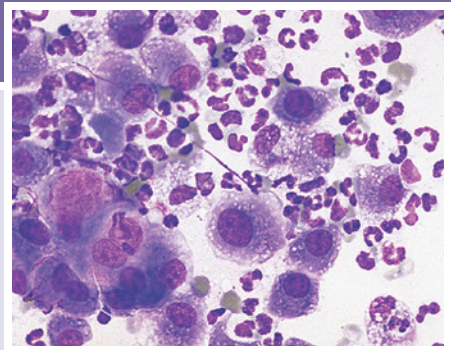
Chapter eview uestions ppendix

Problems That Can Occur With Romanowsky Stains and Proposed Solutions

Excessive Blue Staining (RBCs May Be Blue-Green)

Precipitate on Preparation

- Commonly performed methods for preparation of for cytologic evaluation include compression smear, smear, combination methods.
- Fluid be prepared with compression or smear.
- Preparation methods are based on characteristics of volume of obtained.
- A variety of preparation methods may be needed, and multiple preps are usually made.
- The compression preparation commonly used method.
- Line smears are preferred when volume small cellularity low.
- Cytology slides are fixed with methanol before they are stained.
- Most cytology be stained with Romanowsky



After studying this chapter, you will be able to:

- Describe eneral rocedure or valuation cytology
- Describe eneral pearance om inflammatory
- Describe eneral pearance om neoplastic
- State uclear iteria nancy.
- Differentiate etween uppurative, ranulomatous, pyogranulomatous, osinophilic ion.
- Describe eneral umor ypes.
- State haracteristics om ch general egories umors.

Inflammation,
Neoplasia,

Key Points,

Anisokaryosis
Anisonucleoliosis
Benign
Carcinoma
Discrete round cell tumors
Eosinophilic
Epithelial cell tumors
Granulomatous
Histiocytoma
Karyolysis
Karyorrhexis
Lymphoma
Malignant
Mast cell tumors
Melanoma
Mesenchymal cell tumors
Neoplasia
Nuclear molding
Plasma cell tumors
Pleomorphism
Pyknosis
Pyogranulomatous
Sarcoma
Suppurative
Transmissible venereal tumors

The primary purpose of cytology evaluation to differentiate inflammation from neoplasia. The evaluation proceed systematic manner focused initially on determining predominant ell ypes resent. ny rphologic normali ties resent ed uantified. resence infectious ents ed. ummarizes eps valuation tology ecimens. valuation of he ytology reparation hould erformed ith ow ag nification o etermine hether dequately stained to detect any localized areas of increased cellularity. To rove esolution ecreasing efraction, op oil e ced overslip dded. on sistent proach xamination nsure h-quality results. bjects uch ell lusters, asites, ystals, al yphae rmally vident uring w-power examination. valuation harac terize ellularity omposition ecord ypes ells resent elative umbers ch type. h-power xamination o be erformed valuate ompare vidual ells further haracterize ypes ells resent. rsion must e d entify ecific uclear iteria cytoplasmic abnormalities are indicative of malignancy various inflammatory reactions. The cytology report indicate cell types present, their appearance, their relative proportions.

Inflammation normal physiologic response to tissue damage or invasion by microorganisms. This damage releases substances that ave hemotactic ffect ertain hite lood ells. hese chemotactic ctors refore volved racting hite blood ells ion. hite lood cells to arrive are neutrophils. Neutrophils phagocytize dead tissue microorganisms. The process of phagocytosis creates hanges utrophils hanges, utrophils ecome hagocytize further, ells uickly oint, crophages move to site pick phagocytic activity. Cytology om ory refore haracterized resence hite lood ells, ticularly utrophils macrophages. Occasionally eosinophils or lymphocytes may be resent. n ucleated ell re e ommon ion. ften urbid, hite ellow. total rotein ften re Inflammation egorized uppurative ulent), granulomatous, pyogranulomatous, or eosinophilic on of elative umbers arious ell ypes resent.

Samples from inflammatory lesions are characterized

Suppurative (purulent) inflammation Fig. character ized by presence of large numbers of neutrophils usually make more of total nucleated cell count. hen significant numbers of macrophages are present more of total count), classified granulomatous or pyogranulomatous Fig. Fungal parasitic infections often est resentation. resence re f osinophils ddition eased umbers neutrophils osinophilic ion This ually asitic ections, ut be resent oplastic rders.

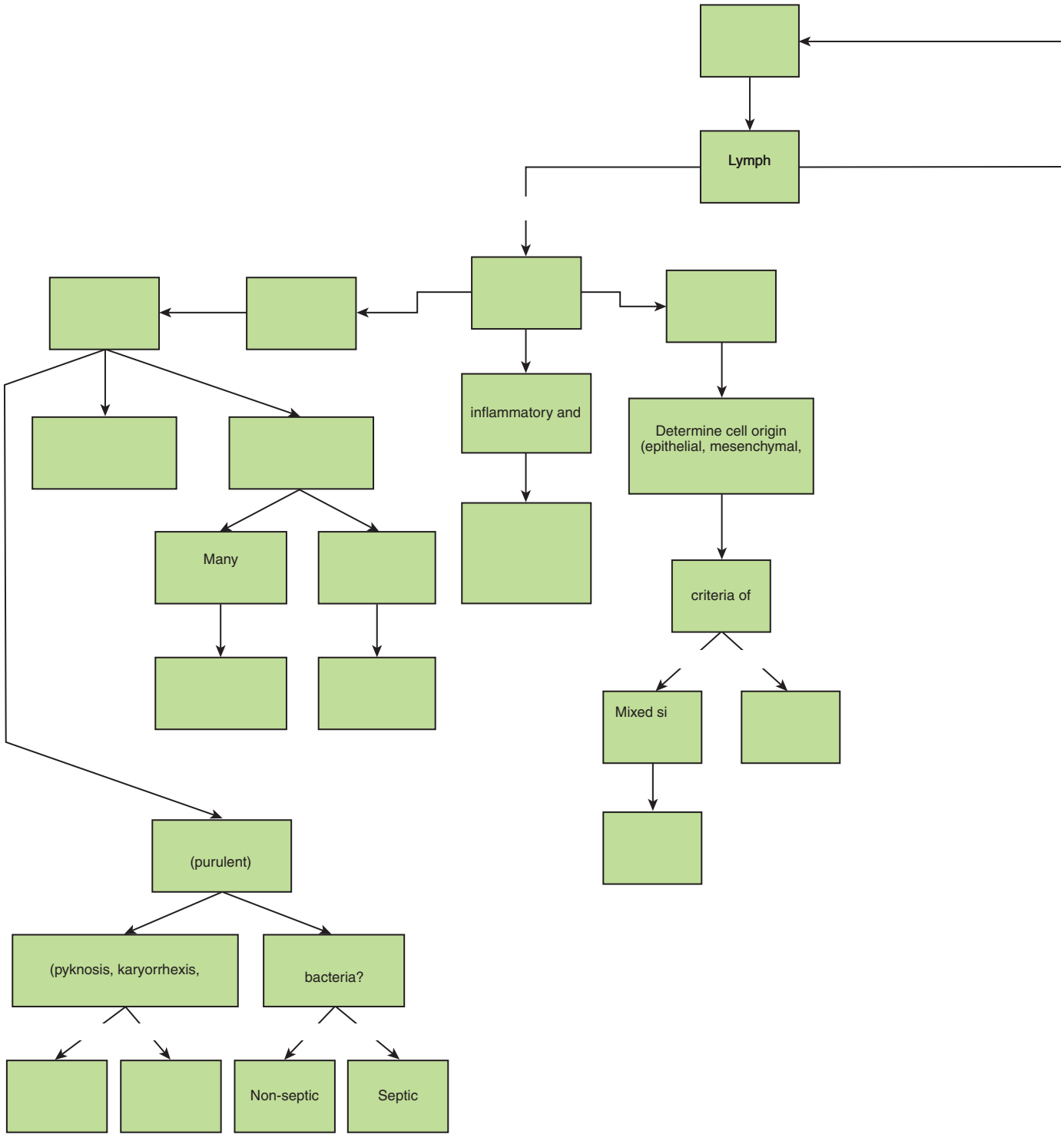
After y ve een esignated ory, ells must e valuated or vidence egeneration or resence oorganisms. uclear hanges found ory ells utrophils) lude olysis, yorrhexis, yknosis, yolysis ving greatest significance. Pyknosis represents slow cell death (aging) efers o ondensed, ucleus ment yorrhexis). aryolysis epresents ell occurs ith ptic cterial) ory eactions. appears ollen, agged ucleus ithout ct uclear membrane and with reduced staining intensity. Cells be valuated or resence cteria. nflammatory ells ontain hagocytized oorganisms eferred septic Fig. 3.5). dditional hagocytized aterial ay nclude erythrocytes, asites, rganisms

Unlike ion, oplastic ecimens rmally ontain rather homogeneous populations of single cell type. lthough mixed ell opulations re ometimes een, hese sually nvolve oplastic oncurrent ion. eoplasia indicated hen ells resent issue origin. fter ells ve een entified oplastic, echni entify issue rigin valuate ells or resence **malignant** haracteristics Table

Neoplasia erentiated ither enign malignant. enign oplasia epresented yperplasia iteria resent ucleus ells. The ells e ype, elatively orm in ppearance. ells hat isplay east hree bnormal uclear configurations entified uclear iteria of nancy lude ollowing:

- Anisokaryosis: ny usual ariation verall ell ucleus
- Pleomorphism: ariability cell ype
- High r ariable ucleus-to-cytoplasm atio
- Increased mitotic activity: Mitosis rare normal tissue, cells ually vide venly o. ny res ence f otic ells viding qually onsidered iterion.
- Coarse hromatin ern: hromatin ern oarser rmal pear opy ordlike.
- Nuclear eformation uclei uclei within ell djacent ells
- Multinucleation: ultiple uclei ell
- Nucleoli vary size (anisonucleoliosis), shape nucleoli), umber ultiple ucleoli)

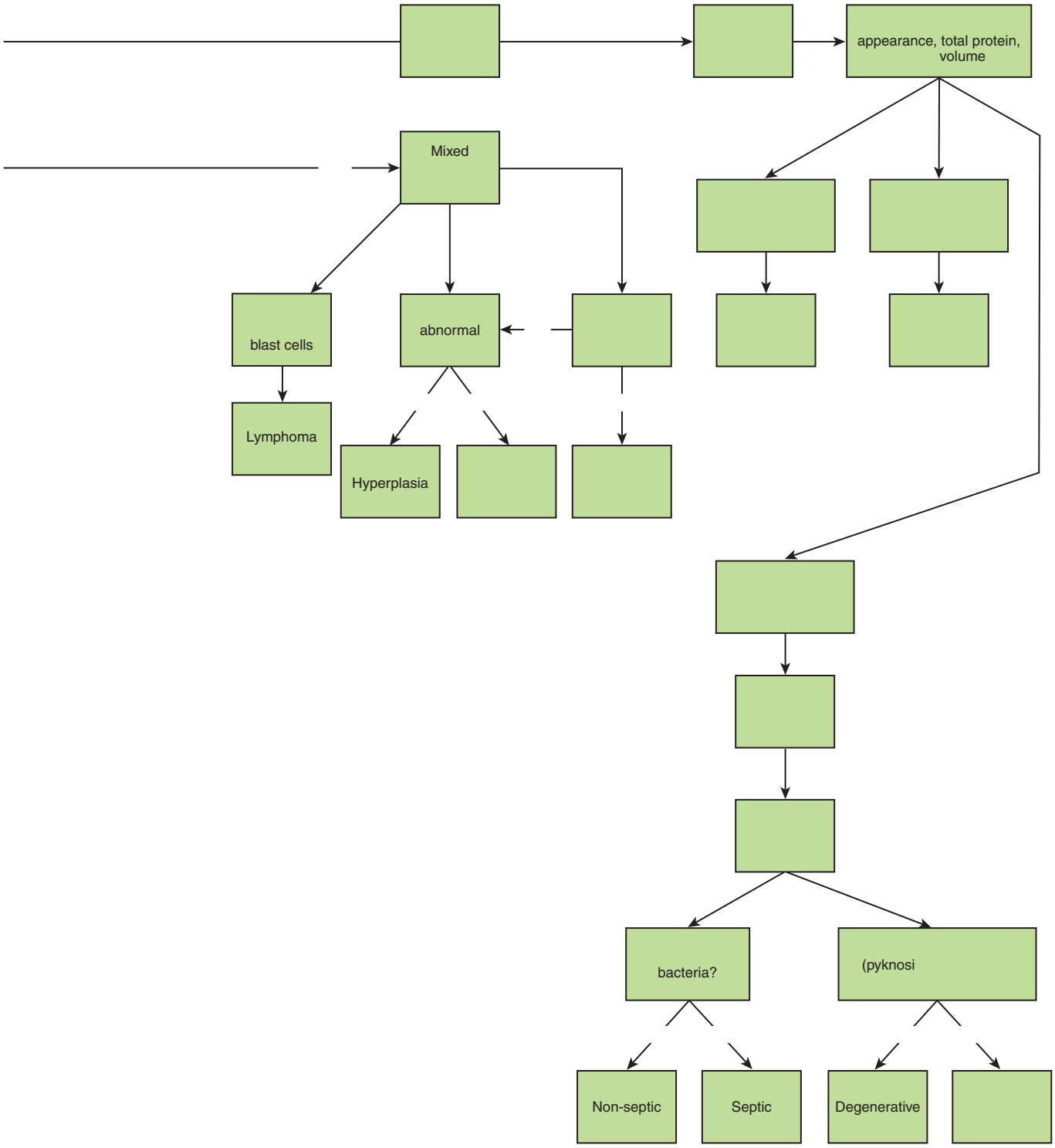
In general, three or more nuclear criteria of malignancy are present, specimen identified malignant. Exceptions to general rule are indicated inflammation present or only few cells display malignant characteristics. The histopathologic verification tologic ortant or umors, whether they are cytologically benign or malignant. In addition, cytologically benign cells may be obtained from malignant tumors. Histopathologic examination offers advantage of enabling assessment ctors, uch issue ration essel or ymphatic vasion umor ells. haracteristics malignant umors vident tologically.



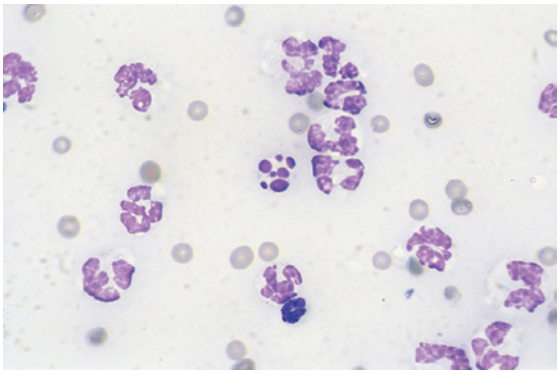
Flow chart for the examination of cytology specimens.

Specimens ve een lassified further evaluated to determine cell type involved. The primary types of tumors are encountered veterinary medicine are categorized epithelial cell tumors, mesenchymal cell tumors, discrete round cell tumors. The overall characteristics of from ch f ell ypes ummarized able

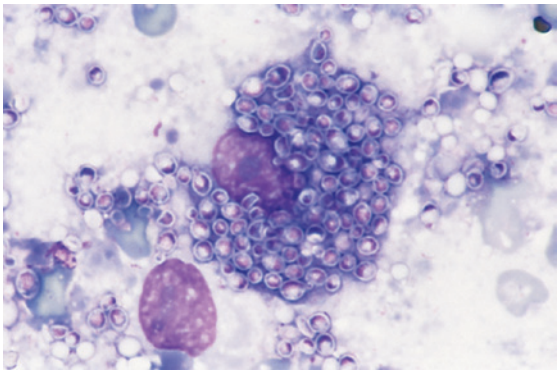
Epithelial ell umors eferred cinoma adenocarcinoma. The tend to be highly cellular, they



often xfoliate lumps heets Fig. 3.7). esenchymal ell tumors e eferred coma, ually cellular. The cells tend to exfoliate singly or wispy spindles Fig. iscrete ell umors xfoliate ery ell, ut are usually clumps or clusters. Round cell tumors include histiocytoma, lymphoma, cell tumors, cell tumors, transmissible venereal tumors, melanoma. Histiocytoma transmissible enereal umors pear mewhat xcept histiocytoma usually highly cellular Fig. cell tumors be recognized by presence of large numbers of ells ith ccentrically cated ucleus rominent perinuclear clear zone Fig. Mast cells be recognized



of neutrophils. Note the presence of karyorrhexis in the center cell.



organisms. Numerous organisms are also free in this sample.

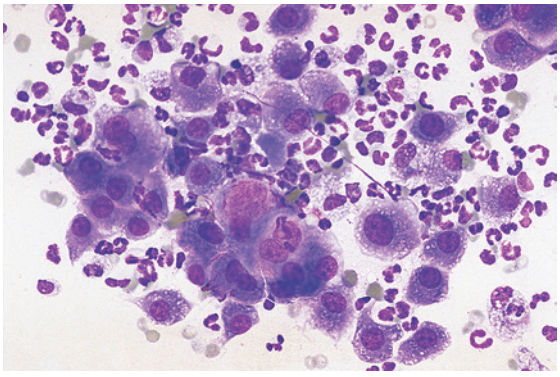
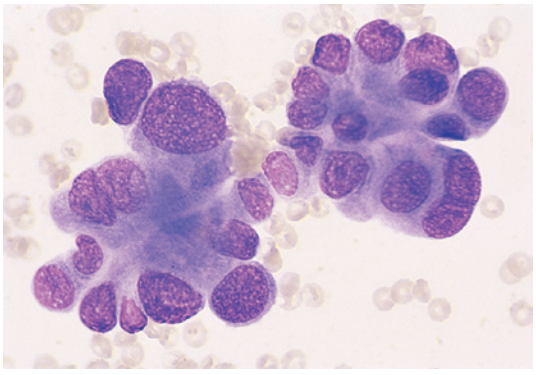
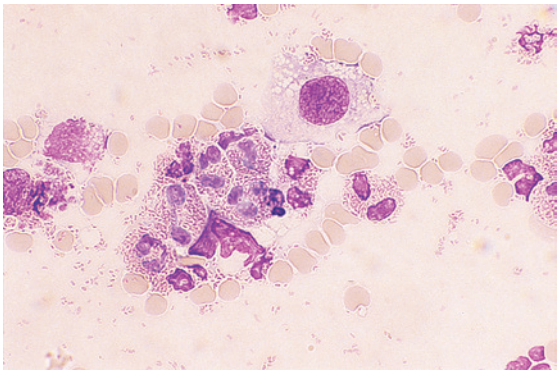


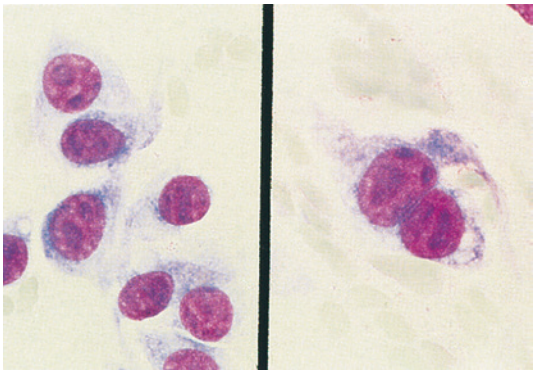
Fig. 53.3 Pyogranulomatous inflammation. Macrophages represent more than 15% of the cells present.



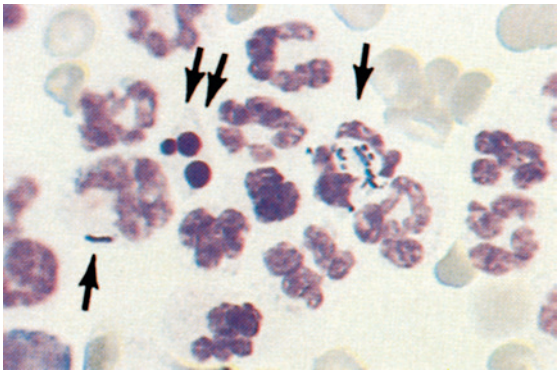
ation, and high and variable nucleus-to-cytoplasm ratios are present.



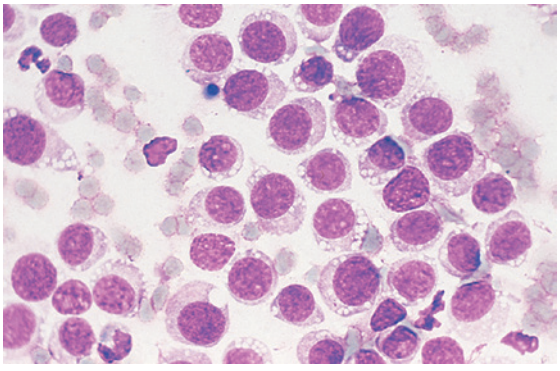
Eosinophilic inflammation. Note the single macrophage and the numerous free eosinophilic granules.



Sarcoma. The aspirate shown here from a malignant spindle cell

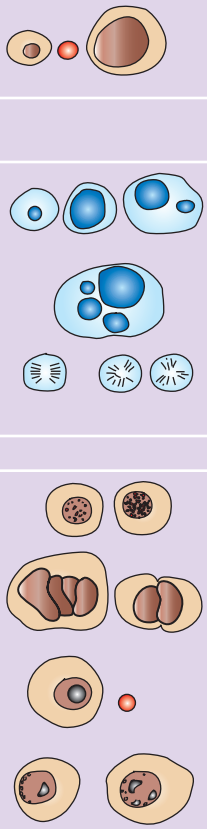


phagocytized bacterial rods. A pyknotic cell *(double arrow)* is also present.

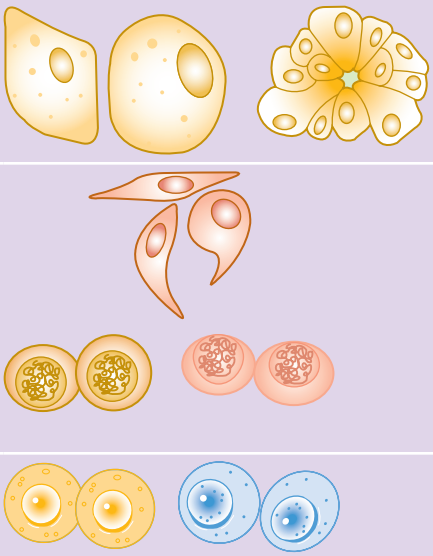


sible venereal tumor.

