1

Getting Started with Clinical Pathology

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KEY TERMS

Clinical pathology
Laboratory
Equipment
Supplies
Maintenance
Safety

LEARNING OBJECTIVES

1) Become familiar with the equipment used to perform clinical pathology testing.
2) Understand when to use different types of blood collection tubes.
3) Know the sample types needed for clinical pathology tests.
4) Be able to process and store samples for clinical pathology tests.
5) Follow basic laboratory safety procedures.

Case example 1

A cytology sample slide was placed onto a microscope. Cells were visible using the 10× objective lens but could not be focused on using the 40× objective lens. What troubleshooting can you do to resolve this issue?

Clinical Pathology and Laboratory Techniques for Veterinary Technicians, Second Edition.
Edited by Amy L. MacNeill and Anne M. Barger.

Companion website: www.wiley.com/go/macneill_barger_clinicalpathology2e

Case example 2

A urine sample needs to be centrifuged for 5 minutes at a speed of 450×*g* to collect a concentrated urine sediment for microscopic analysis without damaging the cells in the urine. Your centrifuge settings are reported as rpm, not *g*. How do you determine what rpm setting is needed to process the urine sample?

Case example 3

The total protein concentration of a patient's serum needs to be measured. You place a small amount of patient serum onto a refractometer and close the lid. When you look through the instrument, you cannot see a clear line that determines the protein concentration. What do you do?

Case example 4

A feline blood sample was collected into a blood tube containing EDTA, but the amount of blood in the tube was below the volume indicator on the side of the tube. The veterinary technician loaded the appropriate amount of the sample into the automated hematology analyzer. Results indicated that the cat had low erythrocyte and platelet counts. The technician recorded that the tube was underfilled before the results were reported. Why is it critical that the technician recorded the fact that the sample was underfilled?

Case example 5

A glass slide with dried blood on it was dropped on the floor and shattered. The animal caretaker saw the mess and began to pick up the pieces of glass with her bare hands. What should you do?

Introduction

Clinical pathology evaluates disease in animals using *laboratory* data collected during analysis of blood, urine, body fluids, and tissue aspirates. Laboratory data sets collected in sick animals typically include hematology data, serum or plasma chemistry concentrations, urinalysis results, and cytology interpretations. This chapter introduces the *equipment* used to collect accurate laboratory data.

Standard Equipment

A) Microscope.

A well-maintained and properly aligned microscope is an important tool for analysis of blood smears, fecal samples, and urine sediment samples. This is an expensive tool that requires proper training to maximize the full potential use of the instrument.

- Types of microscopes.

  There are several types of microscopes available (e.g., upright binocular light microscopes, inverted binocular fluorescent microscopes, and dissection monocular light microscopes). The most commonly used microscope in a veterinary clinic is an upright binocular light microscope. This type of microscope has a light source located below the sample stage, an objective lens located above the sample stage, and two eyepiece lenses that the user looks through simultaneously to visualize the sample. Figure 1.1 is a diagram of a typical upright binocular light microscope.