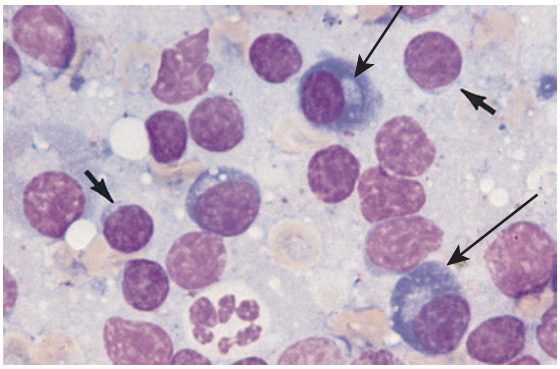
Nuclear Criteria of Malignancy



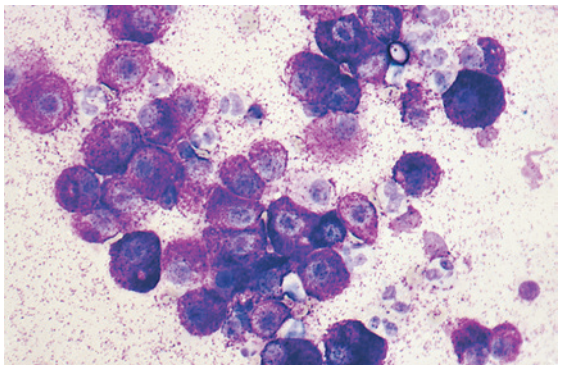
General Appearance of the Three Basic Tumor Categories



From Sirois M: *Principles and practice of veterinary technology,*

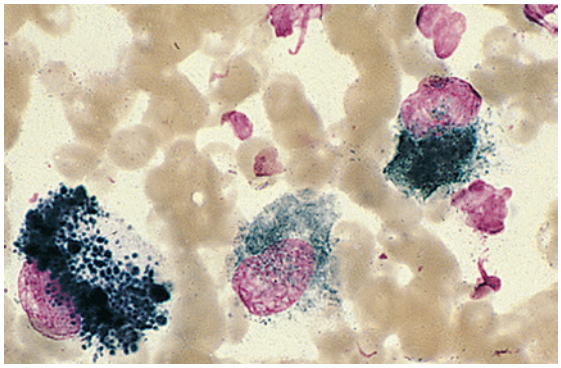


Several plasma cells are evident in this sample taken from a



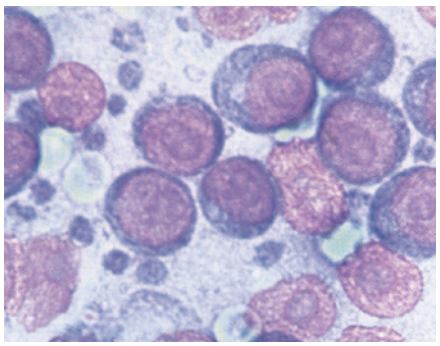
eosinophils are also present.

by their prominent purple/black granules Fig. Melanoma characterized by cells with prominent dark-black granules Fig. Occasionally, cells from poorly differentiated tumors may contain few or granules (amelanotic melanoma). variety of terminology escribe arious umor ypes, some eferences er egard lassification specific ypes umors.



Chapter eview uestions ppendix

- Samples pear ory egorized suppurative ulent), ranulomatous, yogranulomatous, or osinophilic elative umbers various ell ypes resent.
- Neoplastic specimens normally contain homogeneous popula tions f ell ype.
- Benign oplasia escribed yperplasia iteria of nancy resent ucleus ells.
- Nuclear iteria lude yosis; pleomorphism; high or variable nucleus-to-cytoplasm ratio; increased mitotic figures; coarse chromatin pattern; nuclear multinucleation; nucleoli vary with regard to e, e, umber.
- Samples from epithelial cell tumors tend to be highly cellular, y ften xfoliate lumps ets.
- Samples om nchymal ell umors end ve cellularity, xfoliate ly ispy
- Samples om ete ell umors end xfoliate very ell, ut ually lumps lusters.
- Plasma cell tumors be recognized by presence of large numbers f ells ccentrically cated ucleus prominent erinuclear one.



Cytology of Specific Sites

After studying this chapter, you will be able to:

- Describe haracteristics rmal peritoneal ural
- State iteria exudate, ransudate, dified ransudate.
- List escribe ell ypes en rmal lymph des.
- Describe pearance om eactive ymph node.
- Describe haracteristics rmal vial
- Describe pearance ollected racheal
- Describe pearance aginal tology om rmal emale.
- Describe valuations erformed men

Cornified
Curschmann's spirals
Exudate
Lymphoma
Modified transudate
Parabasal
Peritoneal fluid
Pleural fluid
Reactive lymph node
Synovial fluid
Transudate
Wave motion

Under ormal ircumstances, eritoneal horacic avities contain nly nough dequately ubricate urfaces of rgans vity ollected ylenediaminetetraacetic ubes or ucleated cell counts (TNCCs), cytologic examination, refractometric protein urements ubes or etermination of total protein concentration. Other clinical chemistry determinations erformed equently **peritoneal pleural fluids**

Normal eritoneal nd leural uids re olorless traw ellow transparent to slightly turbid. Both be odorless. Gross discoloration increased turbidity may be result of increased ell umbers rotein oncentrations. ollec tion f dorous eritoneal om ominocentesis may e crotic gment owel eritoneal cavity, uptured gment owel ee ontents vity, r ccidental nterocentesis. onditions be inguishable tologically eference cal ndings. lthough ommonly een, hylothorax ay evident milky" ecially ecently eaten, because of chylous effusion's high content large number of mature lymphocytes. In fasted may be Unlike with high leukocyte counts (which may have hitish olor), hylous oes ve uper after centrifugation. The chylous present small droplets (chylomicrons), which be stained with Sudan r . he hylous ved ther after een ed dium ydroxide sodium icarbonate. umbers rythrocytes present, ve eddish olor.

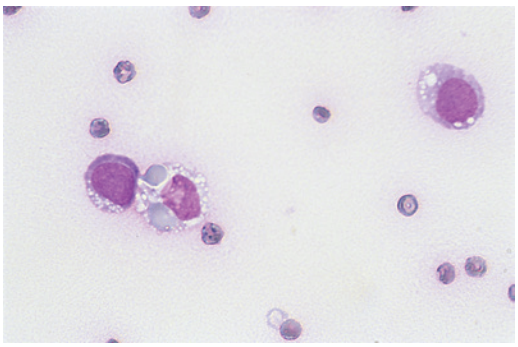
performed methods are used for complete blood count (see Unit cross-species generaliza tion, rmal eritoneal ural ve nucleated ells/ ually o ononuclear cells may be visible clusters of cells, which make counting individual ells erential ucleated ells performed, ith ell ypes nd orphologic haracteristics oted. Nucleated cells are categorized neutrophils, large mononuclear cells collective grouping of mesothelial cells macrophages), lymphocytes, osinophils, ucleated ells. otes on ell rphologic haracteristics lude omments about uclear toplasmic pearance. cteria present, ir rphologic eatures cilli, occobacilli, cocci) location free or phagocytized) must be recorded.

In uch ram ured.

TECHNICIAN NOTE Peritoneal and pleural fluids are evaluated for color,

Normal eritoneal ural ontain ew rythrocytes. The umber resent imated. uitable categories lude are, ew, y, umbers. number resent aries thod reparation. Iatrogenic ontamination cute hronic morrhage are distinguished grossly, previously outlined. If erythrocytes have een resent or veral phagocytized by macrophages (erythrophagocytosis) Fig.

Peritoneal nd eural fluid mples should also be valuated for cellularity. The total protein values for allow o lassified ransudate, dified ransudate, exudate Table Published normal values for peritoneal ural tology ogs ce. ormal horses enerally ve verage proximately neutrophils, nonuclear ells, lymphocytes, occasional eosinophil alues for e mewhat ut rmal ften ve comparable umbers utrophils ymphocytes.



Variable numbers.

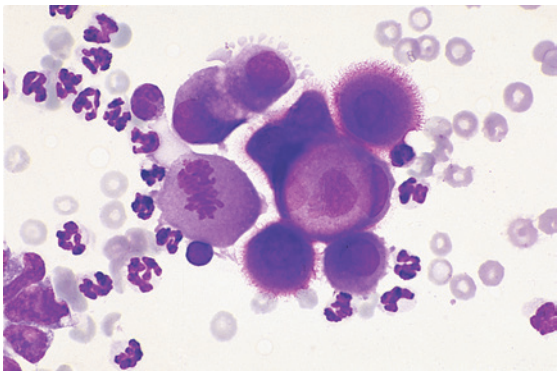
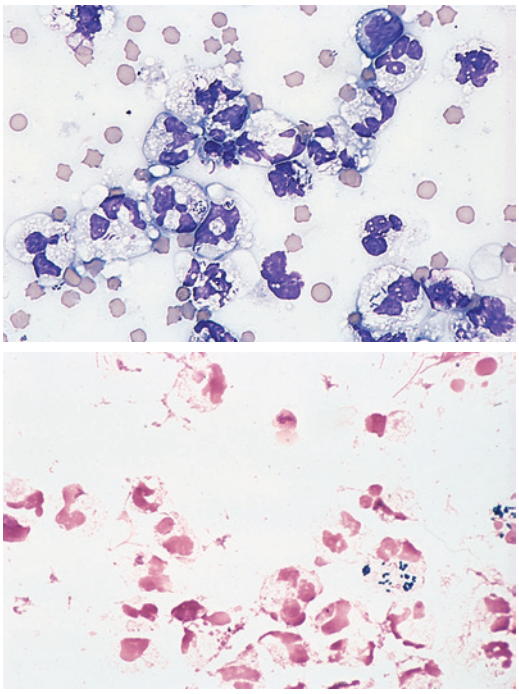
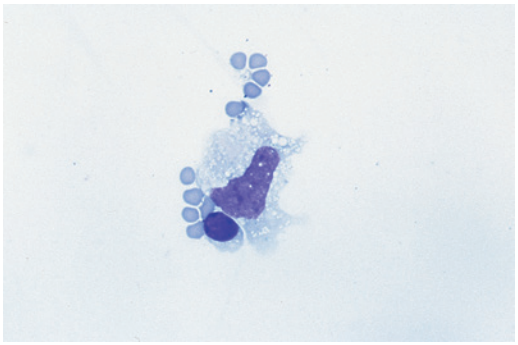


Fig. 54.2 A cluster of reactive mesothelial cells. Note the mitotic figure.





This sample is characteristic of normal fluid and transudates.

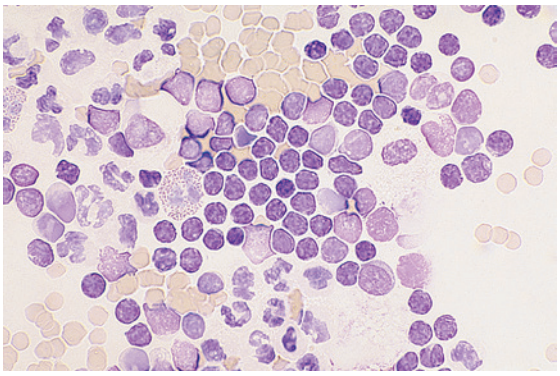
Exudates e eased ellularity rotein concentration esult ion. ollowing species eneralization de. uppurative ory reactions ell ercentage, absolute umbers utrophils re nucleated ells. uppurative ory eactions ually h-normal levated levated utro phil ercentages umerous ells, crophages, or oth. esothelial ells ody vities. resence of eased vities, ells ecome reactive ultinucleate, cytosis yo rominent ucleoli, philic toplasm) Reactive ells om some oplastic ells. ome ells resent clusters or rafts of cells. These clusters result from prolifera tion xfoliation ells om eritoneal mesothelium, esponse ecrease ontact ition between cells on opposing surfaces of peritoneum result of ffusion. acrophages hagocytize egenerate ells or ellular ebris. igrating asite vae eased neutrophil osinophil ercentages, ithout elevated

Cellular rphologic eatures epend oorgan present. They may vary from cytoplasmic vacuolation with few nuclear changes evident to marked cytoplasmic vacuolation, marked nuclear swelling disruption (karyolysis), general cellular degeneration or fragmentation. Bacteria may be evident within cytoplasm of neutrophils macrophages Fig. Cases f eritonitis ve ype cterium evident, r cterial opulation ed esult devitalization or rupture of bowel. ccidental penetration of owel uring ominocentesis esult ed population cteria owever, er leukocyte umbers rphologic haracteristics ually normal, bacteria are frequently phagocytized. Large ciliated rganisms ed e-bowel nterocen tesis rses.

Transudates (ascitic effusions) typically have low protein con centrations low with fairly normal differential ossibly ercentage large nonuclear ells nonuclear ells principally ells, hich lusters and hich be uite eactive ppearance. ransudates re frequently condary ongestive ure, occur lood oncentrations.

Modified transudates e haracterized elatively moderate redominantly esult lymphatics. This leakage responsible for high total protein concentration dified ransudates. ells resent include w umbers ory ells ndegenerate), mostly l ure ymphocytes, ew crophages, mesothelial ells

Intra-abdominal tumors may exfoliate cells into peritoneal The cytologic diagnosis of such neoplasia may be ften or ecialist tologist. owever, technician be able to recognize abnormal lymphocytes by criteria of malignancy previously outlined be suspicious of clusters of pleomorphic, secretory-type cells. The presence of unexpected cells (e.g., cells) must be noted.

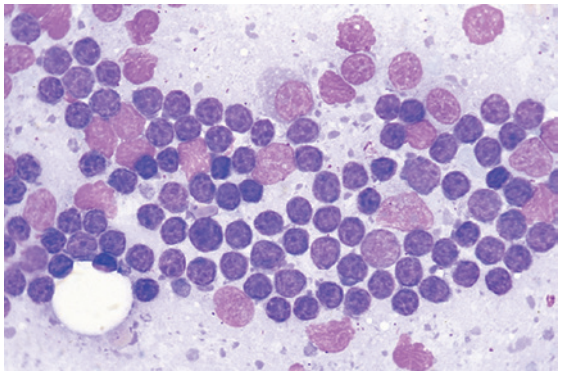


cytes, neutrophils, and an eosinophil are present. This is characteristic

The tologic valuation ymph de issue erformed to nose ymph de nlargement er entiate ng yperplasia, ion, rimary oplasia **(lymphoma)** tastatic oplasia. ymph des show vidence ion ymphadenitis), yperplasia (benign oplasia), ed ory oplastic cells present), neoplasia (lymph node cells with abnormal nuclear features), metastasis (neoplastic cells from other body tissues that pread o ymph odes). ach hese as pecific ell ypes e ciated normality.

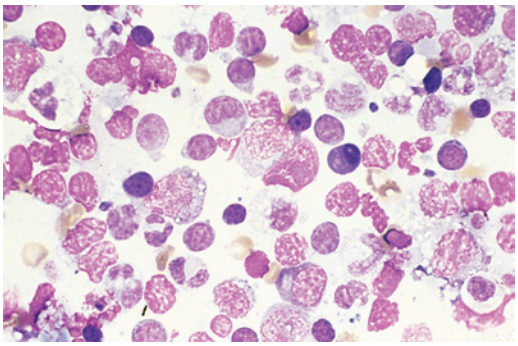
Lymph de issue rmally ollected om eriphery of nlarged ymph de edle iopsy. ients with generalized lymphadenopathy, be obtained from two lymph nodes. Because lymph nodes drain oral cavity gastrointestinal tract are antigenically stimulated under rmal onditions, voided. then repared ompression echnique standard omanowsky-type iety of cell types may be found in lymph de aspirates. These lude ymphocytes, ells, hite lood ells, neoplastic ells. icroorganisms, ymphoglandular odies, bacteria may be present. Lymphoglandular bodies are small cytoplasmic agments en etween ells are gic eature. able ontains ummary ell ypes ymph de ates.

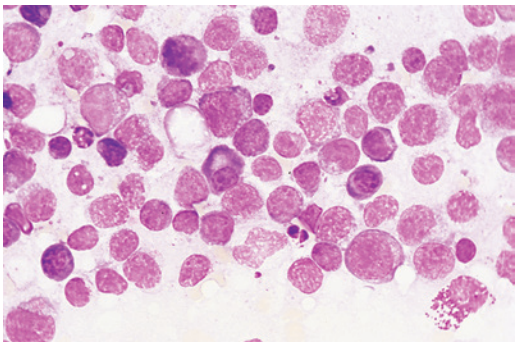
In rmal ymph de, redominant ell ype small, ure ymphocyte. end omprise re three fourths of total cells present. Smaller numbers of inter mediate ymphocytes ymphoblasts ell crophages are resent. ells ccasionally en ast ells ually are tologic reparations lymph de issue. ymph des vidence tion ymphadenitis) ill ve redominance hagocytic leukocytes



Aspirate from a normal lymph node. Small, mature lympho

Lymph des esponding igenic imulation contain redominantly mall, ature ymphocytes nd re eferred to **reactive lymph nodes** However, cells, lymphoblasts, ermediate ymphocytes re are rmal ymph de casional ells





tion of small, medium, and large lymphocytes, plasma cells, and a mast

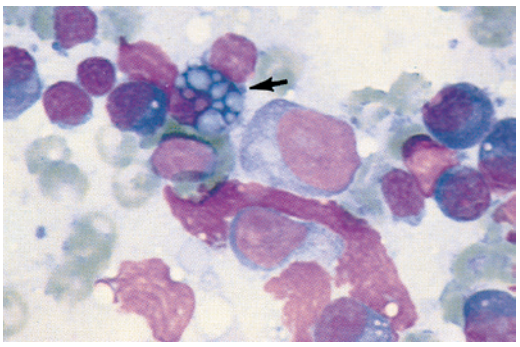
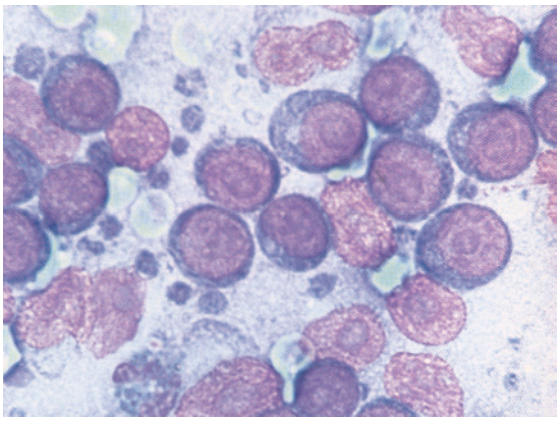


Fig. 54.9 The plasma cell that appears vacuolated is a Mott

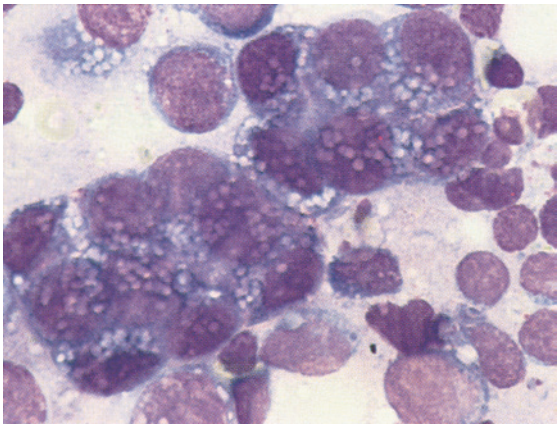
(plasma ells hat ontain ecretory esicles mmunoglobulin) may be seen Fig. ntigenic stimulation ory esponse haracterized presence f utrophils, crophages,

Reactive lymph nodes contain predominantly small,

Primary ymphoid oplasia, ymphoma, haracterized by redominance ymphoblasts, otic common. acrophages resent, ells scarce. Other neoplastic cells may be present lymph node



Immature, neoplastic lymphocytes are present in this sample from a dog with malignant lymphoma.



that metastasized to a local lymph node.

aspirates lude ells, cinoma ells, coma ells, histiocytes. ells ee normal uclear configurations ually entified Lymph de ontain tastatic ells om other ody

As oss-species eneralization, rmal erebrospinal ontains rythrocytes ucleated ells per microliter (usually to per Pleocytosis elevated ucleated ell ormal ontains mononuclear ells, lmost ll hich re ymphocytes. acterial infections volve enerally ked ocytosis, mostly esult utrophils. nflammation ciated viruses, oplasia, egenerative onditions enerally dramatic pleocytosis, with significant proportion of mononuclear ells ften ymphocytes). osinophils times seen, especially with parasitic inflammatory responses. In general, ive ent ften tologically parent. Neoplastic ells ldom bserved

Normal contains virtually erythrocytes. Erythrocytes may be counted by charging hemocytometer with well-mixed f uted ll ells ntire

boxed ea ounted. thod, erythrocytes ucleated ells bserved. istinguishing between roups ells ually ossible, ut to ubcategorize ucleated ells. ell or uted e ultiplied ive umber ells er microliter. thanol-diluted ell multiplied rythrocytes om ucle ated ells umber ells counted by latter method be subtracted from number of ucleated ells ounted rythrocyte

The f arious orrection ctors een dvocated to djust ucleated ell or eripheral lood leukocytic ontamination. ecause rmal ontains erythrocytes, bserved ucleated ell or rected umber rythrocytes er oliter known. he proach onsider ch rythrocytes ccompanied ukocyte.

In ddition tology valuation, ariety hemical unologic erformed

Fluid from eye to low cellularity. It omposed nonuclear ells tially rythrocytes, rotein oncentration. The erpretation hanges ueous umor f .

If nly ne ops btained, rmal joints f ogs, ross olor turbidity ell tologic xamination ect (possibly with concurrent subjective assessment of viscosity) may be l ractical. ollected, refractometric protein measurement (on EDTA-preserved y e dded ollection er volumes lows or erformance dditional uch ucin lot hapter

Normal **synovial fluid** lear o raw ellow nturbid. Yellow vial ommon ecially horses. urbidity, hen resent, ells, rotein fibrin), r tilage.

Normal vial ontains ew rythrocytes. atrogenic contamination uring throcentesis ommon. eren tiation etween ontamination ecent morrhage erformed reviously escribed.

Viscosity reflects uality oncentration of hyaluronic acid, which part of synovial fluid–mucin complex. The function of mucin joint lubrication. iscosity may be quantitated with viscometer; however, subjective assessment often used.

Normal vial icky. op ced etween thumb forefinger, digits are separated, forms o rand efore reaking. imilarly, hen ently expressed through needle on horizontally held syringe, hangs o rand efore parates om edle ip.

In eneral, iscosity ecreased rmal from patients with degenerative problems. It frequently ecreased cterial ion esult mucin degradation by bacterial hyaluronidase joints with significant ffusion luding ydrarthrosis) esult dilution f yaluronic

Because egrade yaluronic iscosity mucin clot formation usually are assessed on to which anticoagulant been added. If anticoagulation necessary esult f rinogen oncentration, parin referred icoagulant.

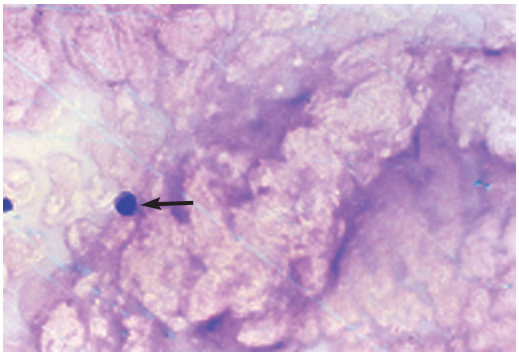
Slides or ell repared A-preserved or without anticoagulant latter especially only ew ops btained de diately). Thin s de wly dvancing reader Because of high viscosity of normal synovial cells usually do accumulate feathered edge of smear. The gination f ells iscosity ecreases. At low cell counts concentration of cells by entrifugal dimentation ubsequent esuspension of ells olume upernatant roduces re cellular lides ually omanowsky able ummarizes lassification vial

Normal synovial generally contains mono nuclear ells ess han eutrophils Fig. 4.12). osino phils re arely bserved. ononuclear ells omprise bout qual numbers of lymphocytes monocytic/macrophage-type cells, which e nvacuolated nphagocytic. acuolated or hagocytic nonuclear ells omprise differential count of normal synovial Macrophages become vacuolated rmal vial rocessed on after ollection; refore, rompt reparation important o revent tificial

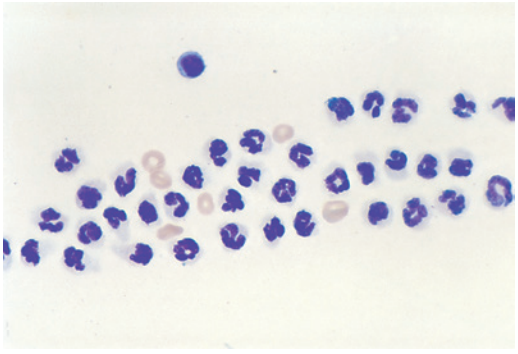
Cells vial ood iscosity end n ection iving indrow" appearance Fig. 54.13). Mucin precipitation produces eosino philic ranular ackground omanowsky-stained mears, density f hich eflects ells om viscous read ell hich make ir entification uch uted ith econstituted yaluronidase decreases viscosity after few minutes, allowing for more accurate ell rphology hen ed.

As generalization, mononuclear cells predominate from ients raumatic egenerative thropathies, usually with increased numbers of large vacuolated or phagocytic cells. casionally, hen rosion rogressed ough to ubchondral one, eoclasts bserved. ontrast, neutrophils redominate om ients ec tious thropathies esult cteria, iruses,

Canine and feline cytology



(From Raskin R, Meyer D:



Synovial fluid from a patient with inflammatory joint disease.

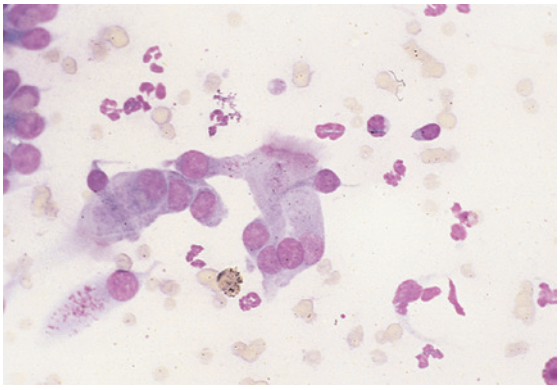


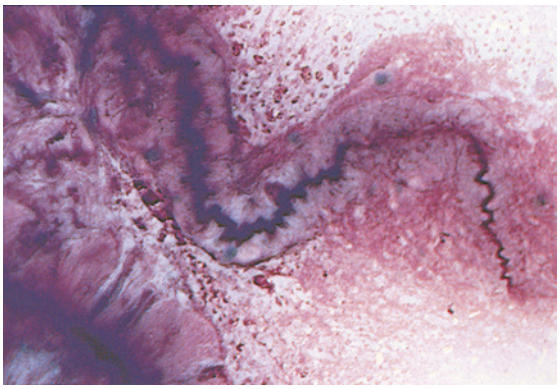
Fig. 54.14 Normal ciliated columnar epithelial cells in a normal tracheal

infectious process suspected. chronic–active type of arthropa thy uggested hen utrophils acuolated/phagocytic macrophages are both increased number. Lupus erythematosus cells, hich utrophils ontain hagocytized uclear chromatin, are seen occasionally synovial of with stemic rythematosus.

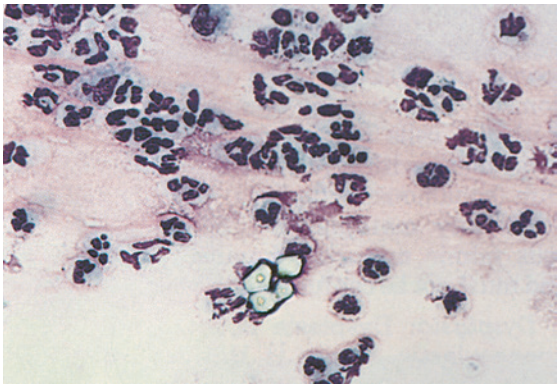
mycoplasmas) ninfectious onditions heu matoid arthritis, systemic lupus erythematosus). hen cells are clumped ogether thylene lue demonstrate erlocking rin rands. arely ive organism ptic bserved tologically, especially when phagocytized. Culture recommended when

e ually erformed racheal ell numbers e ubjectively ecorded om valuation smear. racheal om rmal ontains few ells, ually often pears oscopically osinophilic rands y nmesh ells. pithelial ells rincipal ell type. f ollected om vel rachea, ciliated pithelial ells redominate. ells olumnar to oidal, ucleus order pposite . ecimen ollected om ronchi, bronchoalveolar pithelial ells re lso airly ommon. hey re round, nonciliated cells with basophilic cytoplasm, they may occur lumps. ew oblet ells cretory pithelial ells) may e bserved.

If bronchoalveolar alveolar macrophages may redominate. vidual ells



present. The eosinophilic background represents mucus.



round o val ucleus derate philic cytoplasm. f ecome eactive ctivated, toplasm increases volume becomes more granular vacuolated. Neutrophils, ymphocytes, osinophils, lasma ells, ast ells, nd erythrocytes re arely een pecimens rom ormal nimals.

Abnormal racheal enerally xudates. contain numerous mucus strands, they are cellular. Eosinophilic spiral from small bronchioles **(Curschmann's spirals)** suggest chronic bronchiolar problem Fig. Cell morphology highly variable both among within Many cells may be unidentifiable. Neutrophils macrophages are umerous. cute ion, utrophils predominant ell ype, epresent re of ucleated ells rocess ecomes re chronic, nonuclear crophages umber. causative ent, hich ossibly cterial noted—whether free, phagocytized, or both—in smear. Tra cheal be cultured routine microbiologic procedures.

The presence of bacteria or fungi tracheal does necessarily organism pathogenic. or fungal spores sometimes contaminate tracheal from herbivores

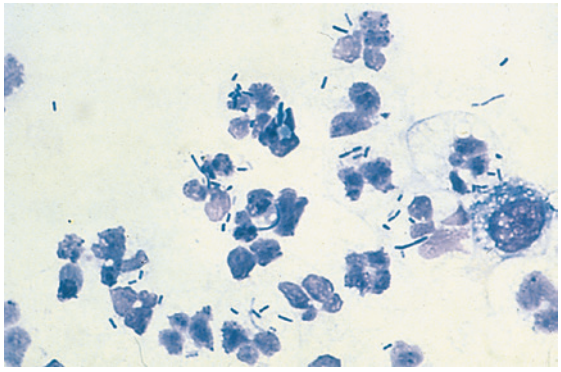


Fig. 54.17 Nasal wash from a patient with bacterial rhinitis. Note the both intracellularly and extracellularly.

inhaled from feed), they may be phagocytized by mac rophages. haryngeal ontamination ollection apparatus or increased inspiratory effort with contamination of upper tracheal mucosa by pharyngeal microflora may inclusion of bacteria tracheal specimen. Such bacteria are frequently associated with or adherent to squamous epithelial cells f haryngeal ucosa.

Eosinophils rominent ossibly re nucleated ells) ory eactions lergic parasitic component. Because cell preservation often only fair, free osinophil ranules ather ct osinophils noted. Rarely, parasite eggs or larvae may be noted smear.

Erythrocytes arely en rmal racheal eci mens. Recent hemorrhage may be evidenced by numerous intact erythrocytes ontrast, morrhage, few rythrocytes ed crophages may ontain mosiderin ranules lue ed to ranules).

Neoplastic cells may be detected tracheal specimens. Criteria or reviously escribed. eoplastic cells are frequently found clusters. They are generally epithelial rigin, equently cretory pearance their toplasm philic acuolated).

from normal contains **cornified** non cornified squamous epithelial cells, often with adherent bacteria and egligible vidence emorrhage nflammation. arious abnormalities may be demonstrated with procedure, such inflammation econdary epsis, ungi nd easts, nd eoplasia Fig. onfused love owder, which y resent ecimens

Evaluation om of itis xterna. ollected rior iation treatment otton parate or each . he roduced ither ectly through oscope cretions ollected then olled nto deally,

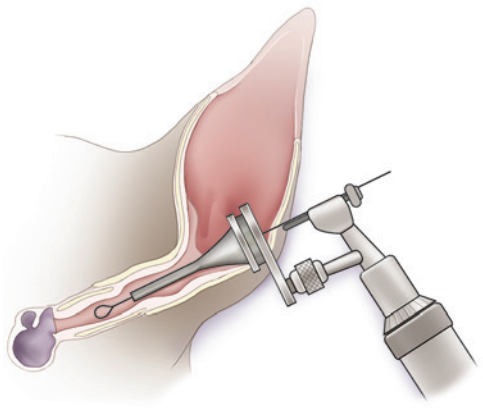


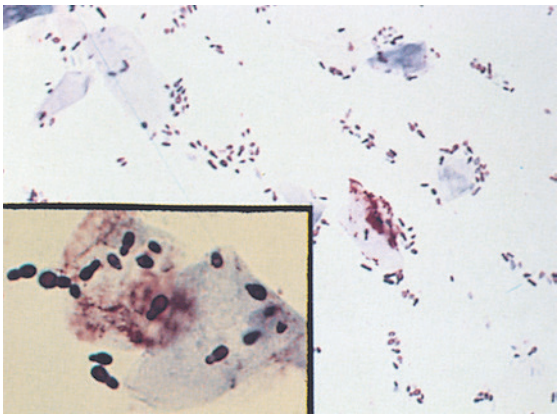
Fig. 54.18 Smears of horizontal ear canal secretions may be collected

Cowell and Tyler's

prepared om ch
small ral dded overslip
dded valuated er ower
to entify asites resent. cond -dried
d outine matology ram
f xcessive erumen resent, ently
ed o tially emove erumen,
requirement. The stained slide best used for identification of bacteria yeasts. Separate jars be maintained for tology cteria om oesn't contaminate ars or matology.

Samples om rmal ients ontain ornified epithelial cells, with negligible evidence of inflammation few microorganisms. ommon normal cteria yeasts, ith ithout ory ells. nfections volving cocci primarily involve *Staphylococcus* spp while infections with bacterial ods ually volve *Pseudomonas* ommon asites that ay e ound nclude *Otodectes cynotis* see ig. 8.24 nd *Otobius megnini*

The organism *Malassezia,* which potential of chronic ns ections, ill ram r ven thylene lue or uick et rep." ook for characteristic peanut-shaped organisms Fig. Some controversy exists among specialists regarding whether pres ence of *Malassezia* small numbers significant. Some believe y rganisms rounds or reatment, hereas others laim umbers rmal When cteria entified, eterinary echni eport esults verage umber en er power ld.



organisms.

cycle bitches queens. It with optimal timing of mating r tificial mination ut of ractical alue or poses ows, oes, wes, or ws. ytologic erpreted onjunction with ory ch rous cle etailed ollowing ctions. These stages are convenient divisions of continuum of change, they are brought about by variations blood estrogen progesterone oncentrations. ecause etermination stage of estrous cycle may be on of single examination, repeat examinations every few days may be neces sary. Unlike bitch, queen ovulates after coital stimulation. Cytologic erent rous cle o itch or pithelial ells utrophils; however, rythrocytes resent

Some riation xists he erminology sed escribe he ell types are commonly seen vaginal cytology preparations. In addition o neutrophils and erythrocytes, variety of squamous epithelial ells en aginal tology reparations Fig. ells ther egorized their size degree of cornification. The epithelial cells present may lude ells, htly er epithelial ells est-sized ncornified squamous pithelial ells, hich metimes eferred intermediate rous cle, intermediate cells may contain pyknotic nuclei Fig. Cornified epithelial cells are appearance. They usually have uclei ontain yknotic nuclei. acteria resent aginal of rous cle ecially uring rus), ut ually have pathologic significance they are part of normal vaginal oflora).

Exfoliative vaginal cytology useful adjunct to history clinical xamination or etermining rous

The rual itch ulvar elling oes ract dogs. vaginal reveals predominantly noncornified

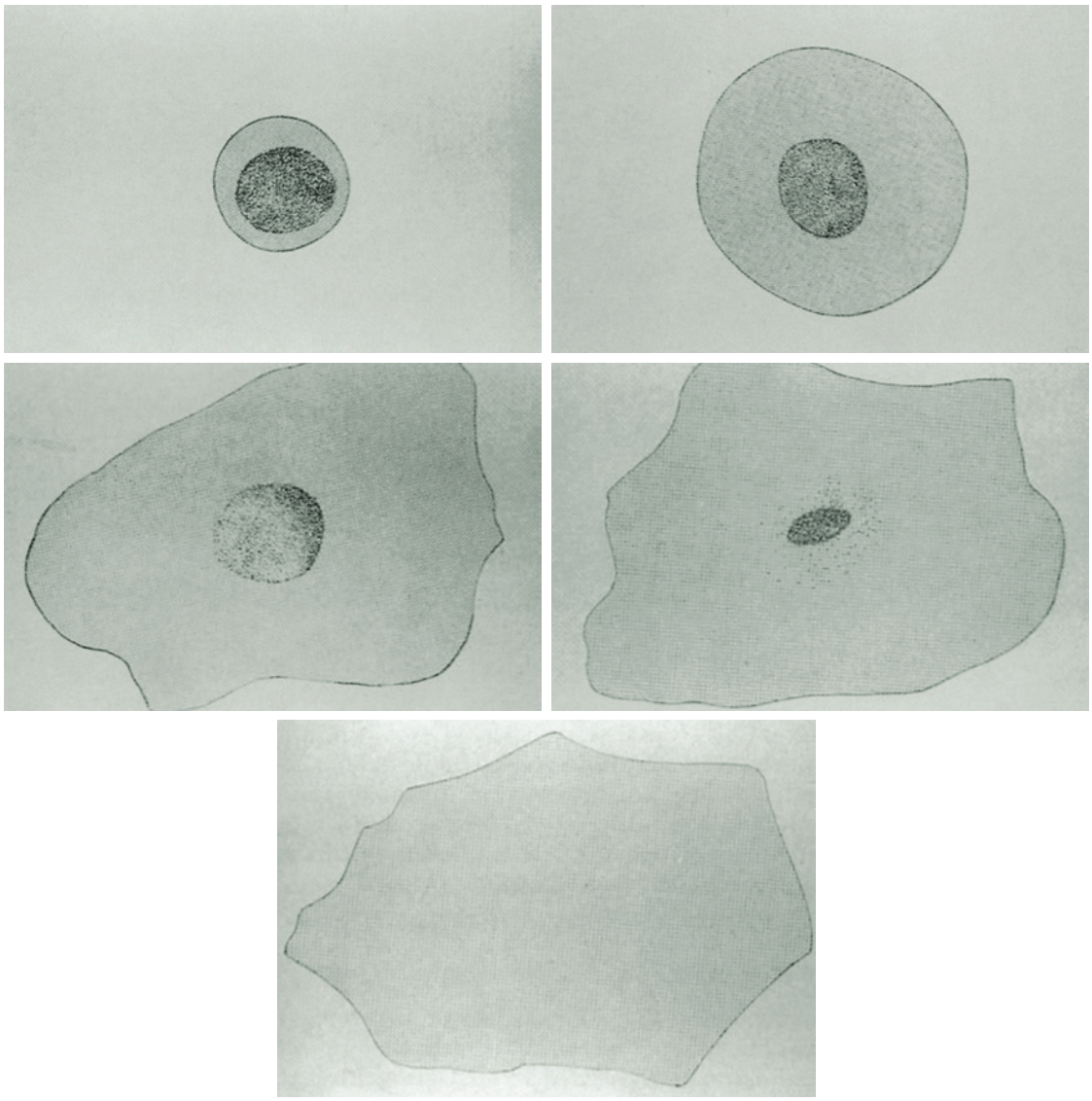
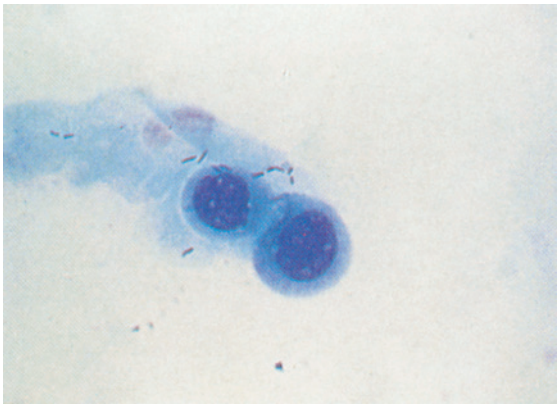


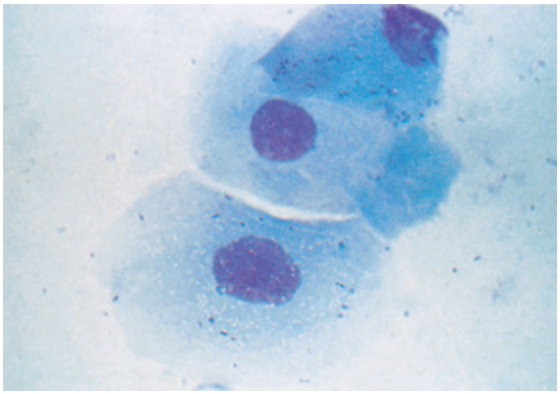
Fig. 54.20 Diagrams of cells from the canine vagina. Parabasal epithelial cell. Small intermediate cell. Large intermediate cell. Superficial cell with pyknotic nucleus. Anuclear superficial cell.

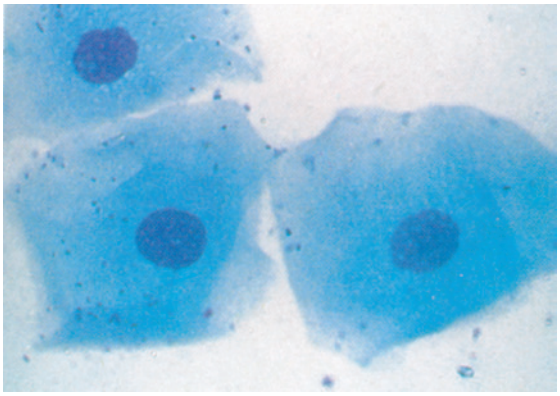


Parabasal vaginal epithelial cells from a dog.

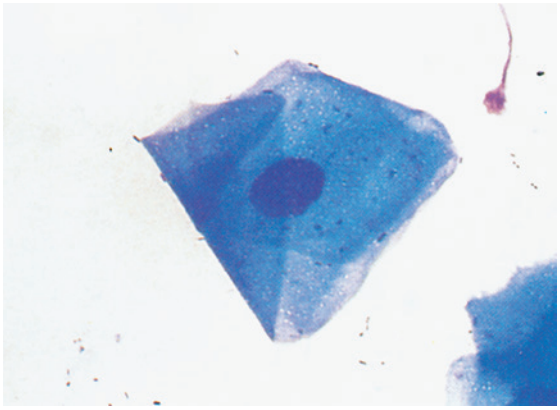
squamous epithelial cells large cells with rounded border, abundant asophilic ytoplasm, nd arge ound ucleus). f ells egorized ermediate, **parabasal** r pithelial ells. ontain some utrophils ut rythrocytes. nestrus ariable length, but generally months. Some reference materials efer rus.

itch roestrus ollen ulva, eddish ulvar discharge. The bitch attracts but does accept dogs are empting reed. roestrus ys, verage f ys. roestrus ften ther ubdivided early proestrus late proestrus. Gradual changes physiology cellular morphology are seen stages progress. During early proestrus, high numbers of erythrocytes are present along with parabasal epithelial cells Fig. proestrus continues, numbers of erythrocytes gradually decrease,

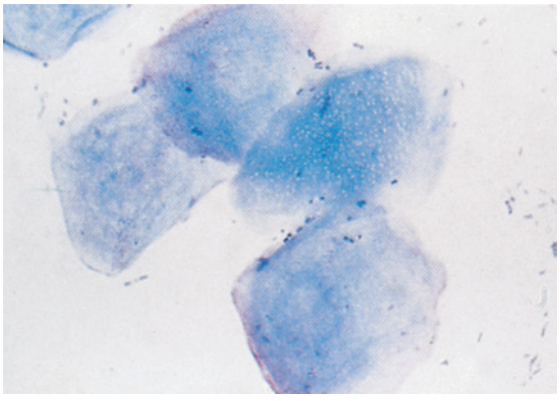




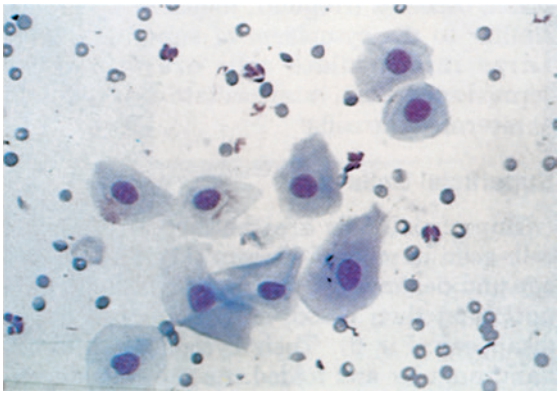
Small and large intermediate vaginal epithelial cells from a dog.



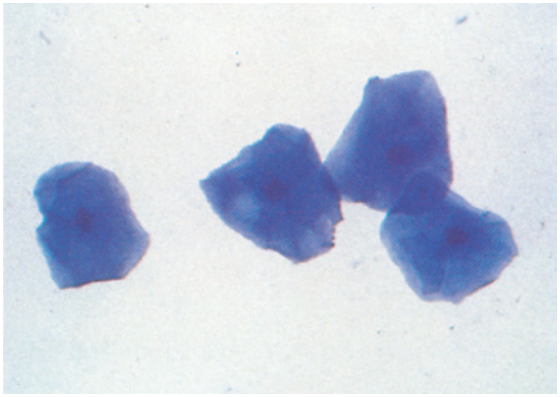
Superficial epithelial cell with a slightly pyknotic nucleus and



Anuclear superficial (cornified) vaginal epithelial cells from



Vaginal smear from a dog in proestrus. Intermediate epithelial cells predominate. Red blood cells and a few neutrophils are also present.