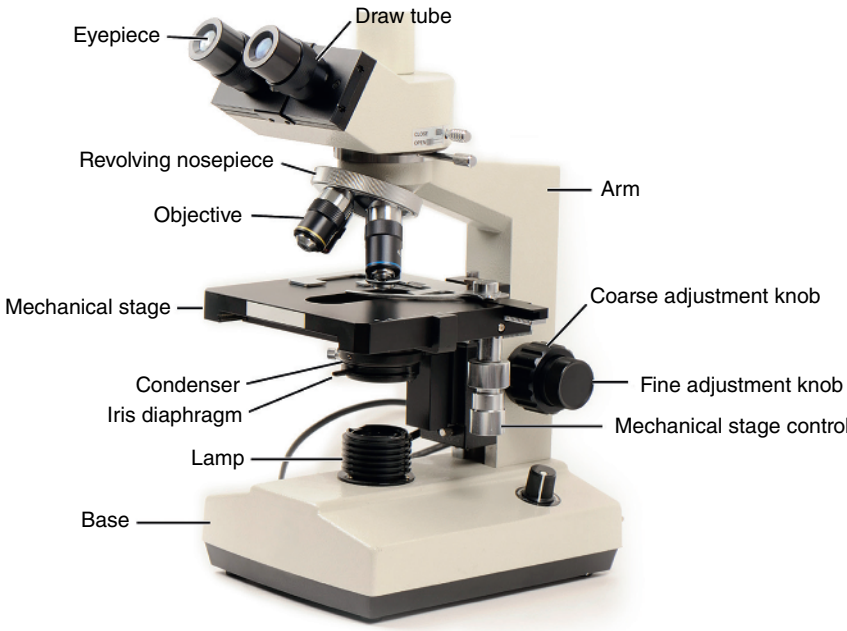


Figure 1.1 Microscope diagram. Components of an upright binocular light microscope are indicated. Common microscope components include two eyepieces, two draw tubes, a body tube, an arm, objective lenses, a revolving nosepiece, the microscope stage, stage clips, a mechanical stage control, a course adjustment knob, a fine adjustment knob, a condenser, an iris diaphragm, a lamp, and a rheostat (*Source:* David Ahn/Getty Images).

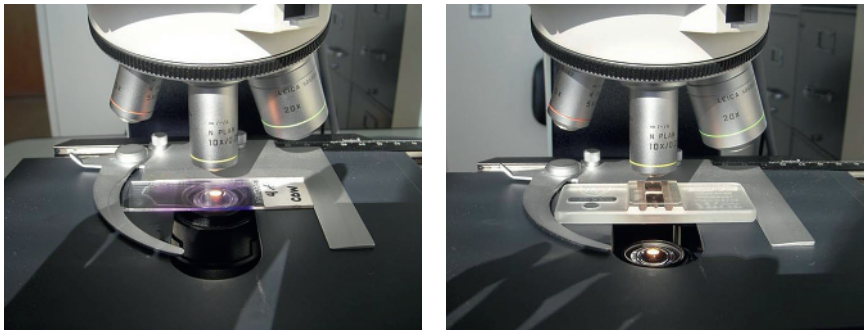
- Components of an upright binocular light microscope.
  - Eyepieces typically hold lenses that magnify the sample by 10-fold (10×).
  - Draw tubes may or may not be present, depending on the brand of microscope. Draw tubes allow for minimal adjustment of sample focus to accommodate differences in visual acuity between each eye.
  - The body tube is the hollow section of the microscope between the eyepieces and the objective lenses.
  - The arm of the microscope supports the body tube and connects the eyepieces to the base of the microscope.
  - Objective lenses magnify the sample. The strength of magnification and whether or not the lens requires immersion oil to focus on the sample are indicated on the side of the objective lens. For most clinical pathology tests performed in veterinary clinics, at least three objective lenses are recommended: a low-power lens, a dry high-power lens, and a high-power lens that requires immersion oil for fine focusing.
    - Objective lenses are attached to a revolving nosepiece so that the user can easily switch between objective lenses while looking at a sample. Properly aligned microscopes require very minimal movement of the fine adjustment knob to sharpen the focus between the different objective lenses.
  - The microscope stage holds the sample under the objective lens and over the light source. A stage may have stage clips that secure the sample onto the stage if the microscope has a mechanical stage control.
  - A mechanical stage control allows the user to move the sample around the stage via a joystick.
  - A course adjustment knob allows the user to focus the sample using scanning and low-power lenses. This knob should not be adjusted when using high-power lenses.
  - The fine adjustment knob is used to make subtle changes that bring the sample into fine, crisp focus when using high-power lenses.
  - A condenser is located between the bottom of the stage and the light source so that light scatter can be minimized.
    - There is a condenser height adjustment knob in front of the coarse adjustment knob that changes the location of the condenser relative to the stage.
    - Condenser adjustment knobs are also located just below the condenser and are used to center the light source on the sample and align the light with the field of view through the eyepieces.
  - An iris diaphragm is found over the light source of a microscope.
  - The lamp of the microscope is the light source. Most microscopes have a rheostat located on the base that can be used to adjust the brightness of the light source.
  - The microscope base should be placed on a clean, flat surface for proper use of the instrument.