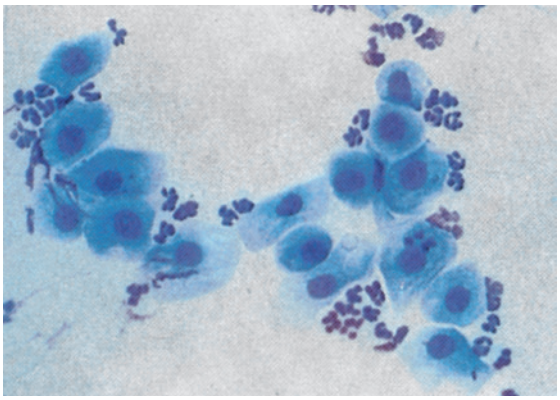


angular cytoplasm from a dog in estrus.

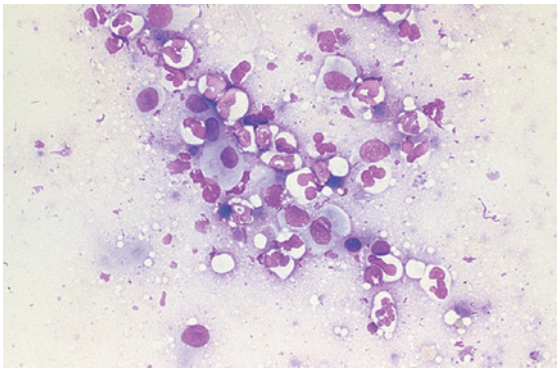
epithelial cells begin to show signs of cornification (e.g., pyknotic nuclei). uring roestrus, ly pithelial ells resent are ermediate ells yknotic uclei. mall umbers neutrophils are sometimes seen proestrus especially during lier

The estrual bitch history of recent proestrus swollen vulva, ith ossibly raw-colored harge becomes whiter metestrus approaches. Bitches estrus accept ogs empting aginal eveals all squamous epithelial cells are cornified usually anuclear Fig. 4.26 hat eutrophils re bsent, hat all umbers of rythrocytes resent. rus, rythro cyte umbers ecrease urther, nd eutrophil umbers ncrease rapidly. rus enerally ys, verage ys.

itch testrus ory ecent rus. ulvar swelling and discharge have ecreased, and she no longer attracts or receptive to dogs. Cornified squamous epithelial cells are replaced by noncornified squamous epithelial cells abundant cytologic ebris. proximately er rus, epithelial cells are noncornified. Neutrophils increase number until approximately third day of metestrus then decrease



Numerous neutrophils and intermediate cells from the vaginal



to ew y out y. ythrocytes enerally throughout metestrus Fig. Metestrus may for to months. Cytologically, metestrus anestrus are often to erentiate. regnancy tologically inguishable from testrus rus.

TECHNICIAN NOTE

Inflammation agina terus esults hite vulvar discharge, usually without vulvar swelling or linical signs of proestrus or estrus. vaginal reveals noncornified squa epithelial cells massive numbers of neutrophils, possibly with ee r hagocytized cteria

Dry-mount ecal tology metimes onjunction with ecal ion nostic or valuation patients ith astrointestinal evaluated ithin utes er ollection. ype collected ary ut lude oided eces, ectal vage, ectal aping. ecal op

to ollect oided esirable ecause they tend to only provide represents intestinal lumen ather ucosal urface. light ution feces may be needed be accomplished by placing drop of erile oscope l ecal erial erile wooden plicator. ectal erformed sterile lunt

Dry-mount fecal cytology are prepared They must be thoroughly dried before staining. ny standard Romanowsky

The slide examined under oil immersion objective. Fecal cytology lides rom ormal amples enerally ontain riety of bacilli rare cocci. may be present normal ell. he xamined or gens uch *Cryptosporidium, Giardia, Entamoeba, Campylobacter, Trichomonas, Balantidium Clostridium perfringens* or *Clostridium difficile* may e resent umbers ecal tology om normal ncreased umbers cterial organisms equire dditional nostic esting.

Campylobacter e ram-negative, nder, curved ods orm heir resence onsid ered normal. resence ukocytes ecal normal equires ther nostic esting. Epithelial cells are present when are collected traumati cally. hen are collected atraumatically, presence of large numbers or sheets of epithelial cells may indicate mucosal pathology.

The valuation emen mportant art he ssessment of for breeding void exposing semen o ked hanges emperature ecially water, ectants, ariations ll oratory quip ment used for semen collection examination be clean y med proximately equipment ludes oscope overslips, ettes. uents med proximately amples rocessed oom on possible er ollection.

The ollowing haracteristics eadily etermined laboratory: volume of ejaculate, gross appearance, wave motion, microscopic motility, spermatozoal concentration, ratio of live-to-dead ermatozoa, rphologic eatures, presence f oreign ells erial. ortant ecord animal's species, breed, age, brief history with salient clinical findings, uspected normalities ell thod semen ollection e.g., rtificial ina, lectroejaculation, assage).

The olume jaculate ured olumetric which may be incorporated into collection receptacle. Marked species ariations ccur, thod ollection reatly influences olume btained, ross pearance, spermatozoal concentration. generalization, ejaculate volume larger but spermatozoal concentration lower specimen

apparently more dilute) when collected by electroejaculation when collected with artificial vagina. In addition, repeated ejaculation—whether associated with semen collection or sexual activity—decreases the volume and concentration of semen obtained subsequent collections. Semen volume tends to be greater collection preceded by period of sexual arousal "teasing").

The ejaculate composed of three portions: sperm-free watery secretion, sperm-rich fraction, sperm-poor frac tion. The first third fractions are derived from accessory sex glands. In bucks, bulls, rams, toms, all three fractions are collected together. However, with boars, dogs, stallions, third fraction conveniently may be collected separately, which advisable, because third fraction voluminous three therefore unnecessary encumbrance during subsequent evaluation of semen In three species, first two fractions (collected together) are used other procedures follow.

The approximate average total ejaculate volumes (all three fractions) are follows: boar, buck ram, bull, dog, stallion, tom, Ejaculate volume does necessarily correlate with fertility. In general, spermatozoal number, motility, morphologic characteristics are better guides to fertility. However, small ejaculates may be of concern species have voluminous ejaculates. Knowledge of ejaculate volume necessary to determine total spermatozoal numbers to be divided possibly diluted) for artificial insemination procedures.

The opacity color of be recorded. Opacity subjectively reflects the concentration of spermatozoa. Categories used include thick, creamy, opaque; milky opaque; opalescent milky; watery white. This generalization works best for semen from bucks, bulls, rams, which normally have opaque, creamy-white semen because of high spermatozoal concentration. As density of spermatozoa decreases, specimen becomes more translucent and milkier in appearance. Semen from boars, dogs, stallions normally fairly translucent white to gray. Contaminants, especially intact or degenerate erythrocytes, discoloration of semen.

Sperm motility (movement) is subjectively assessed and depends on careful handling of for meaningful results. Variations temperature exposure to nonisotonic or destructive chemicals (including detergents) must be avoided. Motility correlated with fertility; however, improper specimen handling adversely affects assessment. If other tests (especially sperm morphology) suggest semen normal but sperm motility poor, another be examined to ensure technical errors were responsible for poor motility. Motility may be conveniently assessed two ways.

Wave motion subjective assessment of gross motility of sperm. Four general classifications are used—very good, good, fair, poor—on of of swirling activity observed drop of semen on microscope slide low power magnification. These categories respectively correspond to distinct vigorous swirling, moderate slow swirling, barely cernible swirling, lack of actual swirling but with motile sperm present, which may to have irregular oscillating appearance. Wave motion depends on high sperm density therefore best from bucks, bulls, rams, which normally have high sperm concentrations. Wave motion decreases sperm concentration decreases. Consequently, normal boars, dogs, stallions, toms may have or poor wave motion. As guide, wave motion very good or good, be diluted for evaluation of percentage of motile sperm their rate of motility.

The progressive motility of individual spermatozoa determined on relatively dilute drop of semen under coverslip examined magnification. Because motility of vidual spermatozoa to appreciate dense such concentrated be diluted before examination. Warm physiologic or fresh buffered sodium citrate solutions are suitable diluents.

drop of semen placed on slide diluted until satis factory concentration of spermatozoa observed. coverslip placed on top to produce monolayer of cells. Excessive dilution of makes evaluation of motility The rate of motility generally subjectively classified very good, good, fair, or poor, which corresponds to rapid activ ity, moderate activity, slow or erratic activity, very slow erratic activity, respectively. The percentage of motile spermatozoa broadly categorized very good, good, fair, or poor, which corresponds to approximately to to to motile cells, respectively. Satisfactory have moderately active spermatozoa.

Several solutions are satisfactory for semen dilution before sperm numbers are counted, including of sodium bicarbonate or of sodium chloride with of formalin of distilled water; chlorazene; or of sodium sulfate with of glacial acetic acid of distilled water (Gower's solu tion). dilution made, counted hemocytometer. The number of spermatozoa central grid area of one side of chamber counted nification. The number of spermatozoa per milliliter of semen calculated by multiplying number observed by million. If spermatozoal concentration high (e.g., bucks, bulls, toms), fewer squares may be counted multiplica tion factor adjusted accordingly. Spermatozoal concentration may be determined by colorimetric electronic particle counter techniques.

Depending on collection method, average sperm con centrations millions per milliliter) are approximately for boars stallions, for bucks rams, for bulls, for dogs, for toms.

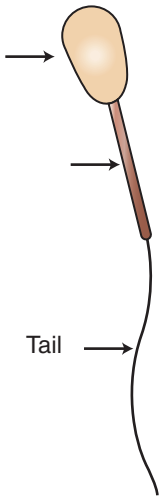
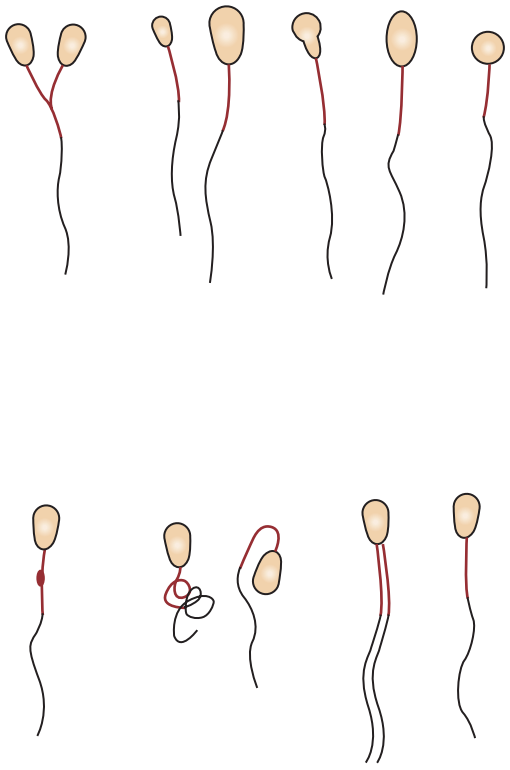


Diagram of a normal spermatozoa.



ith ye lows or imination etween ve ead ermatozoa. osin/nigrosin ure opular for pose, ermits xamination erm morphologic eatures. repared eosin rosin ution dium rate dihydrate. his ution or ear.

small op warm stain gently xed with small op of men n oscope fter veral conds contact between specimen dye, mixture smeared hen lood apidly ied. fter ied, oscopic xamination elayed. Live erm pear hite blue-black rosin ckground. ontrast, ead erm sively e ed. atio of ve-to-dead erm, hich xpressed ercentage, determined by examination or magnification preferably er bservation ells.

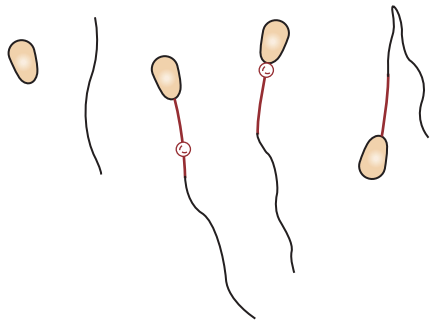
Unfortunately, procedure susceptible to technical prob lems. Conditions kill sperm, especially temperature changes, produce misleading results. Findings always be interpreted with regard to other results, such sperm concentration, motil ity, rphology.

Sperm rphology eadily Wright's or Romanowsky Species differences exist regard points of sperm morphology, but all sperm have ructure ercentage normal spermatozoa ypes ecorded er bserving to ells. ounting wer umber ells usually dequate er echnician ecome roficient. Abnormalities are conveniently divided into head, midpiece, problems. bnormalities are often categorized primary or secondary.

Primary efects ccur uring ermatozoal roduction include ds ouble, oo oo ddly shaped ., yriform, isted, bby) midpieces are swollen, kinked, twisted, double, or eccentri cally ttached ead abaxial); ails hat re oiled Fig. rimary normalities enerally onsidered re serious condary heir ercentage ly onsis tent men ollected veral ys. Slightly ce chment oars robably nificant ecies, ecause umerous ermatozoa parently rmal oars.

Secondary defects may occur any time from storage epididymis until made. Therefore, because secondary abnormalities tifactual, eful ecimen mandatory. ion echnique-induced condary abnormalities lows or erpretation. econd ary efects lude rotoplasmic oplets midpiece, bent or broken Fig. For every tailless head, re dless er ed ounted. Protoplasmic oplets inct om ollen ces. Protoplasmic oplets rmally resent ermato zoa e pididymis. oplets rate ly ce erm ells ure pididymis. The oplets ually efore ermatozoa ve epididymis.

As road eneralization, ually ermatozoa normal rmal Higher percentages of abnormal spermatozoa may compromise fertility. owever, umber rmal ermatozoa important ather ercentage normal erm.



Normal emen ontains ew if ny) eukocytes, rythrocytes, epithelial cells bacteria or fungi. If present, their approxi mate quantity be noted. If bacteria or fungi are observed without ory esponse, ontamination normal preputial microflora be suspected. ttention to reputial ion efore ollection emedy problem. If indicated, semen may be submitted for microbiologic xamination.

Cells om erminal yers estis usual men epresent vere esticular uch cells lude ermatids, ermatocytes, ed ells (often called medusa heads). recise tegorization is import ng ells lassified ure erm ells.

Disorders f rostate ommon ogs, ut are rare other domestic Cells of prostatic origin may be ollected ethral terization ombination prostatic massage (or penile massage) performed per rectum stimulate rostatic cretion. rostatic issue ated by ranscutaneous edle iopsy.

An enlarged prostate may be result of prostatic hypertro phy, yperplasia, oplasia, ion. ros tatic ells ccur ly lusters. ermatozoa present some especially are collected by enile

Normal rostatic ells ve orm ly h ucleus-to-cytoplasm atio, ransparent ray cytoplasm, mogeneous uclear hromatin, bvious nucleoli. ormal rostatic issue ontains ew uko cytes. rostatic ypertrophy nlargement esults from increased size of individual cells without increased cell numbers. hypertrophic prostate cytologically distinguish able from normal prostate. The distinction made on of land e ion adiography. rostatic yperplasia land nlargement esults om eased umbers cells. he ells orm pearance ve high ucleus-to-cytoplasm atio; philic toplasm often acuolated; ucleus roughened" hromatin pattern uniform, small, single nucleolus. Few leukocytes are present. Metaplasia change (from normal) population of rostatic ells. xfoliated iopsied ells ave he ppearance of ncornified pithelial ells. onsequently, have low nucleus-to-cytoplasm ratio somewhat pyknotic nucleus. rostatic oplasia haracterized omorphic population ells ucleus-to-cytoplasm atio very philic toplasm. uclear aries ells, nuclei ontain ariable umbers regular omorphic ucleoli. tologic rostatic abscessation umbers utrophils r issue acrophages ymphocytes e resent ariable umbers.

Subclinical clinical bovine mastitis (mammary gland infec tion) important economic concern for dairy farmers. Mastitis may e etected veral oratory rocedures. frequently ectly ectly eflect matic ell counts or bacterial counts. Tests are performed on from ndividual uarters, ilk amples rom ll our uarters pooled ogether, om veral ows ogether, usually rd).

When vidual eing eened, oremilk btained efore egins) enerally re ells btained but ewer ells ollected ell counts ary ctation. om normal ows eek ctation have her ell btained erim.

Somatic ell rmal enerally ells/mL ells/mL. re ells/mL itis. nformation alifor astitis cated hapter

Differential ell metimes erformed. ucleated cells are categorized neutrophils or mononuclear cells. Normal obtained during mid-lactation generally neutrophils, hereas, vere cute itis, have o utrophils.

Chapter eview uestions ppendix

- Peritoneal pleural are evaluated for color, transpar ency, dor,
- Cells e ounted lassified om eritoneal ural

- The evaluation of cellular elements allows to be classified exudates, transudates, or modified transudates.
- In normal lymph node, predominant cell type small, mature lymphocyte.
- Reactive lymph nodes contain predominantly small, mature lymphocytes well cells, lymphoblasts, inter mediate lymphocytes.
- Evaluations performed on synovial include ment of color turbidity; cytologic examination of direct smear; the subjective assessment of viscosity; and mucin test, refractometric protein measurement.
- Abnormal tracheal are generally exudates.
- Yeasts, squamous epithelial cells, *Malassezia* organisms are commonly isolated from may indicate pathology.
- Vaginal may contain variety of epithelial cells addi tion to neutrophils erythrocytes.
- Epithelial cells are present vaginal cytology may include small cells, slightly larger parabasal epithelial cells, noncornified squamous epithelial cells (intermediate cells), cornified epithelial cells.

here are hazards associated with specific chemicals described?
aterial Safety Data Sheets
. azard Communication Standard
athogen Standard
IM Guidelines

he bacterial agent that causes toxoplasmosis is classified as having which biohazard level?
.
. II

hat agency mandates regulations related to the safe ship ment of potentially hazardous or infectious materials?
ederal Aviation Administration
. .S. Department of Agriculture
cupational Safety and Health Administration
.S. Department of Transportation

4. rue or False: Chemicals transferred into secondary contain ers always require special hazard labeling.
True
. False

rue or False: The use of personal protective equipment (e.g., lead-lined x-ray gloves) is optional.
True
. False

hich regulation describes the scope and extent of worker training and the documentation of that training?
rocedural Control Listing
. C Biosafety Standard
hemical Hygiene Plan
E Standard

rue or False: Most diagnostic samples from veterinary patients sent to outside laboratories for analysis fall into Category B.
True
. False

nfectious canine hepatitis is classified as which biohazard level?
.
. II

9. hich government agency is responsible for enforcing safety regulations in the workplace?
CDC
.
. DOT
FAA

he use of a fume hood when handling chemicals is an example of which approach to minimizing workplace hazards?
ineering controls
. dministrative controls
rocedural controls
ersonal protective equipment

hich is used to calibrate the refractometer?
ontrol serum
b. efractometer standard
istilled water
ormal saline

hat designation is used to describe a pipette that has a double-etched or frosted band at the top?
To contain"
. To deliver with blow-out"
To deliver"
To contain with rinsing"

hich type of pipette is used only to add liquid to another liquid and must then be rinsed with the first liquid?
To contain"
. To deliver with blow-out"
olumetric
ransfer

4. hat is used to clean the optical surface of the refractometer immediately after use?
oap and water
b. ylene
imwipes
ens paper

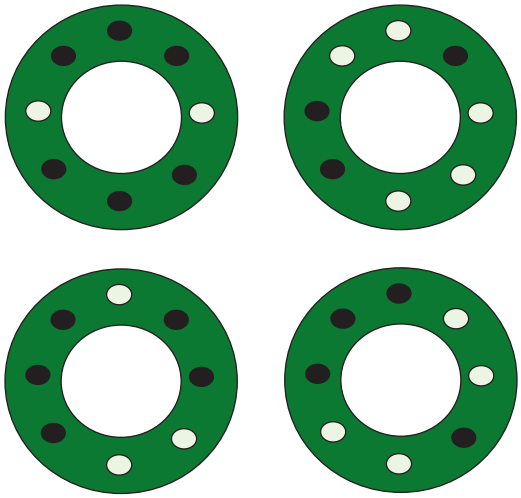
hat type of tube is used to prepare a urine sample prior to centrifugation?
erile
b. lood collection

onical
icrohematocrit

6. hat item is required for the processing of blood components?
d clinical centrifuge
b. ngled-head centrifuge
efrigerated centrifuge
d centrifuge

hich of the following does *not* require an adapter for use with different sizes of test tubes?
imple standard water baths
b. eat blocks
irculating water baths
aterless bead baths

8. hich of the following diagrams depicts a properly balanced centrifuge?



hat is the final magnification of a specimen being viewed via a 10 ocular objective and a 40 objective lens?

b. 0
c.

hich term is used to describe flat field objective lenses?
a. bbe
b. chromatic
pochromatic
igh-eyepoint

hich component of the microscope functions to aim and focus the light that is illuminating the specimen?
a. ondenser
b. osepiece
ris diaphragm
perture diaphragm

rue or False: Mineral oil can be used in place of immersion oil with the oil-immersion lens.
rue
b. alse

hich component of the microscope regulates the amount of light that is illuminating the specimen?
a. ondenser
b. osepiece
ris diaphragm
oarse adjustment knob

hat is the final magnification of a specimen being viewed via a 10 ocular objective lens and a 100 objective lens?

b.

hat is the preferred cleaning solution to be used for the routine cleaning of microscope lenses?
ethanol
b. ylene
ineral oil
mmonia

hat is the preferred cleaning solution to be used for the removal of excess oil from the microscope lenses?
ethanol
b. ylene
ineral oil
mmonia

hich measurement is equal to 1

.

2. hat is the final dilution of a solution that contains 20 dL of a substance that is diluted 1

.

hich number is equal to the number that is written in scientific notation as 10

.

ater freezes at _____° according to the Fahrenheit scale and at _____° according to the Celsius scale.
32
. 212
0
d. 273; 0

ow is the number 624,012 written in scientific notation?

.

ow is the number 0.024 written in scientific notation?

.

ng (nanogram)

.

ow many milliliters are in a 1-L bag of 0.9% NaCl solution?

.

o prepare a 1 ution of a patient sample, combine _____ of the sample with _____ of distilled water.
L; 90
. L; 100
mL; 90
mL; 100

ow is the number 9700 written in scientific notation?