- Magnification (TECHNICIAN TIP 1–1).
  - 2×, 4×, and 5× dry lenses are considered scanning lenses.
  - 10× and 20× dry lenses are examples of low-power lenses.
  - 40× and 60× lenses are high-power lenses that usually are dry lenses. A cover slip should be used with these objectives to prevent contaminating the lens with the sample.
  - 50× and 100× lenses are high-power lenses that usually require immersion oil to allow the user to focus on the sample.
  - Most microscopes in veterinary practices have 10× and 40× dry lenses and a 100× oil lens.

TECHNICIAN TIP 1–1 TOTAL MAGNIFICATION

The total magnification of a sample equals the product of the objective lens magnification and the magnification of the eyepiece lens. For example, if you are looking at a sample at "high power" (40× objective lens) and you have a 10× eyepiece lens, the total magnification of the sample is (40 × 10) = 400×.

- Specimen evaluation.
  Different sample types are prepared for microscopic examination in different ways. Whole blood typically is smeared onto a glass slide, dried, and stained before examination. Urine sediment, on the other hand, is examined as a wet mount after the sample has been centrifuged, concentrated, resuspended in a smaller volume of urine, dropped onto a slide, and then covered with a cover slip. Feces can be examined as a direct smear after being stained or as a wet mount after fecal flotation has been performed. These processes are described in detail in other chapters of this book. The focus of this section is to describe how to examine dry, stained smears, and wet mounts using a microscope. Additional tips for adjusting a microscope are listed in TECHNICIAN TIP 1–2.
  - The microscope needs to be on a flat surface near access to an electrical power outlet to plug in the light source.
  - Microscope lenses should be properly installed and clean.
  - Typically, the iris is kept opened when examining a sample for clinical pathology tests but may be closed slightly to reduce the amount of light reaching the sample or to minimize light scatter, if needed.
  - Samples must be thin enough to allow light to shine through the specimen.
  - A scanning objective lens or 10× low-power lens should be in place, aimed toward the microscope stage.
  - A glass microscope slide containing the sample is placed onto the microscope stage carefully. This often requires fitting the slide between stage clips.



(a) (b)

Figure 1.2 Microscope condenser settings. (a) Dried samples are examined with the condenser close to the microscope stage to reduce light scatter. (b) Fluid samples (including wet-mounted samples and hemocytometers) are examined with the condenser lowered away from the microscope stage to improve visualization of objects that are floating in solution.

- To examine dry, stained smears, the condenser is raised up, close to the stage (Figure 1.2a).
- To examine a wet mount, the condenser should be lowered down, away from the stage (Figure 1.2b).

– Look into the eyepieces, turn on the light source, and adjust the light using the rheostat so that it is not painful to the eyes. Focus on the sample using a scanning or 10× lens and the coarse adjustment knob. If draw tubes are present, the focus can be adjusted individually to each eye.
– Switch to a dry high-power objective lens by moving the revolving nosepiece to the proper position. Make small adjustments to the focus using the fine adjustment knob. Complete any analysis you can while focused on the sample with the dry objective lens.
  - The 40× objective lens is the highest magnification used for wet mounts.
  - DO NOT use the course adjustment knob to focus on the slide using a high-power objective lens; it is highly likely that the slide will crack or the lens will be damaged.
– If the sample is a dry, stained smear, place a small drop of immersion oil onto the sample and use the revolving nosepiece to move the high-power oil lens in place for final data collection.
– *NOTE*: You cannot switch back to a dry objective lens once oil has been placed on the sample.

TECHNICIAN TIP 1–2 WAYS TO FIX COMMON PROBLEMS WITH THE MICROSCOPE

- The viewing field is too dark.
  - Open the iris diaphragm.
  - Increase the light intensity.
  - Be sure that the condenser is up near the slide if the sample is a dry, stained smear.
- There is a stationary spot in the field of view that does not move when the slide is moved.
  - Use lens cleaning solution and lens paper to clean the eyepieces.
  - Use lens cleaning solution and lens paper to clean the objective lens.
  - Remove the eyepieces and clean any debris at the bottom of the draw tube with lens paper.
  - Wipe off the condenser with lens paper.
  - Wipe off the light source with lens paper.
- The view field is cloudy or cannot be perfectly focused using the 40× objective lens.
  - Use lens cleaning solution and lens paper to clean the objective lens.
  - Place a coverslip on the slide to improve the performance of the 40× lens.
    - A coverslip can be placed onto a slide using immersion oil or mounting medium.
- The view field is partially lit and partially black.
  - Check the positioning of the objective lens.
  - Be sure that the light is centered on the sample (see Microscope maintenance).
- A sample can be focused on using the 10× objective lens but not the 40× objective lens.
  - Ensure that the slide is right-side-up with the sample facing toward the objective lens.
  - Use lens cleaning solution and lens paper to clean the objective lens.
  - Place a coverslip on the slide to improve the performance of the 40× lens.
    - A coverslip can be placed onto a slide using immersion oil or mounting medium.

- Microscope maintenance.
  Microscopes should be serviced at least once a year by a professional microscopist. Daily maintenance must be performed by clinic staff.
  - Daily maintenance includes the following:
    - Keeping the lenses, condenser, and light source clean of debris using lens cleaning solution and lens paper. Avoid scratching the surface of the lenses.
    - Centering the light on the sample (Köhler illumination).
      - Set the scanning lens in place using the revolving nosepiece.
      - Close the iris diaphragm.
      - Lower the condenser. A small spot of light should be visible in the field of view.
      - Center the spot of light using the condenser adjustment knobs.
      - Raise the condenser.
      - Open the iris diaphragm.
  - To safely move a microscope, hold onto the arm of the microscope with one hand and support the base of the microscope with the other hand.
  - DO NOT get oil on a dry lens. If oil does get on a dry lens, it will need to be carefully and thoroughly cleaned using lens cleaning solution and lens paper.
  - Be sure to turn off the light source and cover the microscope with a plastic bag or microscope cover after use.

B) Centrifuge.

Working centrifuges are needed to process samples for clinical pathology testing. It is imperative that centrifuges are used correctly and cleaned routinely to avoid damage to the machine. To clean a centrifuge, wipe all surfaces with dilute soapy water, rinse with water, and allow the parts to dry completely before using the centrifuge. Do not use metal brushes or sharp objects to remove debris from rotors or sample holder; if these components are scratched, they will need to be replaced.

Centrifuges are designed to spin a rotor, which holds the samples, at a specific set speed measured in revolutions per minute (rpm). The length of the rotor arm varies in different centrifuges. The length of the rotor arm and speed of the spin determine the gravitational force (*g*) that a sample is subjected to (TECHNICIAN TIP 1–3). The *g* force causes larger, heavier components of the sample to gravitate to the bottom of the sample tube and lighter, less dense components to remain at the top of the sample. For example, in a blood sample, cells will pellet at the bottom of a centrifuged tube and lipids will remain near the top of the sample.

TECHNICIAN TIP 1–3 HOW TO CONVERT BETWEEN REVOLUTIONS PER MINUTE (rpm) AND GRAVITATIONAL FORCE (*g*)

- Determine the radius (r) of the centrifuge rotor in centimeters (cm).
- $\text{rpm} = \sqrt{g \div \left[r \times \left(1.118 \times 10^{-5} \right) \right]}$
- $g = \text{rpm}^2 \times r \times (1.118 \times 10^{-5})$
  - Note that *g*, g-force, and relative centrifugal force (rcf) are synonymous terms.

Centrifuge rotors should be examined yearly for pitting or warping of the metal. Damaged rotors are not safe to use and need to be replaced. There are two common types of rotors, fixed rotors and swinging bucket rotors. An example of a fixed rotor is found in a microhematocrit centrifuge. In a swinging bucket rotor, all buckets *always* should be placed into the rotor before using the machine to avoid warping the rotor. Either type of rotor must have a balanced load before the centrifuge is turned on. This means if a sample is placed in one sample holder, an equal weight must be put in the sample holder located directly across from the sample (Figure 1.3).

C) Refractometer.

Refractometry is an analytical method that correlates the degree of light refraction (refractive index) in a liquid with the amount of solids in the liquid.