.

hich term refers to how close a test result is to the actual patient value?
ensitivity
b. ecificity
recision
ccuracy

hich term refers to the reproducibility of a test result?
ensitivity
b. ecificity
recision
ccuracy

hich term describes a product that is analyzed in the same manner as a patient sample and used to verify test results?
ontrol
b. d
eagent
alibrator

hich term describes the ability of a testing method to be accurate and precise?
eproducibility
b. eliability
recision
ccuracy

5. ollecting a blood sample from a patient that is not properly fasted is an example of which type of error?
reanalytic
b. nalytic
ostanalytic
onbiologic

6. n improperly maintained analyzer can introduce which kind of error into a test result?
reanalytic
b. nalytic
ostanalytic
onbiologic

---

1. emoglobin formation begins during the _____ stage of erythrocyte maturation and ends during the _____ stage.
ubricyte; metarubricyte
. ubricyte; reticulocyte
ubriblast; reticulocyte
etarubricyte; reticulocyte

hat is the primary cytokine involved in the stimulation of erythrocyte production?
Leukopoietin
. Erythropoietin
Thrombopoietin
Hematopoietin

hat is the primary cytokine involved in the stimulation of platelet production?
Leukopoietin
. Erythropoietin
Thrombopoietin
Hematopoietin

hat is the primary cytokine involved in the stimulation of leukocyte production?
Leukopoietin
. Erythropoietin
Thrombopoietin
Hematopoietin

hat is the primary site for the production of blood cells in the neonatal and juvenile animal?
Liver
. ed bone marrow
Spleen
ellow bone marrow

hat is the primary site for the production of blood cells in the adult animal?
Liver
. ed bone marrow
Spleen
ellow bone marrow

7. he cells in the erythrocyte maturation series in order from most immature to most mature are:
ubricytes, rubriblasts, proreticulocytes, reticulocytes
. roreticulocytes, metarubricytes, rubricytes, reticulocytes
ubriblasts, prorubricytes, metarubricytes, rubricytes, reticulocytes
ubriblasts, prorubricytes, rubricytes, metarubricytes, reticulocytes

8. he cells in the granulocyte maturation series in order from most immature to most mature are:
romyeloblast, myeloblast, myelocyte, metamyelocyte, band cell
. yeloblast, promyelocyte, metamyelocyte, myelocyte, band cell

yeloblast, promyelocyte, myelocyte, metamyelocyte, band cell
yeloblast, myelocyte, promyelocyte, metamyelocyte, band cell

9. fter a granulocyte matures to the _____ stage, it begins to exhibit granules that are characteristic of the neutrophil, the eosinophil, or the basophil.
Myeloblast
. Promyelocyte
Myelocyte
Metamyelocyte

hich cells are part of the granulocyte proliferation pool?
etamyelocytes and band cells
. yeloblasts, promyelocytes, and myelocytes
yeloblasts and metamyelocytes
romyelocytes and metamyelocytes

11. hich term describes a decrease in the numbers of all blood cells and platelets?
Pancytopenia
. Erythropenia
Reticulocytosis
Polycythemia

hat is meant when a sample is described as having a left

Neutrophilia
. shift in the neutrophil–lymphocyte ratio in favor of neutrophils
n increase in the number of immature neutrophils in the blood
ukemoid response

he Vacutainer that is most suitable for collection of blood for hematology usually has a _____ colored top.
. Red
. urple or lavender
. Green
Blue

hich anticoagulant is preferred for routine hematologic studies because it preserves cell morphology?
Heparin
. odium citrate
EDTA
mmonium oxalate

3. hat is the preferred site for venipuncture in large animals?
ammary vein
. ugular vein
nfraorbital sinus
ail vein

4. hich site is preferred for venipuncture in dogs when small volumes are needed?
ephalic vein
. ugular vein
aphenous vein
emoral vein

hich term describes the fluid portion of the blood that contains no formed elements or fibrinogen?
hole blood
. lood plasma
nticoagulated blood
Serum

hich anticoagulant provides the best preservation of glucose?
Heparin
. odium fluoride
EDTA
mmonium oxalate

hat can occur if a tourniquet is left in place for an excessive amount of time?
Hemolysis
. Icterus
Hemoconcentration
Lipemia

hen collecting samples from a patient who requires hematology, chemistry, and coagulation testing, the first sample collected will be the one that requires _____ anticoagulant.
. No
. Fluoride
EDTA
Citrate

9. hen collecting samples from a patient who requires hematology and chemistry testing, the first sample collected will be the one that requires _____ anticoagulant.
. No
. Fluoride
EDTA
Citrate

hat is contained in the "tiger-top" blood collection tube?
EDTA
. Oxalate
l separator
Silicone

hich type of analyzer counts particles on the basis of their size?
mpedance counter
. itative buffy coat analyzer
efractometer
r flow cytometer

hich type of instrument counts particles on the basis of their relative size and density?
mpedance counter
. itative buffy coat analyzer
efractometer
r flow cytometer

3. hich instrument provides an estimate of cell counts on the basis of differential centrifugation?
mpedance counter
. itative buffy coat analyzer
efractometer
r flow cytometer

unning the electrolyte solution through the impedance counter to identify the presence of small particles so that the analyzer does not count them is referred to as the:
ackground count
. gglutinin zero point
hreshold control test
istogram output

amples from _____ may not be adequately evaluated with impedance analyzers as a result of the size similarities between red blood cells and platelets.
a. ogs
b. ats
orses
d. ows

6. hat is the volume of each of the nine squares of the hemo cytometer with the Neubauer ruling?

b.

hich term describes an increase in the number of circulat ing erythrocytes?
ancytopenia
b. ythropenia
eticulocytosis
olycythemia

hich of the following is a possible cause of relative polycythemia?
umors
b. ung disease
nic contraction
xcess EPO in the blood

hich of the following is a common cause of increased plasma protein levels?
Overhydration
. enal disease
ver disease
Dehydration

hich term describes plasma that appears to have a red tinge in a spun microhematocrit tube?
Icteric
. Hemolyzed
Lipemic
Polycythemic

hich term describes plasma that appears to have a yellow tinge in a spun microhematocrit tube?
Icteric
. Hemolyzed
Lipemic
Polycythemic

hich term describes plasma that appears milky in a spun microhematocrit tube?
Icteric
. Hemolyzed
Lipemic
Polycythemic

5. hich instrument is used to measure the total protein content of a sample?
mpedance counter
. HemoCue
Refractometer
Centrifuge

6. hich of the following causes a false decrease in packed cell volume?
rtened centrifuge time
. Icterus
ow blood-to-anticoagulant ratio
lots in the blood sample

he normal packed cell volume in dogs is about:

.

hat is the MCV in a canine patient with a PCV of 38% and a RBC count of 6.5

.
pg
pg

hat is the unit of measure for MCHC?

. pg

.

10. n estimate of the total red blood cell count can be obtained by dividing the _____ by 6.

.
emoglobin level

1. hen making a blood smear with a sample that is very thick and that contains some small clots, you should do which of the following?
ncrease the spreader slide angle to about 45 degrees.
. ecrease the spreader slide angle to about 20 degrees.
ilute the sample 2 ith EDTA.
ake the smear from a fresh sample.

2. hat is the predominant white blood cell type in ruminants?
Neutrophil
. Lymphocyte
Eosinophil
Monocyte

3. hich cell type is characterized by amoeboid nuclear mate rial and abundant, blue-gray, vacuolated cytoplasm?
Neutrophil
. Lymphocyte
Eosinophil
Monocyte

4. hich white blood cell is the predominant one that responds to allergies and parasitic disease?
   Neutrophil
   . Lymphocyte
   Eosinophil
   Monocyte
5. hich cell type can be identified by its dark, irregular, lobu lated nucleus and its colorless or pale-pink cytoplasm?
   Neutrophil
   . Lymphocyte
   Eosinophil
   Monocyte

he cell with a rounded or slightly indented nucleus that almost completely fills the cell is the mature _____.
   Neutrophil
   . Lymphocyte
   Eosinophil
   Monocyte

hich of the following best describes the granules of feline eosinophils?
   mall and round
   . arying size
   Rod-shaped
   e and oval

hich is the largest white blood cell in the peripheral blood?
   Neutrophil
   . Lymphocyte
   Eosinophil
   Monocyte

f a patient has 70% neutrophils on the differential blood cell film and a white blood cell count of 12,000, what is the absolute number of neutrophils present per

.

ocytosis is the primary function of which cell?
   Eosinophil
   . Lymphocyte
   Basophil
   Neutrophil

hich of the following best describes the morphology of canine erythrocytes?
   Elliptical
   . Nucleated
   iconcave disc
   Round

hich of the following best describes the morphology of avian erythrocytes?
   Elliptical
   . Nucleated
   iconcave disc
   Round

elet estimate that is performed with the use of a dif ferential blood cell film requires the counting of platelets in a minimum of _____ microscopic fields.

.

ultiply the platelet estimate obtained from the blood film by _____ to get the platelet estimate per L of blood.

.

hich of the following can be seen in lead poisoning?
   asophilic stippling
   . Spherocytosis
   Echinocytosis
   Anisocytosis

hich abnormality is characterized by grapelike clusters of red blood cells that do not break up when the sample is diluted with saline?
   owell-Jolly bodies
   . einz bodies
   Rouleaux
   Agglutination

hich red blood cell abnormality is often seen in healthy horses?
   owell-Jolly bodies
   . einz bodies
   Rouleaux
   Agglutination

hich term describes pale or bluish round areas attached to the red blood cell membrane that are caused by chemi cal- or drug-induced oxidative injury?
   owell-Jolly bodies
   . einz bodies
   Rouleaux
   Agglutination

hich abnormality is characterized by coinlike stacks of red blood cells?
   owell-Jolly bodies
   . einz bodies
   Rouleaux
   Agglutination

hich term describes round basophilic nuclear remnants in the red blood cells of animals with regenerative anemia?
   owell-Jolly bodies
   . einz bodies
   Rouleaux
   Agglutination

7. hich of the following is a toxic change that may be observed in neutrophils?
   odies
   . owell-Jolly bodies
   einz bodies
   uclear hyposegmentation

8. he presence of basophilic macrocytes on a peripheral blood cell film usually indicates increased numbers of:

Codocytes
. Reticulocytes
Metarubricytes
Dacrocytes

9. hich term describes cells with regular cytoplasmic projections around the periphery that give the cell the appearance of a scalloped border?
Acanthocytes
. Keratocytes
Echinocytes
Schistocytes

mall round or rod-shaped structures that are found as single organisms or pairs on the periphery of a red blood cell are:
einz bodies
. odies
c. *Ehrlichia*
d. *Mycoplasma*

hich type of stain is used to prepare a reticulocyte count

Supravital
. Romanowsky
Diff-Quik
Wright's

2. ow is the reticulocyte count recorded if a reticulocyte smear from a cat has 5 punctate reticulocytes and 15 aggregate reticulocytes per 1000 red blood cells?

.

3. ow is the reticulocyte count recorded if a reticulocyte smear from a dog has 25 reticulocytes per 1000 red blood cells?

.

4. hat is the absolute value of reticulocytes if 200 reticulocytes are counted per 1000 red blood cells and the total red blood cell count is 2.2

.

hat is the corrected reticulocyte percentage if a canine patient has 10% reticulocytes and a packed cell volume of

.

6. hat is the corrected reticulocyte percentage if a feline patient has 10% reticulocytes and a packed cell volume of 32%?

.

hat is the minimum number of nucleated cells that are counted and classified to calculate the ratio of myeloid cells to erythroid cells in bone marrow?

.

ow would a bone marrow sample from an adult animal be described if it contains 80% fat?
Aplastic
. Hypocellular
Hypercellular
Normal

ow would a bone marrow sample from a juvenile animal be described if contains 50% fat?
Aplastic
. Hypocellular
Hypercellular
Normal

hat stain is used to evaluate the presence of hemosiderin in a bone marrow sample?
Wright-Giemsa
. russian blue
Diff-Quik
Wright's

hat is the common term that is used to describe the presence of neoplastic blood cells in the bone marrow and the peripheral blood?
eukemia
ymphoproliferative
yeloproliferative
yogranulomatous

hich of the following is likely to cause microcytic anemia?
lichiosis
b. ug toxicity
ypothyroidism
ron deficiency

hich term describes chronic bone marrow inflammation that is characterized by increased numbers of macrophages?
ypoplastic
b. ranulomatous
yogranulomatous
rinous

hich term describes chronic bone marrow inflammation that is characterized by increased numbers of macrophages and neutrophils?
ypoplastic
b. ranulomatous
yogranulomatous
rinous

he classification of anemia according to _____ distinguishes between regenerative and nonregenerative anemia.

one marrow response
b. ythrocyte indices
tiology
orphology

hat is the molecule that platelets express on their surface when they are activated?
hrombin
b. on Willebrand factor
hatidylserine
icroparticles

2. hich molecule binds to tissue factor in the plasma to initiate the coagulation reactions?
actor VIII
b. hrombin
on Willebrand factor
rothrombin

hich of the following functions to stabilize the platelet plug?
actor VIII
b. hrombin
on Willebrand factor
rothrombin

4. hich factor is activated as a result of the formation of coagu lation complexes?
actor II
b. actor VIII
actor IX
d. ctor X

hich of the following is involved in the breakdown of the clot?
hrombin
b. issue factor
issue plasminogen activator
imers

hich anticoagulant is preferred for platelet testing?
eparin
b. odium citrate
A
mmonium oxalate

hich is the preferred anticoagulant for most plasma assays of coagulation?
eparin
b. odium citrate
A
mmonium oxalate

hat is the proper ratio of blood to citrate anticoagulant?

.

hat type of technology is used in the Coag Dx™ analyzer to evaluate coagulation?
detector
b. echanical
ire movement
ollagen-coated membrane

hat type of technology is used in the PFA-100 analyzer to evaluate coagulation?
detector
b. echanical
ire movement
ollagen-coated membrane

1. hich term describes the average size of the individual plate lets in a sample?
eletcrit
. ean platelet volume
elet–large cell ratio
elet distribution width

2. hich term refers to the percentage of the total blood volume that is comprised of platelets?
eletcrit
. ean platelet volume
elet–large cell ratio
elet distribution width

hich test evaluates the variability in the size of platelets?
eletcrit
. ean platelet volume
elet–large cell ratio
elet distribution width

hich test provides a measure of the percentage of platelets that are larger than normal?
eletcrit
. ean platelet volume
elet–large cell ratio
elet distribution width

hich term describes newly released platelets that contain high levels of RNA?
elet bands
b. ranulated platelets
A-rich platelets
eticulated platelets

1. hich coagulation test uses a tube containing diatomaceous earth?

b. leeding time
T
CT

hich coagulation test evaluates the extrinsic coagulation pathway?

b. leeding time

T
CT
hat are D-Dimer and FDP tests used to evaluate?
rimary (mechanical) hemostasis
. econdary (chemical) hemostasis
ertiary hemostasis
nticoagulant rodenticide toxicity
hat is the PIVKA test used to evaluate?
rimary (mechanical) hemostasis
. econdary (chemical) hemostasis
ertiary hemostasis
nticoagulant rodenticide toxicity
hich test represents a primary assay for the evaluation of platelet number and function?
elet estimate
. uccal mucosa bleeding time
ee–White bleeding time
lot retraction

hich coagulation assay is a good screening test for roden ticide ingestion?
ctivated partial thromboplastin time
b. rothrombin time
on Willebrand antigen assay
elet count

2. hat is the most common inherited coagulation disorder of domestic animals?
hrombocytopenia
b. emophilia
nticoagulant rodenticide toxicity
on Willebrand disease
hich term refers to the presence of pinpoint hemorrhage?
chymoses
b. etechia
urpura
pistaxis

4. hich of the following is often found on a blood smear from patients with DIC?
histocytes
b. s
olycythemia
yeloproliferation

5. eficient or defective production of which coagulation factor results in hemophilia A?
actor VII
b. hristmas factor
actor VIII
rothrombin
hat is the most common coagulation disorder of domestic

hrombocytopenia
b. emophilia
nticoagulant rodenticide toxicity
on Willebrand disease

7. hich of the following are the vitamin-K–dependent coagu lation factors?
actors I, VII, X, and XII
. actors II, VII, IX, and X
actors II, VIII, XI, and XII
actors IV, IX, X, and XX
hat is the primary site for the production of coagulation factors?
one marrow
b. en
ile duct
ver

1.
2.
3.
4.
5.
6.
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8.
9.

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APPENDIX

The data on the following pages were compiled from a variety of sources, including personal notes, workshops, conference pre sentations, and the references listed at the end of each chapter. These values can be affected by a large number of factors, such as the test methods used, the type of equipment used, and the characteristics of the patient. Each laboratory should establish its own reference ranges for the methods used in that particular laboratory.

Hematology Reference Ranges

Coagulation Reference Ranges

Clinical Chemistry Reference Ranges

Urinalysis Reference Ranges

High-power field.

Blood Gas and Acid Base Measurements (Mean
Unanesthetized Dogs

	Mixed Venous Blood

From DiBartola SP: *base disorders in small animal practice*

Bacterial Pathogens of Veterinary Importance

The following table is a summary of characteristics of and dis eases produced by microbial pathogens seen in mammals and birds. A comprehensive bacteriology text should be consulted for additional characteristics used to identify these species defini tively and for additional information about less common species.

- Gram-negative
- Facultative

- Pus

- Identification/differentiation
  - Growth
  - CAMP
  - Esculin
  - Acid

  - Oxalase
  - Catalase
  - Urease

- Gram-positive,
- Non–spore-forming
- Poor
- Facultative

- Usually
- Often
- Identification/differentiation
  - Colony
  - Hemolysis
  - Esculin
  - Acid

  - Nitrate
  - Catalase
  - Urease

- Gram-negative,
- Located

- Identification/differentiation
- Demonstration
- Immunology

- Gram-negative,
- Microaerophilic
- Identification/differentiation
  - Colony
  - Nitrate
  - Catalase
  - Growth
  - Hydrogen

- Gram-positive,
- Identification/differentiation
  - Colony
  - Hemolysis

- Gram-positive,
- Identification/differentiation
  - Colony
  - Hemolysis
  - Motility
  - Nitrate
  - PCR

- Gram-negative,
- Anaerobic
- Identification/differentiation
  - Cellular
  - Colony
  - Bile
  - Esculin
  - Indole
  - Fermentation

- Gram-negative
- Identification/differentiation
  - Colony
  - Growth
  - Slide
  - Urease
  - Oxidase
  - Nitrate
  - Citrate
  - Motility

		• Coiled
		• Microaerophilic
		• Identification/differentiation
		  • Demonstration
		
		  • Immunologic

		• Gram-negative,
		• Identification/differentiation
		  • Cellular
		  • Colony
		  • Hemolysis
		  • Indole

		• Gram-negative
		• Nonmotile
		• Facultative
		• Identification/differentiation
		  • Hemolysis
		  • Indole
		  • Oxidase
		  • Catalase
		  • Nitrate
		  • Urease

	orchitis, posterior paralysis	

		• Gram-negative
		• Identification/differentiation
		  • Cellular
		  • Colony
		  • Growth
		  • Motility
		  • Oxidase
		  • Catalase
		  • Nitrate
		  • Carbohydrate

		• Gram-negative

C. jejuni jejuni		• Microaerophilic
		• Motile
		• Identification/differentiation
		  • Colony
		  • Hydrogen
		  • Oxidase
		  • Catalase
		  • Nitrate
		  • Carbohydrate

	Chlamydophila psittaci	• Gram-negative
		• Identification/differentiation
		  • Cytology
		  • Immunology
		
		  • Cell

- Gram-negative
- Identification/differentiation
  - Cytology
  - Immunology

  - Cell

- Identification/differentiation
  - Colony
  - Hydrogen
  - Motility
  - Acid

- Gram-positive,
- Oxygen
- Produce
- Identification/differentiation
  - Colony
  - Hemolysis
  - Immunology
  - Histology
  - Cytology

- Gram-positive,

- Aerobic
- Most
- Non–acid-fast
- Identification/differentiation
  - Colony
  - Catalase
  - Hemolysis
  - Nitrate
  - Esculin
  - Acid
  - Urease

- Gram-positive,
- Aerobic
- Motile
- Non–acid-fast
- Identification/differentiation
  - Cellular
  - Colony
  - Catalase
  - Hemolysis
  - Acid
  - Nitrate

Organism	Primary Species Affected	Disease or Lesion	Characteristics
***Dichelobacter* Species**			

D. nodosus	Sheep, cattle, pigs, goats	Foot rot	• Gram-negative, pleomorphic, slightly curved bacilli
			• Anaerobic
			• Identification/differentiation
			  • Cellular morphology
			  • Colony characteristics
			  • Esculin hydrolysis test
			  • Acid production in carbohydrate substrates
			  • Indole production test

***Ehrlichia* Species**			

E. bovis	Cattle	See *Anaplasma bovis*	• Gram-negative, coccoid to ellipsoid forms
			• Located within cytoplasmic vacuoles of endothelium, myeloid cells, granulocytes, or thrombocytes
			• Identification/differentiation
			  • Demonstration of organisms on blood film
			  • Immunology

E. canis	Dogs, other canids	Monocytic ehrlichiosis	
E. chaffeensis	Dogs	Monocytic ehrlichiosis	
E. ewingii	Dogs	Granulocytic ehrlichiosis	
E. muris	Mice	Ehrlichiosis	
E. platys	Dogs	See *Anaplasma platys*	
E. ruminantium	Ruminants	Heartwater	
***Enterobacter* Species**			

E. aerogenes	Most mammals	Mastitis, neonatal septicemia, metritis, urinary tract infection, wound infection	• Gram-negative, motile bacilli
			• Identification/differentiation
			  • Colony characteristics
			  • Cellular morphology
			  • Citrate utilization test
			  • Hydrogen sulfide production
			  • Acid and gas production from lactose

E. cloacae	Birds		
***Eperythrozoon* Species**			
See *Mycoplasma*			
***Erysipelothrix* Species**			

E. rhusiopathiae	Cattle, pigs, sheep, turkeys	Diamond skin disease, arthritis, endocarditis	• Gram-positive, non–spore-forming bacilli
			• Facultative anaerobes
			• Nonmotile
			• Identification/differentiation
			  • Cellular morphology
			  • Colony characteristics
			  • Hemolysis pattern
			  • Catalase test
			  • Esculin hydrolysis test
			  • Acid production from carbohydrates
			  • Hydrogen sulfide production
			  • CAMP test
			  • Immunology (agglutination)

***Escherichia* Species**			

E. coli (numerous pathotypes)	Most vertebrates	Enteritis, septicemia, ruminant mastitis, canine pyometra, cystitis, calf scours	• Gram-negative bacilli
			• Most are motile
			• Identification/differentiation
			  • Cellular morphology
			  • Colony characteristics
			  • Hemolysis pattern
			  • Growth on MacConkey agar
			  • Catalase test
			  • Oxidase test
			  • Acid and gas production from glucose
			  • Hydrogen sulfide production
			  • Immunology (agglutination, ELISA, PCR)
			  • Histology

Organism	Primary Species Affected	Disease or Lesion	Characteristics
Francisella **Species**			

F. tularensis	Rabbits, most other mammals	Pneumonia, fever, lymphadenitis, ulcerative dermatitis	• Gram-negative coccobacilli
			• Identification/differentiation
			  • Cellular morphology with fluorescent antibody stain
			  • Immunology (agglutination, ELISA, antibody titer)
			  • Histology

Fusobacterium **Species**			

F. equinum	Horses	Lower respiratory tract disease	• Gram-negative, non–spore-forming fusiform bacilli
			• Identification/differentiation
			  • Cellular morphology
			  • Colony characteristics
			  • Hemolysis pattern
			  • Catalase test
			  • Nitrate reduction test
			  • Esculin hydrolysis test
			  • Fermentation of glucose
			  • Indole production test