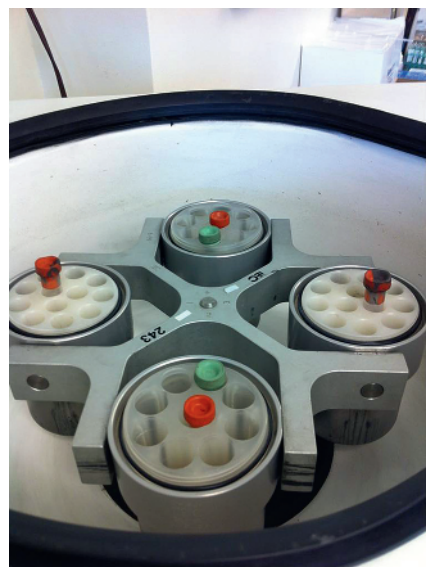


Figure 1.3 Balanced centrifuge. The same sample weight is placed on opposite sides of a centrifuge rotor to balance the sample load during centrifugation. This prevents damage to the rotor.

Figure 1.4 Refractometer measurement. The proportion of solids in a fluid sample is calculated by a refractometer. This value is read by looking through the eyepiece of the instrument and adjusting the eyepiece to fine focus the scale present in the refractometer.

Refractometers are available in most veterinary practices and commonly are used to determine protein concentration and specific gravity of fluid samples.

To use a refractometer (TECHNICIAN TIP 1–4), lift the clear lid and fill the refractometer with the liquid. Close the clear lid and angle the refractometer toward a light. Look through the eyepiece of the refractometer and locate the scale (Figure 1.4). Adjust the eyepiece by turning it back and forth to bring the scale into focus. Read the scale with the appropriate units for the solid or property you are measuring. For example, the units for the total protein of a sample are grams per deciliter. Because this technique relies on light to be able to pass through the liquid, both hemolysis and lipemia can interfere with accuracy.

TECHNICIAN TIP 1–4 WAYS TO FIX COMMON PROBLEMS WITH A REFRACTOMETER

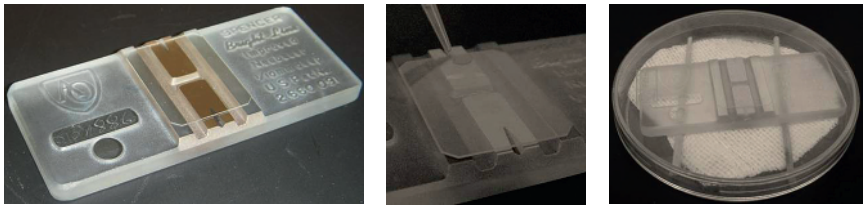
- A clear line is not observed when looking through the refractometer to read the instrument scale.
  - Ensure that enough sample fluid was placed onto the refractometer.
    - The sample fluid must extend across the entire width of the refractometer.
    - Load additional sample fluid onto the refractometer.

  - Ensure that the clear lid of the refractometer is completely closed.
    - Glass shards, hair, or other debris can prevent the lid of the refractometer from closing completely.
    - Remove the debris manually or by rinsing the refractometer off with distilled water.
    - If distilled water was used to remove the debris, gently dry the refractometer with low-lint tissue paper and then reapply the patient fluid sample.
  - Adjust the angle of the refractometer and the light source to increase the amount of light focused on the sample.
  - Determine if the instrument is damaged.
    - Scratches on the refractometer (where the fluid sample is placed) can interfere with sample measurement. If this occurs, the refractometer might need to be replaced.
- The fluid sample is difficult to expel from the microhematocrit tube that the sample is in.
  - Hold the microhematocrit tube over the refractometer without touching the glass.
  - Use a clean, empty syringe that is filled with air to force air into the top end of the microhematocrit tube so that the fluid in the tube is pushed out of the bottom end onto the refractometer.

D) Hemocytometer.

A hemocytometer is a specialized chamber with a small precise grid used to perform manual cell counts when cells are suspended in a liquid medium (Figure 1.5a). A specific type of coverslip must be used with the hemocytometer to ensure that the proper volume of fluid is loaded onto the chamber; otherwise, cell counts will be inaccurate.

- Using a hemocytometer.
  - Before using a hemocytometer, be sure that the hemocytometer and coverslip are clean.
  - Place the coverslip onto the chamber.
  - Add 10 µL of well-mixed sample fluid onto each side of the hemocytometer (Figure 1.5b).
- Allow the cells to settle by incubating the hemocytometer for 10 minutes in a petri dish containing a wet piece of absorbent paper (Figure 1.5c).
- Using the microscope with the condenser lowered (Figure 1.2b), determine the average number of cells present in 1-mm grid section on the hemocytometer (Figure 1.6).