F. necrophorum	Cattle, sheep, horses, pigs, rabbits	Foot rot, mastitis, liver abscess, metritis, calf diphtheria, thrush (equine), abortion, ulcerative stomatitis, "bull nose"	
F. nucleatum	Cattle, sheep	Abortion	
Haemobartonella **Species**			
H. canis	Dogs	See *Mycoplasma haemocanis*	
H. felis	Cats	See *Mycoplasma haemofelis*	
Haemophilus **Species**			

H. felis	Cats	Rhinitis, conjunctivitis	• Gram-negative, pleomorphic bacilli or coccobacilli
			• May form filaments
			• Nonmotile
			• Facultative anaerobes
			• Growth on chocolate agar
			• Identification/differentiation
			  • Catalase test
			  • Indole production test
			  • CAMP test
			  • Acid production in carbohydrate substrates
			  • Urease test
			  • Immunology (immunohistochemistry, PCR)

H. haemoglobinophilus	Dogs	Vaginitis, cystitis	
H. influenzaemurium	Rodents	Respiratory disease, ocular disease	
H. paragallinarum	Chickens	Infectious coryza	
H. parasuis	Pigs	Glasser's disease, meningitis, myositis, pneumonia, septicemia	
Helicobacter **Species**			

H. bilis	Mice	Hepatitis	• Gram-negative helical, curved, or unbranched bacilli
			• Motile
			• Microaerophilic
			• Identification/differentiation
			  • Colony characteristics
			  • Catalase test
			  • Oxidase test
			  • Urease test

H. canis	Dogs	Gastroenteritis	
H. cholecystus	Hamsters	Cholecystitis, pancreatitis	
H. felis	Cats, dogs	Gastritis	
H. hepaticus	Mice, rats	Hepatitis	
H. muridarum	Mice, rats	Gastritis	
H. mustelae	Ferrets	Gastritis	
H. nemestrinae	Macaques	Gastritis	
H. pullorum	Poultry	Gastroenteritis, hepatitis	
H. pylori	Monkeys, cats	Gastritis	
H. rappini	Mice, rats, dogs, sheep	Abortion	
Histophilus **Species**			

H. somni	Cattle	Bronchopneumonia, "honker syndrome," myocarditis, otitis, conjunctivitis, myelitis, vaginitis, orchitis, thromboembolic meningoencephalitis	• Gram-negative, nonmotile, pleomorphic bacilli
			• Capnophilic
			• Identification/differentiation
			  • Colony characteristics
			  • Hemolysis pattern
			  • Catalase test
			  • Oxidase test
			  • Nitrate reduction test
			  • Immunology

Organism	Primary Species Affected	Disease or Lesion	Characteristics
Klebsiella **Species**			

K. pneumoniae pneumoniae	Cattle, horses, sheep, dogs, birds	Metritis, mastitis, neonatal septicemia	• Gram-negative, nonmotile, encapsulated bacilli
			• Identification/differentiation
			  • Colony characteristics
			  • Cellular morphology
			  • Citrate utilization test
			  • Hydrogen sulfide production
			  • Acid and gas production from lactose
			  • Urease test
			  • Indole production test

K. oxytoca	Horses	Vaginitis, metritis, abortion, infertility	
Lawsonia **Species**			

L. intracellularis	Pigs, hamsters, cats, dogs, horses, ferrets	Proliferative enteritis, "wet tail," ileitis	• Gram-negative, curved intracellular body
			• Motile
			• Identification/differentiation
			  • Cellular morphology with silver staining
			  • Immunology (ELISA, immunofluorescence)

Leptospira **Species**			

L. bratislava	Horses, pigs	Abortion	• Spiral bacteria
			• Aerobic
			• Motile
			• Identification/differentiation
			  • Cellular morphology with dark field microscopy
			  • Immunology (agglutination, PCR, fluorescent antibody stain)

L. canicola	Cattle, pigs, dogs	Uremia, abortion	
L. grippotyphosa	Cattle, pigs, horses	Fever, jaundice, uremia	
L. hardjo	Cattle	Abortion, infertility	
L. icterohaemorrhagiae	Dogs, cattle, rats	Septicemia, abortion	
L. kennewicki	Horses	Abortion	
L. pomona	Pigs, cattle, horses	Abortion	
Listeria **Species**			

L. monocytogenes	Cattle, sheep, goats, horses, birds, dogs, rodents, pigs	Central nervous system infection, abortion, mastitis, septicemia	• Gram-positive, non–spore-forming bacilli
			• Facultative anaerobes
			• Motile
			• Identification/differentiation
			  • Cellular morphology
			  • Colony characteristics
			  • Hemolysis pattern
			  • Catalase test
			  • Esculin hydrolysis test
			  • Acid production from carbohydrates
			  • Hydrogen sulfide production
			  • CAMP test

Mannheimia **Species**			

M. haemolytica	Cattle, sheep	Pneumonia, septicemia, mastitis	• Gram-negative bacilli and coccobacilli
			• Nonmotile
			• Facultative anaerobes
			• Identification/differentiation
			  • Colony characteristics
			  • Hemolysis pattern
			  • Oxidase test
			  • Acid production from glucose
			  • Nitrate reduction test

M. granulomatis	Cattle	Panniculitis	
M. varigena	Cattle	Pneumonia, septicemia, mastitis	
Moraxella **Species**			

M. bovis	Cattle	Infectious keratoconjunctivitis	• Gram-negative coccobacilli
			• Nonmotile
			• Identification/differentiation
			  • Colony characteristics
			  • Growth on MacConkey agar
			  • Hemolysis pattern
			  • Oxidase test
			  • Catalase test
			  • Nitrate reduction test
			  • Immunology (fluorescent antibody stain, ELISA)

M. canis	Dogs	Bite-wound infections	
M. ovis	Small ruminants	Pinkeye	

Organism	Primary Species Affected	Disease or Lesion	Characteristics
Morganella Species			

M. morganii	Dogs	Otitis externa, cystitis	• Gram-negative bacilli
			• Identification/differentiation
			  • Colony characteristics
			  • Oxidase test
			  • Indole production test

Mycobacterium Species			

M. avium avium	Birds	Tuberculosis	• Gram-positive, non–spore-forming bacilli
			• Nonmotile
			• Aerobic
			• Acid fast
			• Identification/differentiation
			  • Colony characteristics
			  • Cellular morphology
			  • Intradermal skin test
			  • Carbohydrate utilization test

M. avium paratuberculosis	Ruminants	Johne's disease	
M. bovis	Ruminants, dogs, cats, pigs, goats, nonhuman primates	Tuberculosis	
M. fortuitum	Cattle, cats, dogs, pigs	Mastitis; joint, lung, and skin disease	
M. intracellularae	Pigs, cattle, nonhuman primates	Tuberculosis, granulomatous enteritis	
M. lepraemurium	Cats, rats	Leprosy	
M. porcinum	Pigs	Lymphadenitis	
M. smegmatis	Cattle, cats	Mastitis, ulcerative skin disease	
M. vaccae	Cattle	Skin disease	
M. xenopi	Cats, pigs	Nodular skin lesions, lymphadenitis	
Mycoplasma Species			
Nonhemotropic Mycoplasmas			

M. agalactiae	Goats, sheep	Contagious agalactia	• Identification/differentiation
			  • Colony characteristics
			  • Colony stain with Diene stain
			  • Urease test
			  • Immunology (immunodiffusion, immunofluorescent assay, agglutination, ELISA)

M. alkalescens	Cattle	Arthritis, mastitis	
M. bovigenitalium	Cattle	Infertility, mastitis	
M. bovis	Cattle	Arthritis, mastitis, pneumonia, abortion, abscesses, otitis media, genital infections	
M. bovoculi	Cattle	Conjunctivitis	
M. californicum	Cattle	Mastitis	
M. canadense	Cattle	Abortion, mastitis	
M. capricolum	Goats	Abortion, mastitis, septicemia, polyarthritis, pneumonia	
M. conjunctivae	Sheep	Infectious keratoconjunctivitis	
M. cynos	Dogs	Pneumonia	
M. dispar	Cattle	Respiratory disease	
M. felis	Cats, horses	Conjunctivitis, pneumonia	
M. gallisepticum	Chickens, turkeys	Airsacculitis, sinusitis	
M. gatae	Cats	Arthritis	
M. hyopneumoniae	Pigs	Pneumonia	
M. hyorhinis	Pigs	Polyarthritis	
M. meleagridis	Turkeys	Airsacculitis, skeletal abnormalities	
M. mycoides capri	Goats	Arthritis, mastitis, pleuropneumonia, septicemia	
M. mycoides mycoides	Cattle, goats, sheep	Pleuropneumonia, mastitis, septicemia, polyarthritis, pneumonia	
M. ovipneumoniae	Goats, sheep	Pleuropneumonia	
M. pulmonis	Rats, mice	Murine respiratory mycoplasmosis	
M. synoviae	Chickens, turkeys	Infectious synovitis	
Hemotropic Mycoplasmas			

M. haemocanis	Dogs	Haemobartonellosis	• Coccoid organisms
			• Obligate intracellular parasites
			• Attach to red blood cell surface
			• Identification/differentiation
			  • Cellular morphology
			  • Immunology (PCR)

M. haemofelis	Cats	Haemobartonellosis, feline infectious anemia	
M. haemomuris	Rats, mice	Haemobartonellosis	
M. ovis	Sheep, goats	Eperythrozoonosis	
M. suis	Pigs	Eperythrozoonosis	
M. wenyonii	Cattle	Eperythrozoonosis	

Organism	Primary Species Affected	Disease or Lesion	Characteristics
Neisseria **Species**			

N. canis	Dogs	Bite-wound infections	• Gram-negative coccobacilli
			• Nonmotile
			• Identification/differentiation
			  • Colony characteristics
			  • Hemolysis pattern
			  • Oxidase test
			  • Catalase test
			  • Acid production from carbohydrates

N. weaveri	Dogs	Bite-wound infections	
Neorickettsia **Species**			

N. helminthoeca	Dogs, other canids	"Salmon poisoning"	• Gram-negative, coccoid to ellipsoid forms
			• Located within cytoplasmic vacuoles of myeloid cells or enterocytes
			• Identification/differentiation
			  • Demonstration of organisms on blood film
			  • Immunology

N. risticii	Horses	Potomac horse fever, monocytic ehrlichiosis	
Nocardia **Species**			

N. asteroides	Dogs, cats, cattle, horses, pigs	Lymphadenitis, subcutaneous abscess, stomatitis, mastitis, pleuritis, peritonitis, abortion	• Gram-positive, pleomorphic, non–spore-forming bacilli
			• Nonmotile
			• Aerobic
			• Acid-fast
			• Identification/differentiation
			  • Colony characteristics
			  • Cellular morphology
			  • Nitrate reduction test
			  • Esculin hydrolysis test
			  • Urease test

N. brasiliensis	Horses	Pneumonia, pleuritis	
N. otitidiscaviarum	Cattle, guinea pigs	Ear infections, mastitis	
Pasteurella **Species**			

P. caballi	Horses	Respiratory infection, metritis	• Gram-negative bacilli and coccobacilli
			• Nonmotile
			• Aerobes
			• Identification/differentiation
			  • Colony characteristics
			  • Hemolysis pattern
			  • Growth on chocolate agar and MacConkey agar
			  • Catalase test
			  • Urease test
			  • Indole production test
			  • Oxidase test
			  • Acid and gas production from carbohydrates
			  • Nitrate reduction test

P. canis	Dogs	Puppy septicemia	
P. gallinarum	Chickens, turkeys	Fowl cholera, salpingitis	
P. haemolytica	Cattle, sheep	See *Mannheimia haemolytica*	
P. lymphangitidis	Cattle	Lymphangitis	
P. mairii	Pigs	Abortion, septicemia	
P. multocida	Ruminants, pigs, rodents, dogs, cats, cattle	Pneumonia, fowl cholera, rhinitis, mastitis, hemorrhagic septicemia, bite-wound infections	
P. pneumotropica	Rodents, rabbits	Pneumonia	
P. trehalosi	Sheep	Septicemia, pneumonia	
Porphyromonas **Species**			

P. levii	Cattle, most mammals	Bovine summer mastitis, pleuritis	• Non–spore-forming, pleomorphic bacilli
			• Nonmotile
			• Obligate anaerobes
			• Identification/differentiation
			  • Colony characteristics
			  • Hemolysis pattern
			  • Acid production on carbohydrate substrates
			  • Indole production test

P. gingivalis	Numerous	Periodontitis, gingivitis	

Organism	Primary Species Affected	Disease or Lesion	Characteristics
***Prevotella* Species**			

P. melaninogenica
P. heparinolytica
	Cattle
	Horses
		Foot rot
		Lower respiratory tract disease
			• Non–spore-forming, pleomorphic bacilli
			• Nonmotile
			• Obligate anaerobes
			• Identification/differentiation
			  • Colony characteristics
			  • Hemolysis pattern
			  • Acid production on carbohydrate substrates
			  • Indole production test

***Proteus* Species**			

P. mirabilis
P. vulgaris
	Dogs, horses, calves	Cystitis, pyelonephritis, prostatitis, otitis externa	• Gram-negative bacilli
			• Motile
			• Identification/differentiation
			  • Colony characteristics
			  • Oxidase test
			  • Hydrogen sulfide production
			  • Indole production test

***Pseudomonas* Species**			

P. aeruginosa
P. fluorescens
P. mallei
	Cattle, dogs, horses, sheep
	Cattle
		Mastitis, otitis externa, metritis, corneal ulcer, fleece rot
		Mastitis
		See *Burkholderia mallei*
			• Gram-negative, non–spore-forming bacilli
			• Aerobic
			• Identification/differentiation
			  • Colony characteristics
			  • Oxidase test
			  • Growth on MacConkey agar

***Rhodococcus* Species**			

R. equi	Horses, pigs	Bronchopneumonia, cervical lymphadenitis	• Gram-positive, pleomorphic coccobacillus
			• Aerobic
			• Partially acid-fast
			• Identification/differentiation
			  • Colony characteristics
			  • Catalase test
			  • Hemolysis pattern
			  • CAMP test
			  • Immunology (immunodiffusion, ELISA)

***Rickettsia* Species**			
R. felis *R. rickettsii* *R. typhi*	Cats Dogs Rats	Flea typhus Rocky Mountain spotted fever Murine typhus	• Intracellular coccobacilli • Located in endothelial cells and smooth muscle cells • Identification/differentiation • Immunology (fluorescent antibody tests, PCR)
***Salmonella* Species**			

S. ser. abortusovis
S. ser. anatum
S. ser. choleraesuis
S. ser. dublin
S. ser. enteritidis
S. ser. newport
S. ser. pullorum
S. ser. typhimurium
	Sheep
	Sheep, goats, horses
	Pigs
	Cattle, sheep, goats
	Horses
	Cattle
	Poultry
	Cattle, sheep, goats, horses, pigs
		Abortion
		Peracute septicemia; acute, subacute, or chronic enteritis
			• Gram-negative, non–spore-forming bacilli
			• Most are motile
			• Nearly 2500 serovars
			• Organisms are referred to by the genus name and serovar
			• Identification/differentiation
			  • Colony characteristics
			  • Growth on MacConkey agar
			  • Growth on Simmons citrate
			  • Urease test
			  • Indole production test
			  • Hydrogen sulfide production

Organism	Primary Species Affected	Disease or Lesion	Characteristics
Staphylococcus **Species**			
S. aureus	Mammals	Wound infections, mastitis, skin infections, vaginitis	• Gram-positive cocci • Aerobic
S. epidermidis	Cattle, other mammals	Mastitis, skin abscess	• Identification/differentiation • Colony characteristics • Hemolysis pattern • Catalase test • Coagulase test • Fermentation of sugars
S. felis	Cats	Otitis externa, cystitis, abscesses, wound infections	
S. intermedius	Dogs, cattle	Skin and ear infections, mastitis	
Streptococcus **Species**			
S. agalactiae	Cattle, horses	Mastitis	• Gram-positive, non–spore-forming cocci • Facultative anaerobes • Identification/differentiation • Colony characteristics • Hemolysis pattern • Catalase test • Esculin hydrolysis test • CAMP test • Fermentation of sugars
S. canis	Dogs, cats	Genital, skin, and wound infections; metritis, mastitis, kitten septicemia	
S. dysgalactiae dysgalactiae	Cattle, dogs	Mastitis, dermatitis, abortion, septicemia	
S. equi equi	Horses	Strangles, genital infection, mastitis	
S. zooepidemicus equi	Rats, cattle, goats, sheep, chickens	Mastitis, lymphadenitis, wound infections, pneumonia, septicemia	
S. porcinus	Pigs	Abscesses, lymphadenitis	
S. suis	Pigs	Encephalitis, meningitis, arthritis, septicemia, abortion, endocarditis	
Taylorella **Species**			
T. equigenitalis	Horses	Contagious equine metritis	• Gram-negative coccobacilli • Identification/differentiation • Colony characteristics • Growth on chocolate agar • Indole production test • Oxidase test • Catalase test • Esculin hydrolysis test • Immunology (PCR)
Treponema **Species**			
T. brennaborense	Cattle, horses	Digital dermatitis, "hairy foot warts"	• Tight spiral bacteria • Motile
T. paraluiscuniculi	Rabbits	Rabbit syphilis	• Identification/differentiation • Cellular morphology with silver staining
Ureaplasma **Species**			
U. diversum	Cattle	Abortion, vulvitis, pneumonia	• Small mycoplasmas • Identification/differentiation • Colony characteristics • Urea hydrolysis • Immunology (PCR, immunofluorescence assay)
Yersinia **Species**			
Y. enterocolitica	Rabbits, dogs, pigs, horses	Ileitis, gastroenteritis	• Gram-negative bacilli • Facultative anaerobes • Identification/differentiation • Cellular morphology • Colony characteristics • Oxidase test • Catalase test • Fermentation of sugars
Y. pestis	Dogs, cats, goats	Plague	
Y. pseudotuberculosis	Rodents, guinea pigs, cats, cattle, goats	Pseudotuberculosis, abortion, epididymitis, orchitis	

D APPENDIX

Professional Associations Related to Veterinary Clinical Laboratory Diagnostics

Academy of Veterinary Clinical Pathology Technicians:
http://avcpt.net/
American Association of Veterinary Laboratory Diagnosticians:
http://www.aavld.org/
American Association of Veterinary Parasitologists:
http://www.aavp.org/
American Board of Veterinary Toxicology:
http://www.abvt.org/
American College of Veterinary Microbiologists:
http://www.acvm.us/
American Society for Veterinary Clinical Pathology:
http://www.asvcp.org/
Association of Veterinary Hematology and Transfusion Medicine:
http://www.avhtm.org/
Veterinary Laboratory Association:
http://www.vetlabassoc.com/

APPENDIX E

Common Parasites of Some Exotic Animal Species

PARASITES OF BIRDS

Nematodes

Ascaridia species
Capillaria species
Dispharynx nasuta
Heterakis gallinarum
Spiroptera incesta
Tetrameres species

Trematodes

Schistosoma species

Protozoans

Aegyptianella species
Atoxoplasma serini
Cryptosporidium species
Eimeria species
Giardia species
Haemoproteus species
Histomonas meleagridis
Isospora species
Leucocytozoon species
Plasmodium species
Trichomonas gallinae
Trypanosoma species

Arthropods

Argas persicus
Cnemidocoptes mutans
Cnemidocoptes pilae
Dermanyssus gallinae
Echidnophaga gallinacea
Goniocotes gallinae
Haemaphysalis leporispalustris
Menacanthus stramineus
Ornithonyssus sylviarum

Annelids

Theromyzon tessulatum

PARASITES OF RABBITS

Nematodes

Obeliscoides cuniculi
Passalurus ambiguus
Trichostrongylus calcaratus

Protozoans

Eimeria irresidua
Eimeria magna
Eimeria media
Eimeria perforans
Eimeria stiedae

Arthropods

Cediopsylla simplex
Cheyletiella parasitivorax
Cuterebra species
Dermacentor variabilis
Haemaphysalis leporispalustris
Hemodipsus ventricosus
Listrophorus gibbus
Odontopsylla multispinosus
Psoroptes cuniculi
Sarcoptes scabiei

PARASITES OF GUINEA PIGS

Nematodes

Paraspidodera uncinata

Protozoans

Cryptosporidium wrairi
Eimeria caviae
Entamoeba caviae
Giardia caviae
Giardia muris
Tritrichomonas caviae

Arthropods

Chirodiscoides caviae
Dermacentor variabilis
Gliricola porcelli
Gyropus ovalis
Notoedres muris
Ornithonyssus bacoti
Sarcoptes scabiei
Trixacarus caviae

PARASITES OF RATS

Nematodes

Aspiculuris tetraptera
Syphacia muris

Syphacia obvelata
Trichosomoides crassicauda

Cestodes

Hymenolepis diminuta
Hymenolepis nana

Protozoans

Eimeria nieschultz
Giardia muris
Spironucleus muris
Tetratrichomonas microti
Tritrichomonas muris

Arthropods

Cuterebra species
Dermacentor variabilis
Notoedres muris
Ornithonyssus bacoti
Polyplax spinulosa
Radfordia ensifera

PARASITES OF MICE

Nematodes

Aspiculuris tetraptera
Syphacia muris
Syphacia obvelata

Cestodes

Hymenolepis diminuta
Hymenolepis nana

Protozoans

Eimeria falciformis
Eimeria ferrisi
Eimeria hansonorum
Eimeria hansorium
Giardia muris
Klossiella muris
Spironucleus muris
Tetratrichomonas microti
Tritrichomonas muris

Arthropods

Cuterebra species
Dermacentor variabilis
Myobia musculi
Myocoptes musculinus
Polyplax serrata
Ornithonyssus bacoti
Radfordia affinis
Radfordia ensifera

PARASITES OF HAMSTERS

Nematodes

Syphacia muris
Syphacia obvelata

Cestodes

Hymenolepis diminuta
Hymenolepis nana

Protozoans

Giardia species
Spironucleus muris
Tetranucleus microti
Tritrichomonas muris (criceti)

Arthropods

Demodex aurati
Demodex criceti
Ornithonyssus bacoti

PARASITES OF GERBILS

Nematodes

Dentostomella translucida

Cestodes

Hymenolepis diminuta
Hymenolepis nana

Arthropods

Demodex aurati
Demodex criceti
Hoplopleura meridionidis

PARASITES OF FISH

Protozoans

Chilodonella species
Cryptocaryon irritans
Ichthyophthirius multifiliis
Piscinoodinium species
Tetrahymena species

PARASITES OF REPTILES

Pentastomes

Armillifer species
Porocephalus crotali
Porocephalus species
Kiricephalus species

Example of a Standard Protocol for Reporting Results of a Urinalysis Laboratory Report

Patient Name: _____ Date: _____

Species: _____ Breed: _____ Age: _____ Gender: _____

Collection Date/Time: _____ Method of Collection: _____

Physical Properties

Volume Collected:	
Color:	
Appearance/Turbidity:	
Odor:	
Specific Gravity:	

Chemical Properties

pH:	
Protein:	
Glucose:	
Ketones:	
Urobilinogen:	
Bilirubin:	
Hemoglobin:	
Blood:	

Urine Sediment

RBC (hpf):	
WBC (hpf):	
Epithelial cells (hpf) (specify type):	
Bacteria (hpf):	
Crystals (lpf) (specify type):	
Casts (lpf) (specify type):	

Comments:	

G APPENDIX

Taxonomic Classification of Parasites

Kingdom: Animalia (Animals)

- Phylum: Platyhelminthes (flatworms)
  - Class: Trematoda (flukes)
    - Subclass: Monogenea (monogenetic flukes)
    - Subclass: Digenea (digenetic flukes)
  - Class: Cotyloda (pseudotapeworms)
- Phylum: Nematoda (roundworms)
- Phylum: Acanthocephala (thorny-headed worms)
- Phylum: Arthropoda (animals with jointed legs)
  - Subphylum: Mandibulata (possess mandibulate mouthparts)
    - Class: Crustacea (aquatic crustaceans)
    - Class: Insecta
      - Order: Dictyoptera (cockroaches)
      - Order: Coleoptera (beetles)
      - Order: Lepidoptera (butterflies and moths)
      - Order: Hymenoptera (ants, bees, and wasps)
      - Order: Hemiptera (true bugs)
      - Order: Mallophaga (chewing or biting lice)
      - Order: Anoplura (sucking lice)
      - Order: Diptera (two-winged flies)
      - Order: Siphonaptera (fleas)
- Phylum: Sarcomastigophora
  - Subphylum: Mastigophora (flagellates)
- Phylum: Sarcomastigophora
  - Superclass: Sarcodina (amoebae)
- Phylum: Ciliophora (ciliates)
- Phylum: Apicomplexa (apicomplexans)
- Phylum: Proteobacteria
  - Class: Alpha Proteobacteria
    - Order: Rickettsiales
      - Family: *Rickettsiaceae*
      - Family: *Anaplasmataceae*

Zoonotic Internal Parasites

PARASITE	HOST	RESERVOIR	INFECTIVE STAGE	CONDITION
Toxocara spp.	Dogs, cats	Dogs, cats	Egg with L2	Visceral larva migrans
Ancylostoma spp.	Dogs, cats	Dogs, cats	L3	Cutaneous larva migrans
Uncinaria stenocephala	Dogs, cats	Dogs, cats	L3	Cutaneous larva migrans
Toxoplasma gondii	Cats	Cats, raw meat	Sporulated oocyst, bradyzoite, tachyzoite	Toxoplasmosis
Strongyloides stercoralis	Dogs, cats, people	People, dogs, cats	L3	Strongyloidiasis
Dipylidium caninum	Dogs, cats, people	Fleas	Cysticercoid	Cestodiasis
Taenia saginata	People	Bovine muscle	Cysticercus	Cestodiasis
Taenia solium	People	Porcine muscle, people	Cysticercus, egg	Cestodiasis, cysticercosis
Echinococcus granulosus	Dogs	Dogs	Egg	Hydatidosis
Echinococcus multilocularis	Dogs, cats	Dogs, cats	Egg	Hydatidosis
Spirometra mansonoides	Dogs, cats	Unknown	Procercoid in arthropod	Sparganosis
Sarcocystis spp.	People, dogs, cats	Cattle, pigs, dogs, cats	Sarcocyst in muscle, oocyst	Sarcocystiasis, sarcosporidiosis
Cryptosporidium	Mammals	Mammals	Oocyst	Cryptosporidiosis
Balantidium coli	People, pigs	People, pigs	Cyst, trophozoite	Balantidiasis
Ascaris suum	Pigs	Pigs	Eggs with L2	Visceral larva migrans
Trichinella spiralis	Mammals	Porcine and bear muscle	Encysted L3	Trichinellosis
Thelazia spp.	Mammals	Flies	L3	Verminous conjunctivitis
Giardia spp.	Mammals	Mammals	Cyst	Giardiasis
Babesia spp.	Rodents, people	Hard ticks	Sporozoite	Babesiosis
Trypanosoma cruzi	Mammals	Reduviids	Trypanosomal form in kissing bug	Chagas disease

From Sirois M: *Principles and practice of veterinary technology,* ed 3, St Louis, 2011, Mosby.

GLOSSARY

Abdominocentesis Paracentesis of the abdomen.

Absolute value The number of each type of leukocyte in peripheral blood; this is calculated by multiplying the relative percentage from the differential count by the total white blood cell count.

Acanthocyte An erythrocyte with spiny projections of varying lengths distributed irregularly over its surface.

Acariasis Infestation with mites.

Accuracy The closeness with which test results agree with the true quantitative value of the constituent.

Acid–base balance A state of equilibrium between the acidity and alkalinity of the body fluids; also called the hydrogen ion (H^+) balance.

Acid-fast stain A staining procedure for demonstrating the presence of microorganisms that are not readily decolorized by acid after staining; this is a characteristic of certain bacteria, particularly *Mycobacterium* and *Nocardia*.

Acidosis A pathologic decrease in the pH of blood or body tissues as a result of the accumulation of acids or a decrease in bicarbonate.

Acinar Pertaining to or affecting an acinus or acini. This term refers specifically to glandular tissue with a structure that is often described as grapelike clusters.

ACTH stimulation test A test designed to test the response of the hormone that stimulates adrenocortical growth and secretion.

Activated clotting time A test of the intrinsic and common pathways of blood coagulation that involves the use of a diatomaceous earth or kaolin tube to initiate clotting.

Activated partial thromboplastin time A test of intrinsic and common coagulation pathways. An intrinsic pathway activator is added to plasma, and the time taken for clot formation is measured.

Active immunity An animal's production of antibody as a result of infection with an antigen or immunization.

Acute-phase proteins Proteins, including serum amyloid A and C-reactive protein, that are produced by hepatocytes immediately following injury or inflammation.

Addison's disease See *Hypoadrenocorticism.*

Adrenocorticotropic hormone A hormone secreted by the anterior pituitary gland that has a stimulating effect on the adrenal cortex. Also referred to as corticotropin and abbreviated as ACTH.

Agar A seaweed extract that is used to solidify culture media.

Agranulocytes The white blood cell group that has no visible cytoplasmic granules.

Alanine transaminase Cytoplasmic enzyme of hepatocytes released when hepatocytes are damaged.

Albumin A group of plasma proteins that comprises the majority of protein in plasma.

Alkaline phosphatase A group of enzymes that functions at an alkaline pH and that catalyzes the reactions of organic phosphates.

Alkalosis A condition in which the blood pH is higher than 7.45.

Allantoin A crystalline substance produced by the oxidation of uric acid by uricase and present in the urine of most mammals, except primates and Dalmatian dogs (which lack uricase).

Alloantibodies A naturally occurring antibody that is produced by an individual and that reacts with antigens of another individual of the same species.

Alpha-hemolysis Characterized by the partial destruction of blood cells on blood agar, which is evident as a greenish zone around the bacterial colony.

Ammonium biurate Brownish crystals seen in the urine of animals with severe liver disease.

Amylase An enzyme derived primarily from the pancreas that functions in the breakdown of starch.

Amyloclastic A method of measuring serum amylase by evaluating the disappearance of starch substrate.

Anion A negatively charged ion.

Anion gap A method that is used to evaluate a patient's acid-base status; the calculation is based on subtracting the sum of measured major serum anions ($Cl^- + HCO_3^-$) from the sum of measured major serum cations ($Na^+ + K^+$).

Anisokaryosis Variation in the size of the nuclei of cells in a sample.

Anisonucleoliosis Variation in the size of nucleoli.

Antibody titer The level of a specific antibody that is present in serum. This is calculated as the reciprocal of the highest dilution at which a sample no longer exhibits a positive reaction for the presence of the antibody. It is often used to help differentiate active infection from prior exposure to an antigen.

Anticoagulant Any substance that inhibits or prevents clotting.

Antimicrobial disks Paper disks impregnated with antibiotic agents and used during the performance of the antimicrobial sensitivity test.

Antimicrobial susceptibility test An in vitro test of the effectiveness of selected antimicrobial agents against microorganisms.

Anuria Absence of urine.

Apoptosis The death of single cells by a process involving shrinkage, rapid fragmentation, and the engulfment of the fragments by neighboring cells and macrophages.

Arachnid A member of the class Arachnida, which includes mites and ticks.

Arthrocentesis The removal of fluid from a joint.

Ascarid Any of the nematodes of the superfamily Ascaridoidea, which includes the genera *Ascaris, Parascaris, Toxocara,* and *Toxascaris.*

Ascospores The sexual spore of *Ascomycetes.*

Aspartate transaminase An enzyme that is present in body serum and in certain body tissues that catalyzes the transfer of an amino group from aspartic acid to alpha-ketoglutaric acid, thereby forming glutamic acid and oxaloacetic acid. Also referred to as aspartate aminotransferase.

Aspiration The removal of fluids or gases from a cavity with the aid of suction; the removal of cells and tissue fluid from a lesion with the use of suction from a needle and syringe.

Atypical lymphocyte A general term used to describe a lymphocyte with morphologic abnormalities, including azurophilic granules, increased cytoplasmic basophilia, overly abundant cytoplasm, or a larger and more convoluted nucleus than seen in normal lymphocytes.

Autoagglutination The clumping or agglutination of an individual's cells by that individual's own serum, usually because of the presence of autoantibodies.

Avidity Refers to the strength of the binding of antigen and antibody.

Azotemia The increased retention of urea in the blood.

Bacilli Rod-shaped bacteria.

Baermann technique A parasitology test that is used to recover larvae.

Base excess The amount of acid or base required to titrate a sample of whole arterial blood to the normal pH of 7.4.

Basidiospores The sexual spore of basidiomycetes.

Basophil A granular leukocyte with an irregularly shaped, relatively pale-staining nucleus that is partially constricted into two lobes and with cytoplasm that contains coarse bluish-black granules of variable size.

Basophilic stippling Erythrocytes that are characterized by small, blue-staining granules; this represents the presence of residual RNA.

Beer's law A principle that describes the relationship between light absorbance, transmission, and the concentration of a substance in solution.

Bence Jones protein A light chain protein of immunoglobulin molecules that readily passes through the glomerulus and into the urine.

Benign A term used to describe a tumor or growth that is not malignant; this word can refer to any condition that is not life threatening.

Beta-hemolysis The complete destruction of red blood cells on blood agar that creates a clear zone around the bacterial colony.

Beta-lactamase Enzyme produced by bacteria that are resistant to beta-lactam antibiotics.

Bicarbonate (HCO_3) An electrolyte in plasma; part of the bicarbonate–carbonic acid buffer system that maintains the blood pH in equilibrium.

Bile acids A group of compounds that are synthesized by hepatocytes from cholesterol that help with fat absorption.

Bilirubin An insoluble pigment derived from the breakdown of hemoglobin, which is processed by hepatocytes.

Bilirubinuria An abnormal increase in the concentration of bilirubin in the urine.

Binocular Having two eyepieces (e.g., a type of microscope).

Biohazard Biological substances that contain infectious agents that pose a threat to human health.

Bladder expression The manual compression of the urinary bladder to cause the release of urine through the urethra.

Blood agar An enriched medium that supports the growth of most bacterial pathogens; usually composed of sheep's blood.

Blood group antigens The antigens that are present on the surface of erythrocytes and antibodies that may be present in serum.

Blood urea nitrogen The principal end product of amino acid breakdown in mammals.

Bloodborne pathogens Infectious agents that are present in the bloodstream.

Bothria Two longitudinal grooves or sucker-like expansions on the scolex of members of the cestode orders Bothriocephalidea, Diphyllidea, Diphyllobothriidea, and Trypanorhyncha.

Buccal mucosa bleeding time A test that uses a standardized shallow incision into the buccal mucosa of the upper lip to evaluate primary hemostasis.

Buffer A substance that increases the amount of acid or alkali necessary to produce a unit change in pH.

Buffy coat The layer of material above the packed erythrocytes after centrifugation; it consists primarily of leukocytes and thrombocytes.

Calcium The most abundant mineral in the body. Calcium is an important cation in intracellular and extracellular fluid. It is essential to the normal clotting of blood, the maintenance of a normal heartbeat, and the initiation of neuromuscular and metabolic activities.

Calcium carbonate A type of crystal that is commonly seen in the urine of rabbits and horses.

Calcium oxalate A crystal that is found in acidic and neutral urine; commonly seen in small amounts in dogs and horses.

California Mastitis Test An indirect test for bovine mastitis that is based on the presence of a high leukocyte count in mastitic milk.

Candle jar A method of producing anaerobic conditions for the growth of anaerobic bacteria.

Capnophilic An organism that requires high levels of carbon dioxide for growth or for the enhancement of growth.

Capsule stain A differential stain that is used to identify the cell capsules of pathogenic bacteria.

Carcinoma A term that describes tumors of epithelial cell origin.

Casts Structures that are formed from the protein precipitates of degenerating kidney tubule cells; may contain embedded materials.

Catalase An enzyme that catalyzes the breakdown of hydrogen peroxide into oxygen and water.

Catheterization The placement of a catheter in the urethra or the placement of an indwelling catheter in a blood vessel.

Cation A positively charged ion.

Cell-mediated immunity An immune system mechanism that involves actions of the cells of the immune system rather than antibodies.

Cellular cast A formed element in urine that consists of a hyaline cast that contains blood cells or epithelial cells.

Centesis The act of puncturing a body cavity or organ with a hollow needle to draw out fluid.

Centrifugal flotation A method of processing fecal samples for the detection of parasite ova and cysts. It recovers more eggs and cysts in a sample and takes less time than standard flotation.

Centrifuge A piece of equipment that spins samples at high speed.

Cercaria The life-cycle stage of trematodes that develops in the intermediate host.

Cestode An organism in the order Cestoda; a type of tapeworm.

Chemical hygiene plan A document that contains details about the specific chemical hazards present in the workplace.

Chemiluminescence Describes a chemical reaction that results in the emission of light.

Chloride The principal anion in extracellular fluid and gastric juice.

Cholesterol A plasma lipoprotein that is produced primarily in the liver as well as ingested in food; used in the synthesis of bile acids.

Citrate Any salt of citric acid; citrate salts are used as temporary anticoagulants for studies of blood coagulation.

Clot retraction A crude but simple test that allows for the evaluation of platelet number and function and intrinsic and extrinsic pathways.

Clumpelets A made-up word used to describe clumps of platelets seen on a blood smear.

Coagulase A molecule produced by some bacteria that allows for the adhesion of fibrinogen to the cell surface.

Cocci Bacteria with a round shape.

Codocyte An erythrocyte that is characterized by an increased membrane surface area relative to the cell's volume.

Competitive ELISA An immunoassay. Patient antigen, if present, competes with enzyme-labeled antigens for the antibodies that are coating the test wells.

Complement system A group of plasma proteins that function to enhance the activities of the immune system.

Compound light microscope A microscope that generates an image by using a combination of lenses.

Condenser The part of the microscope that consists of two lenses that focus light from the light source on the object being viewed. Light is focused by raising or lowering the condenser.

Conidia An asexual fungal spore that is deciduous (shed at maturity) and formed by budding or splitting off from the summit of a conidiophore. Also called a conidiospore.

Conjugated bilirubin Bilirubin that has been taken up by the liver cells and conjugated to form the water-soluble compound bilirubin diglucuronide.

Control A biological solution of known values that is used for the verification of the accuracy and precision of test results.

Coombs test An immunologic test designed to detect antibodies on the surface of erythrocytes (direct Coombs test) or antibodies against erythrocytes in plasma (indirect Coombs test).

Coracidium The individual free-swimming or free-crawling, spherical, ciliated embryo of tapeworms of the order Pseudophyllidea.

Cornified Keratinized; used to describe vaginal epithelial cells as seen in a vaginal cytology smear from a patient in estrus.

Cortisol A steroid hormone produced by the adrenal glands.

Coverslip smear A method of preparing a blood film with the use of two coverslips.

Creatine kinase An enzyme that is found predominantly in cells of the heart, brain, and skeletal muscle; released when cells are damaged.

Creatinine A waste product that is formed during normal muscle cell metabolism.

Crossmatching A blood test designed to identify compatibility between donor and recipient samples before transfusion.

Culture medium A substrate for the growth of microbiology samples.

Culturette The trade name for a sterile swab in transport media that is used for collection of microbiology samples.

Curschmann's spirals The coiled mucinous fibrils that are sometimes found in cytology preparations of bronchial samples.

Cushing's disease See *Hyperadrenocorticism.*

Cuticle The outer layer or covering of epithelium.

Cystine An amino acid that may be present in the form of hexagonal crystals in the urine.

Cystocentesis The aspiration of fluid from the urinary bladder.

D-Dimer A protein fragment that is formed from the breakdown of fibrin.

Dacryocyte An abnormal erythrocyte that is shaped like a teardrop.

Dark-field microscope A type of microscope that is used primarily in reference laboratories, especially for the viewing of unstained specimens.

Definitive host The host that harbors the adult, mature, or sexual stages of a parasite.

Dermatophyte test medium A differential culture medium designed to support the growth of cutaneous fungal organisms and to inhibit bacterial growth.

Dexamethasone suppression test An endocrine system test designed to detect hyperadrenocorticism.

Differential media A bacterial culture method that allows bacteria to be differentiated into groups on the basis of their biochemical reactions on the medium.

Dilution The process of making a solution weaker or less concentrated.

Direct life cycle The life cycle of an organism that does not require an intermediate host.

Direct sensitivity testing An antimicrobial sensitivity test that involves the application of undiluted samples (e.g., urine) directly to the Mueller-Hinton plate.

Discrete round cell tumors A neoplasia that is characterized by cells with discrete round shapes. Examples of round cell neoplasms include mast cell tumors, histiocytomas, lymphomas, plasmacytomas, and transmissible venereal tumors.

Disseminated intravascular coagulation An acquired secondary coagulation disorder that is characterized by the depletion of thrombocytes and coagulation factors. Also referred to as consumption coagulopathy and defibrination syndrome.

Dog erythrocyte antigen (DEA) A naming convention for canine blood types.

Döhle bodies Small, gray-blue areas that represent ribosomes and that are seen in the cytoplasm of some immature and toxic granulocytes.

Drepanocyte A morphologic abnormality of erythrocytes that is characterized by sickle-shaped cells.

Echinocyte An erythrocyte with multiple small projections that are evenly spaced over the cell circumference.

Ectoparasite A parasite that resides on the surface of its host.

Effective renal plasma flow The effective rate of blood flow through the kidneys; the determining factor relative to the rate of glomerular filtration.

Electrolyte Any substance that dissociates into ions when in solution.

End-point assay A chemical reaction that proceeds to a stable end point.

Endocrine A term that refers to the system of glands and other structures that secrete hormones directly into the circulatory system.

Endoparasite A parasite that resides within a host's tissues.

Endospore The dormant form of a bacterium; intracellular refractile bodies that are resistant to heat, desiccation, chemicals, and radiation; formed by some bacteria when environmental conditions are poor.

Endospore stain A differential stain that has been designed to identify the presence, location, and shape of spores in bacterial samples.

Engineering controls Safety procedures focused on changing the work environment to eliminate or minimize exposure to a hazard.

Enriched media A type of culture media that has been formulated to meet the requirements of the most fastidious pathogens.

Enterotubes A commercially available modular system of culture media that contains media and reagents for numerous bacteriologic tests that can be performed simultaneously.

Enzyme-linked immunosorbent assay (ELISA) An enzyme immunoassay that makes use of an enzyme-labeled immunoreactant (antigen or antibody) and an immunosorbent (antigen or antibody bound to a solid support).

Enzymuria The presence of specific enzymes in urine.

Eosin A type of pink to red acid dye that is a component of differential stains; primarily used for the routine staining of blood films.

Eosinophil A granulocyte with granules that have an affinity for the acidic components of stains.

Eosinophilic A term that refers to an increase in circulating eosinophils or a reddish appearance of cells or components of cells that have a high affinity for stains with acid pH.

Epithelial cell tumors A type of neoplasm associated with a clustered arrangement of cells into ball shapes or monolayer sheets. Examples include lung adenocarcinoma, perianal adenoma, basal cell tumor, sebaceous adenoma, transitional cell carcinoma, and mesothelioma.

Eryptosis The suicidal death of erythrocytes; similar to the apoptosis of nucleated cells.

Erythrocyte indices Calculated values that provide the average volume and hemoglobin concentrations of erythrocytes in a peripheral blood sample.

Erythropoiesis The production of erythrocytes.

Erythropoietin The hormone that stimulates erythropoietic activity in the bone marrow.

Ethylene glycol A solvent with a sweet, acrid taste that is found in many products, such as antifreeze, drying agents, and inks. Ingestion or excessive skin exposure can be toxic.

Ethylenediaminetetraacetic acid An anticoagulant that binds calcium.

Exudate A fluid accumulation that results from inflammatory processes; characterized by increased cellularity and protein concentration.

Facultative anaerobes Bacteria that do not require oxygen for metabolism but that can survive in the presence of oxygen.

Fastidious A term used to describe a bacterial species with complex growth or nutritional requirements.

Fatty casts Formed elements that may be found in urine and that consist of a hyaline cast with embedded globules of fat.

Fecal sedimentation A procedure that is used to prepare samples for examination for parasites; it demonstrates objects that are too heavy or too delicate to evaluate with standard fecal flotation.

Fibrin degradation products Protein fragments formed from the breakdown of fibrin.

Fibrometer An instrument used for the hemostatic evaluation of samples.

Fine-needle biopsy A sample collection method in which tissue is obtained by puncture of a lesion.

Flagella Long, thin, helical structures that function in cell motility.

Flagella stain A differential stain to detect and characterize flagella if present on bacterial cells.

Flea-bite dermatitis The inflammatory lesions and self-trauma caused by a hypersensitivity to flea bites.

Fluorescent antibody A specific antibody that has been labeled with a fluorochrome and that is used in immunoassays.

Fluorescent microscope A type of microscope that is capable of viewing fluorescent particles, such as an antibody labeled with specific fluorescent dye.

Fractional clearance of electrolytes A mathematical manipulation that describes the excretion of specific electrolytes relative to the glomerular filtration rate.

Free catch A method of collecting a urine sample by collecting the sample as the animal voids naturally.

Fructosamine A molecule formed as a result of the irreversible reaction of glucose bound to protein.

Gamma-glutamyltransferase An intracellular enzyme found in high concentrations in liver, pancreatic, and renal tubular cells.

Gamma-hemolysis A term that describes a bacterial sample that produces no hemolysis on blood agar.

Giemsa stain A differential stain that is used for blood and bone marrow smears. Also used to visualize fungal organisms and mast cell granules.

Globulins A complex group of plasma proteins that have been designated as alpha, beta, or gamma; this group includes immunoglobulins, complement, and transferrin.

Glomerular filtration rate The rate at which substances are filtered through the glomerulus and excreted in the urine.