(a) (b) (c)

Figure 1.5 Hemocytometer. (a) Hemocytometer with two chambers for counting the number of cells in a fluid sample. (b) Ten microliters of fluid is loaded into each chamber of a hemocytometer. (c) Hemocytometer incubating in a petri dish over a damp gauze to prevent the sample from drying out and allow the cells to settle.

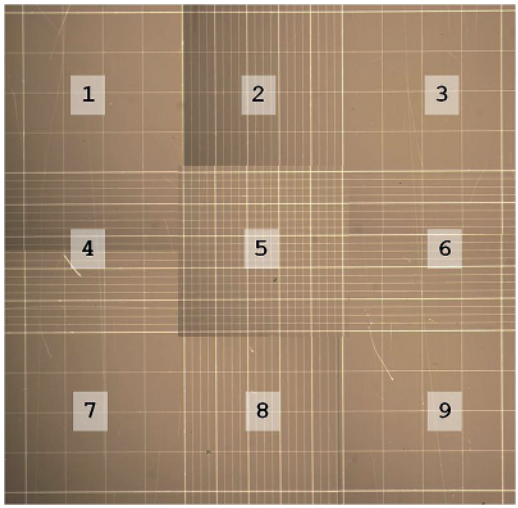


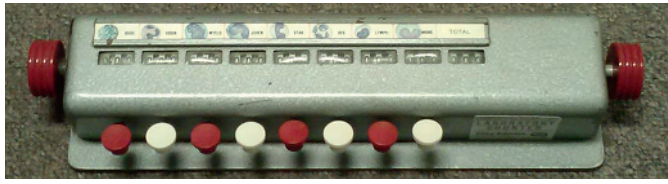
Figure 1.6 Hemocytometer grid. Each chamber of a hemocytometer contains nine 1-mm grid sections. Cells that fall within the grid section and on the upper and left edges of the grid section are counted. Cells that fall on the lower and right edges of the grid section are not counted.

- The average number of cells in one 1-mm grid is the number of cells per 0.1 µL of sample fluid. The following bullet points give an example of how to use a hemocytometer to calculate the number of cells per microliter of sample fluid.
  - *NOTE*: There are nine 1-mm grid sections on each chamber of the hemocytometer.
    - Cells in the middle of a 1-mm grid and touching the top and left edges of a 1-mm grid should be counted.
    - Do not count cells touching the bottom and right edges of a 1-mm grid.
  - At least five 1-mm grid sections from each side of the hemocytometer should be counted to determine the average number of cells/grid.

- Ideally, the variation between cell counts from the two chambers of the hemocytometer should not exceed 10%.
- Calculate cells/μL using the formula:
  - (Cells/grid × sample dilution) ÷ 0.1 μL/grid = cells/μL
  - *Example*: If the sample dilution factor is 1:2000 and the average number of cells in one grid section is 200, then (200 cells/grid × 2000) ÷ 0.1 μL/grid = 4×10^6 cells/μL.

E) Differential cell counter.

A differential cell counter allows a user to keep track of the cell types observed and the total number of cells examined on a sample slide. Both analog (Figure 1.7a) and digital (Figure 1.7b) differential cell counters are available. Different buttons on the cell counter are assigned to different cell types. When a particular cell type is identified microscopically, the user presses a button assigned to that cell type. Each time the button is pressed, the number recorded on the instrument increases by 1. Different numbers are recorded for each button representing each cell type identified. Simultaneously, the total number of cells examined is tallied and recorded. The counters are set to indicate when 100 cells are tallied.



(a)

(b)

Figure 1.7 Differential cell counters. (a) Analog and (b) digital cell counters are shown.