Glomerulus A tuft of capillaries located in the renal cortex.

Glucagon A hormone secreted by the alpha cells of the islets of Langerhans in response to hypoglycemia.

Glucose A monosaccharide that represents the end product of carbohydrate metabolism.

Glucose tolerance test A metabolic test of carbohydrate tolerance.

Glucosuria The presence of glucose in the urine.

Glutamate dehydrogenase A mitochondrial-bound enzyme that is found in high concentrations in the hepatocytes of cattle, sheep, and goats.

Glycosylated hemoglobin The irreversible reaction of hemoglobin bound to glucose.

Gram stain A differential stain that is used to classify bacterial samples on the basis of the chemical structure of their cell walls.

Granular casts A structure that is formed from the protein precipitate of degenerating kidney tubule cells that contain granular material derived from the breakdown of cells incorporated into the cast.

Granulocytes Any cell with distinct cytoplasmic granules.

Granulomatous A term that refers to an inflammatory condition that is characterized by high numbers (more than 70%) of macrophages.

Hanging drop A method of preparing specimens to evaluate motility.

Heinz bodies Round structures of erythrocytes that represent denatured hemoglobin and that appear as a pale area when stained with Wright's stain.

Hematochezia The presence of blood in the feces.

Hematopoiesis The production of blood cells and platelets.

Hematuria The presence of intact erythrocytes in the urine.

Hemoglobin The oxygen-carrying pigment of erythrocytes, which is formed by developing erythrocytes in the bone marrow. It is a type of hemoprotein that contains four heme groups and globin.

Hemoglobinuria The presence of free hemoglobin in urine.

Hemolysis The destruction of erythrocytes.

Hemolyzed Red appearance of a fluid sample (e.g., serum, urine) as a result of the destruction of erythrocytes.

Hemophilia A genetic abnormality of hemostasis that results from the deficient production of certain coagulation factors.

Heparin An acid mucopolysaccharide that is present in many tissues, especially the liver and lungs, and that has potent anticoagulant properties.

Hepatoencephalopathy Severe hepatic insufficiency that may induce a syndrome of excitability, tremor, compulsive walking, head pressing, and apparent blindness, followed by coma and convulsions.

Heterophil A leukocyte of avian, reptile, and some fish species that contains prominent eosinophilic granules; functionally equivalent to the mammalian neutrophil.

Hexacanth The infective stage of some cestodes.

Histiocytoma A tumor that contains histiocytes (macrophages).

Histogram A graphic display of a frequency distribution that is represented by a series of rectangles that divide the data into classes. The height of a rectangle indicates the number of values that are contained in that class (class frequency), and the width of each base represents the size of the intervals into which the classes have been divided.

Howell-Jolly bodies Basophilic inclusions of young erythrocytes that represent nuclear remnants.

Humoral immunity An immune response that involves the production of specific antibody.

Hyaline casts The structures that are formed from protein precipitates of degenerating kidney tubule cells with no embedded materials.

Hyperadrenocorticism The abnormally increased secretion of adrenocortical hormones, as with conditions such as Cushing's syndrome.

Hypercalcemia An increased plasma calcium level.

Hypercapnia An excess of carbon dioxide in the blood that is indicated by an elevated PCO_2 level as determined by blood gas analysis and that results in respiratory acidosis. Also known as hypercarbia or hypercarbemia.

Hyperchromatophilic A term that refers to a cell that appears darker than normal on a peripheral blood sample.

Hypercoagulable Characterized by abnormally increased coagulability.

Hyperglycemia An abnormally increased glucose level in the blood.

Hyperkalemia An increased plasma potassium level.

Hyperlipoproteinemia A condition characterized by excess lipids in the blood. Also referred to as hyperlipidemia and hyperlipemia.

Hypernatremia An increased plasma sodium level.

Hyperphosphatemia An excessive amount of phosphates in the blood.

Hyperproteinemia An increased protein level in the blood.

Hypersegmentation A term that refers to a neutrophil with more than five nuclear lobes.

Hyperthyroidism A condition that is caused by the excessive production of iodinated thyroid hormones.

Hyphae The body of a fungus that is created as a result of the linear arrangements of cells and that forms multicellular or multinucleate growth.

Hypoadrenocorticism A deficiency in the production of mineralocorticoid or glucocorticoid steroid hormones.

Hypoalbuminemia A decrease in the circulating levels of albumin in the blood.

Hypocalcemia A decreased plasma calcium level.

Hypocapnia A deficiency of carbon dioxide in the blood. Also called hypocarbia.

Hypochromasia The presence of erythrocytes with decreased staining intensity as a result of a decrease in hemoglobin concentration.

Hypocoagulable Characterized by abnormally decreased coagulability.

Hypoglycemia A decreased plasma glucose level.

Hypokalemia A decreased plasma potassium level.

Hyponatremia A decreased plasma sodium level.

Hypophosphatemia A decreased amount of phosphates in the blood.

Hypoproteinemia A condition characterized by an abnormally low level of protein in the blood.

Hyposegmentation A term that is used to describe the nucleus of a leukocyte with fewer than the normal number of nuclear lobes.

Hypostome The penetrating, anchor-like sucking organ of the tick.

Icterus Abnormal yellowish discoloration of skin, mucous membranes, or plasma as a result of an increased concentration of bile pigments.

Iditol dehydrogenase An enzyme of the oxidoreductase class that catalyzes the oxidation of l-iditol to l-fructose; it occurs in significant quantities only in the liver, and its increased activity in serum is used as an indicator of parenchymal liver damage. Also referred to as sorbitol dehydrogenase.

Immunodiffusion An immunologic test that is performed by placing reactants in an agar plate and allowing them to migrate through the gel toward each other.

Immunoglobulins Antibodies; plasma proteins produced against specific antigens.

Immunologic tolerance A state of nonresponsiveness to antigens, whether self or foreign.

Impedance analyzer A type of analyzer that counts particles based on their displacement of electrolyte solution when the particles pass through an aperture. The magnitude of the displacement creates an electrical signal that allows particles (e.g., cells) to be classified on the basis of their size.

Incubator A piece of equipment that is used to maintain a constant and suitable temperature for the development of cultures of microorganisms or other living cells.

Indirect cycle The life cycle of an organism that requires one or more intermediate hosts.

Indirect sensitivity testing An antimicrobial sensitivity test that involves the application of diluted samples (e.g., urine) directly to the Mueller-Hinton plate.

Inflammatory response The defensive response of body tissues that is initiated by the release of histamine from damaged cells.

Instar Any stage of an arthropod between molts.

Insulin A protein hormone that is secreted by the beta cells of the pancreatic islets in response to elevated blood levels of glucose and amino acids.

Interferons Small soluble proteins that enhance the function of the immune system.

Intermediate host The host that harbors the larval, immature, or asexual stages of a parasite.

International units The Système International (SI) set of basic units, which is based on the metric system.

Jaundice A condition that is characterized by hyperbilirubinemia and the deposition of bile pigments in the skin, mucous membranes, and sclera.

Karyolysis The degeneration or dissolution of a cell nucleus.

Karyorrhexis The fragmentation of a cell nucleus.

Keratocyte In hematology, an abnormally shaped erythrocyte that appears to have horns.

Ketonuria The presence of detectable ketone bodies in urine.

Kinetic assay A chemical test that measures the rate of change of a substance in the test system.

Kirby-Bauer test A type of antimicrobial susceptibility test in which agar plates are inoculated with a standardized suspension of a microorganism, and then antibiotic-containing disks are applied to the agar surface.

Kovac's reagent A substance used in bacteriology to detect the ability of bacteria to produce indole.

Lactate The anionic form of lactic acid; a salt of lactic acid.

Lactophenol cotton blue A preparation of phenol, lactic acid, glycerin, distilled water, and cotton blue dye that is used to stain fungi in wet preparations.

Left shift The presence of increased numbers of immature cells in a peripheral blood sample.

Leptocyte An erythrocyte that is characterized by an increased membrane surface area relative to the cell volume.

Leukemia A condition characterized by the presence of neoplastic cells in the blood or bone marrow.

Leukemoid response The exhibition of blood counts (particularly leukocytosis) and sometimes other clinical findings that resemble those of leukemia.

Leukocytosis The presence of increased numbers of leukocytes in the blood.

Leukopoiesis The production of leukocytes.

Lipase A pancreatic enzyme that functions in the breakdown of fats.

Lipemia The presence of fatty material in plasma or serum.

Lymphocyte A leukocyte that is involved in the inflammatory process and that also has roles in humoral and cell-mediated immunity.

Lymphoma A neoplastic disorder of the lymphoid tissue.

Lymphopenia The presence of decreased numbers of leukocytes in a peripheral blood sample.

MacConkey agar An agar medium that contains peptone, lactose bile salts, sodium chloride, neutral red, and crystal violet that is used to differentiate lactose fermenters (coliforms) from non–lactose fermenters among the enteric bacilli.

Macrocytosis A condition in which a cell is abnormally large.

Mast cell tumors A benign local aggregation of mast cells that forms a nodular tumor that occurs in the skin of most species (most commonly dogs).

Material Safety Data Sheet (MSDS) Informational material that contains detailed product safety information about hazardous materials found in a particular place of a business; an OSHA mandate.

Mean corpuscular hemoglobin (MCH) An expression of the average hemoglobin content of a single cell in picograms that is obtained by multiplying the amount of hemoglobin (in

grams) by 10 and then dividing that number by the number of erythrocytes (in millions).

Mean corpuscular volume An expression of the average volume of individual red cells in cubic microns that is obtained by multiplying the hematocrit percentage by 10 and then dividing that number by the number of erythrocytes (in millions).

Megakaryocyte The bone marrow cell from which blood platelets arise.

Megathrombocytes Abnormally large platelets that are usually newly formed; seen in greater numbers during an increase in platelet production.

Melanoma A tumor that arises from melanocytes of the skin or other organs.

Mesenchymal cell tumors Tumors of mixed mesenchymal tissues with two or more cellular elements that are not commonly associated (not counting fibrous tissue as one of the elements).

Mesophiles Organisms with optimal growth temperatures of between 25° C and 40° C.

Metacercaria The encysted resting or maturing stage of a trematode parasite in the tissues of an intermediate host or on vegetation.

Methanol Methyl alcohol.

Methemoglobin The form of hemoglobin that contains oxidized iron; inefficient at oxygen transport.

Microaerophilic An organism that requires oxygen for growth at a level that is less than that found in air.

Microcytosis A cell that appears much smaller than normal.

Microfilaria The larval offspring of the group of filarial worms in the phylum Nematoda.

Microhematocrit A term that refers to use of a capillary tube and a high-speed centrifuge to determine the packed cell volume.

Minimum inhibitory concentration The smallest concentration of an antibiotic that regularly inhibits the growth of a bacterium in vitro.

Miracidium The ciliated larval stage of a digenetic trematode.

Modified transudate A transudate with additional protein, cells, or both; it may be a transitional stage that ultimately progresses into an exudate.

Monocyte A precursor cell representing a stage in the development of the tissue macrophage; after a monocyte leaves the bloodstream and enters tissue at a site of inflammation, it becomes an activated macrophage.

Mucin clot test The adding of acetic acid to normal synovial fluid, which causes clot formation; the compactness of the clot and the clarity of the supernatant fluid are the criteria on which the result is based.

Mueller-Hinton medium A standard culture material that is used to evaluate the susceptibility of microorganisms to antimicrobial agents.

Myiasis An infestation with the larvae (maggots) of dipterans.

Natural killer (NK) cells A subpopulation of lymphocytes that is capable of the direct lysis of cells that have been infected with antigen.

Nematode A multicellular parasitic animal of the phylum Nematoda.

Neonatal isoerythrolysis Hemolytic anemia of the newborn.

Neoplasia A generic term that is used to describe any growth; often used to describe a tumor, which may be malignant or benign.

Nephron A structural and functional unit of the kidney that resembles a microscopic funnel with a long stem and two convoluted tubular sections.

Neubauer rulings A specific pattern of precise markings on a hematocytometer slide that facilitates the counting of leukocytes, erythrocytes, and platelets in the blood and of all cells in other fluids.

Neutrophil A leukocyte that functions to phagocytize infectious agents and cellular debris; plays a major role in the inflammatory process.

Neutrophilia An abnormal increase in the number of neutrophils seen in a peripheral blood sample.

Nits The egg stage of lice, which binds to the hair or feather shaft of the host.

Nuclear molding A deformation of nuclei by other nuclei within the same cell or adjacent cells.

Nucleated erythrocyte An immature red blood cell that still contains a nucleus.

Numerical aperture A measure of the efficiency of a microscope objective lens; it is proportional to the square root of the amount of light that enters the instrument.

Nymph A developmental stage of certain arthropods between the larval form and the adult; resembles the latter in appearance.

Objective lens A lens that accepts light from the output phosphor of an image intensifier tube and converts it into a parallel beam to record the image on film.

Obligate anaerobes Organisms that cannot grow in the presence of oxygen.

Occupational Safety and Health Administration (OSHA) A U.S. government agency that mandates specific laboratory practices that must be incorporated into a laboratory's safety policy.

Ocular Pertaining to the eye.

Oliguria A decrease in the volume of urine produced.

Opsonization The complement-mediated adherence of phagocytes to antigens that enhances the phagocytosis of the antigen.

Optical density The degree to which light is transmitted through a medium.

Oxalate An anion of oxalic acid.

Oxidase An enzyme that is present in some groups of bacteria and that is involved with the reduction of oxygen during normal bacteria metabolism.

Packed cell volume The ratio of red blood cells to total plasma volume.

Paracentesis The removal of fluid from a body cavity.

Parthenogenetic A condition in which female organisms produce eggs that develop without fertilization.

Passive immunity A condition that involves receiving antibodies from colostrum or synthesized antibodies.

Pediculosis The term used to describe an infestation with lice.

Pelger-Huët anomaly An inherited anomaly that is characterized by the appearance of bilobed neutrophils in a peripheral blood sample.

Periodic parasite A parasite that lives part of its life cycle on its host and part of its life off of its host.

Peritoneal fluid A naturally produced fluid in the abdominal cavity that lubricates surfaces, thereby preventing friction between the peritoneal membrane and the internal organs.

Personal protective equipment Items such as eye protection and other protective clothing, shields, and barriers that are designed to minimize exposure to hazards in the workplace.

pH A measure of the hydrogen ion concentration of a solution.

Phase-contrast microscope A type of light microscope that involves a special condenser and objective lens with a phase-shifting ring; it is used to visualize small differences in refractive index as differences in intensity or contrast.

Pipette A calibrated, transparent, open-ended tube made out of glass or plastic that is used to measure or transfer small quantities of a liquid or gas. This word can also be used to refer to the use of a pipette to dispense liquid.

PIVKA Proteins induced by vitamin K deficiency or antagonists; the nonfunctional precursor forms of vitamin-K–dependent coagulation factors.

Plasma The fluid portion of the blood.

Plasma cell tumor An extramedullary myeloma; this type of tumor occurs outside of the bone marrow, and it usually affects the visceral organs or the nasopharyngeal and oral mucosa.

Platelets Irregular, disc-shaped fragments of megakaryocytes in the blood that assist with blood clotting.

Pleomorphism A term that refers to something that takes a variety of shapes and forms or that has multiple morphologies.

Plumbism A chronic form of lead poisoning that is caused by the absorption of lead or lead salts.

Pluripotent stem cell A cell capable of differentiating into one of many cell types.

Poikilocytosis Any abnormal cell shape.

Polymerase chain reaction A method that is used to replicate and amplify DNA molecules in a sample.

Polyuria An increase in the total volume of urine produced.

Precision The magnitude of random errors and the reproducibility of measurements.

Prepatent period The time interval between infection with a parasite and the demonstration of that infection.

Proglottid A segment that comprises the body of a cestode.

Proteinuria The abnormal presence of protein in the urine.

Prothrombin time tests A one-stage test for detecting certain plasma coagulation defects that are caused by a deficiency of factors V, VII, or X.

Psychrophiles Organisms that demonstrate optimal growth at cold temperatures (i.e., between 15° C and 20° C).

Punch biopsy The removal of living tissue for microscopic examination with the use of a punch.

Pupa The second stage in the life cycle of certain insects, which occurs between the larval and adult stages. A pupa shows the basic external features

of the adult form, but it does not have expanded wings.

Pyknosis The presence of condensed nuclear chromatin in a degenerating cell.

Pyogranulomatous A term used to describe a cytology sample that is characterized by the presence of macrophages representing more than 15% of total nucleated cells in the sample.

Quadrant streak A technique for microbial inoculation in which a single colony is isolated on a culture plate and divided into four sections.

Quality assurance Any evaluation of services provided and the results achieved as compared with accepted standards.

Radioimmunoassay A technique that is used to determine the concentration of an antigen, antibody, or other protein in the serum. A radioactively labeled substance that is known to react in a certain way with the suspected protein is injected, and any reaction is monitored.

Ratio The relationship of one quantity to one or more other quantities that is expressed as a proportion of one to the others and written either as a fraction or linearly.

Redia A secondary larval form of some digenetic trematodes that develops within a mollusk intermediate host.

Refractive index A measure of the degree that light bends as it passes from one medium to another.

Refractometer A device that measures the refractive index of a solution.

Reliability The ability of a method to be accurate and precise.

Resolution The ability of an imaging process to distinguish adjacent structures in the object; an important measure of image quality.

Rhizoid Resembling a root or serving to anchor.

Ringworm A group of fungal skin diseases that are caused by dermatophytes of several kinds.

Rostellum The anterior of a tapeworm scolex, which commonly features hooklike jaws.

Rouleaux An arrangement of erythrocytes that appears as a column or stack.

Sarcoma A generic term that is used to describe any cancer that arises from cells of the connective tissues.

Schistocytes Fragmented erythrocytes that are usually formed as a result of shearing of the red cell by intravascular trauma.

Scolex The anterior portion of a cestode by which it attaches to its host.

Selective media A type of culture media that contains antibacterial substances that inhibit or kill all but a few types of bacteria.

Serial dilution A laboratory technique in which a substance (e.g., serum) is decreased in concentration in a series of proportional amounts.

Serum The fluid portion of blood after it has clotted; it does not contain cells or coagulation proteins.

Smudge cell A leukocyte that has ruptured.

Specificity The ability of a test to evaluate a given parameter correctly.

Spectrophotometer A piece of equipment designed to measure the amount of light that is transmitted through a solution.

Spherocyte An intensely stained erythrocyte that has reduced or no central pallor.

Spirochete Any bacterium of the genus *Spirochaeta* that is motile and spiral-shaped, with flexible filaments.

Sporocyst The larval stage of a digenetic trematode that develops in a mollusk intermediate host.

Standard solution A nonbiological solution of an analyte, usually in distilled water, with a known concentration.

Stomatocyte An erythrocyte with a linear area of central pallor.

Struvite A common crystal that is seen in alkaline to slightly acidic urine. Also referred to as triple phosphate crystals or magnesium ammonium phosphate crystals.

Sulfhemoglobin A form of hemoglobin that is found in the blood in trace amounts and that contains an irreversibly bound sulfur molecule that prevents normal oxygen binding.

Supernatant The fluid portion of a sample that is present after centrifugation.

Suppurative Containing, discharging, or causing the production of pus; cytology sample characterized by the presence of neutrophils representing more than 85% of total nucleated cells in the sample. May also be described as purulent.

Synovial fluid A transparent, viscous fluid that is secreted by synovial membranes and that acts as a lubricant for many joints, bursae, and tendons. It contains mucin, albumin, fat, and mineral salts.

Target cell A leptocyte with a peripheral ring of cytoplasm surrounded by a clear area and a dense, central, rounded area of pigment.

Thermophiles Organisms that undergo optimal growth at elevated temperatures.

Thoracocentesis The removal of fluid from the thoracic cavity.

Thrombin An enzyme that is formed from prothrombin, calcium, and thromboplastin in plasma during the clotting process. Thrombin causes fibrinogen to change to fibrin, which is essential during the formation of a clot.

Thrombocytes Platelets; cytoplasmic fragments of bone marrow megakaryocytes.

Thrombocytopenia A condition that involves a decrease in the number of circulating platelets.

Thrombocytosis A condition that involves an increase in the number of circulating platelets.

Thrombopathia A condition in which there is a deficiency of clotting ability for reasons other than thrombocytopenia.

Thrombopoiesis The production of platelets.

Thyroid-stimulating hormone A substance secreted by the anterior lobe of the pituitary gland that controls the release of thyroid hormone and that is necessary for the growth and function of the thyroid gland.

Thyroxine A hormone of the thyroid gland that is derived from tyrosine and that influences the metabolic rate.

Tick paralysis A condition that results from the introduction of a neurotoxin into the body during the attachment of and feeding by the female of several tick species.

Transudate An effusion that is characterized by a low protein concentration and a low total nucleated cell count.

Trematode An organism in the phylum Trematoda; commonly referred to as a fluke.

Trypsin A proteolytic digestive enzyme that is produced by the exocrine pancreas and that catalyzes the breakdown of dietary proteins into peptones, peptides, and amino acids in the small intestine.

Trypsinogen The inactive precursor form of trypsin; it is secreted in pancreatic juice and converted into active trypsin through the action of enterokinase in the intestine.

Tyrosine An amino acid that is synthesized in the body from the essential amino acid phenylalanine; it is found in most proteins and is a precursor of melanin and several hormones, including epinephrine and thyroxin.

Undulate To have wavelike fluctuations or oscillations.

Uric acid A metabolic by-product of nitrogen catabolism.

Vaccination Any injection of attenuated microorganisms (e.g., bacteria, viruses, rickettsiae) that is administered to induce immunity or to reduce the effects of associated infectious diseases.

Vacutainer A glass tube with a rubber stopper from which air can be removed to create a vacuum; usually used to draw blood.

Veterinary technician A tireless, dedicated, and vital member of the veterinary health care team. Also referred to as a superhero or saint.

von Willebrand disease An inherited disorder that is characterized by the abnormally slow coagulation of the blood as well as spontaneous epistaxis and gingival bleeding. It is caused by a deficiency of a component of factor VIII. Excessive bleeding is common after injury or surgery.

Warbles The common name for the larva of some species of flies; they are often in swollen, cyst-like subcutaneous sites, with a fistula or pore that communicates with the outside environment.

Wood's lamp An illuminating device with a nickel oxide filter that holds back all light except for a few violet rays of the visible spectrum and ultraviolet wavelengths of about 365 nm. It is used extensively to help diagnose fungal infections.

Yeast Any unicellular (usually oval) nucleated fungus that reproduces by budding.

Ziehl-Neelsen stain One of the most widely used methods of acid-fast staining; it is commonly used during the microscopic examination of a smear of sputum that is suspected of containing *Mycobacterium tuberculosis*.

Zone of inhibition An area of no bacterial growth around an antimicrobial disc that indicates some sensitivity of the organism to the particular antimicrobial.

Zoonoses Diseases that can be transmitted between animals and humans.

Zygospores The spores that result from the conjugation of two isogametes, as occurs with certain fungi and algae.

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