Standard Supplies

A) Stain.
- Cells are more easily identified if they have been stained with dyes. For blood smears and cytology samples, Romanowski-type stains are used most commonly.
- Samples are thinly smeared onto a glass microscope slide so that they are arranged in a single-cell layer. Then, the slides are allowed to dry before they are exposed to cellular dyes.
- There are several types of stains available for microscopic examination of cells. The most common stains used in veterinary practices are Romanowski-type stains and new methylene blue (NMB) stain.
  - Often, cells stained using Romanowski-type stains are fixed in methanol before staining with a cationic dye and an anionic dye. This fixation step allows the dyes to enter the cell membrane and bind intracellular structures.
    - The cationic dye stains acidic cell structures (such as nuclear material and acidic proteins) a blue–purple color.
    - Basic cell structures, including most cellular proteins, bind the anionic dye and stain red.
    - Romanowski-type stains include Diff-Quik®, Wright–Giemsa, May–Grunwald–Giemsa, and Leishman's stains.
  - Diff-Quik® staining procedure.
    - Repeatedly dip a dried sample that has been thinly smeared onto a glass slide in and out of the first staining solution 10–12 times.
      - This solution is clear or light blue.
      - Methanol can be used for this step.
    - Repeatedly dip the sample in and out of the second staining solution 10 times.
      - This solution is red.
    - Repeatedly dip the sample in and out of the third staining solution 8–10 times.
      - This solution is blue.
    - Rinse the slide in deionized water to remove excess stain. Tap water can be used if deionized water is unavailable, but staining characteristics may be slightly altered.
    - Allow the slide to air dry and then examine the sample microscopically.
  - NMB staining procedure: This stain dyes acidic groups (including DNA and RNA) blue. Primarily, NMB is used to detect immature red blood cells (RBCs) and oxidative damage to RBCs.
    - Mix equal amounts of NMB and blood (or other fluid sample) together in a test tube.

- Incubate for 5–10 minutes.
- Prepare a smear of the NMB/sample mixture and allow it to air dry.
- Examine the smear microscopically.

B) Glass slides.

Glass slides used for microscopy need to be precleaned before packaging to avoid glass shards and greasy substances that accumulate on the slide during the manufacturing process. Vendors that sell glass slides will specify if the slides have been precleaned.

All samples, including glass slides, should be labeled well enough to identify the patient that the sample was collected from, the date of collection, and type of sample (TECHNICIAN TIP 1–5). Glass slides should be labeled using a pencil, a marker specifically designated for sample labeling, or a glass etching tool. Otherwise, the label is likely to wash off when the sample is stained. Slides may come with or without a frosted edge. The frosted edge is convenient for labeling the sample. However, if part of the sample is accidentally smeared over the frosted edge, that part of the sample cannot be visualized under the microscope and diagnostic material may be lost.

TECHNICIAN TIP 1–5 LABELING SLIDES

Ink pen and marker will wash off of glass slides when samples are stained. When labeling slides, use a pencil (most useful if the slide has a frosted edge), a marker specifically designated for writing on glass, or a glass etching tool.

C) Coverslips.

Coverslips are needed for wet-mount preparations of fluid samples. In addition, coverslips should be placed over all samples dried onto glass slides to allow for crisp focusing of the microscope when using a high-power objective lens. Coverslips may be placed onto a sample using immersion oil or specialized glue (mounting medium). When glued on, coverslips can preserve samples for a long period of time.

D) Blood tubes.

There are several types of tubes that are used for collection of blood. Specific types of tubes are needed for different types of clinical pathology tests. Avoid positive pressure when filling tubes, forcing the blood into the tube by pressing on the syringe can result in hemolysis. Let the tube fill via vacuum pressure or remove the rubber stopper on the tube to break the vacuum. Tubes should be filled with the appropriate amount of blood as indicated on the side of the tube. This will ensure that the tube contains the correct ratio of the chemical in the tube and the blood.

Figure 1.8 Blood collection tubes. Different blood collection tubes are needed for different clinical pathology diagnostic tests. These tubes come in many different sizes and are color coded for easy identification.

- Types of blood collection tubes (Figure 1.8) and their uses:
  - Striped-red- and gray-topped tubes
    - Used to collect serum
    - Also known as serum separator tubes
    - As the clotted sample is centrifuged, a gel moves between the clot and the serum to prevent alterations in serum chemistry values.
  - Serum separator tubes should not be used for samples drawn to measure drug concentrations because contact with the serum separator gel may falsely decrease results.
- Red-topped tubes
  - Used to collect serum
  - Does NOT contain anticoagulant or serum separator gel
  - Once the sample has clotted, it needs to be centrifuged. Immediately after centrifugation, the serum must be removed from the blood clot and placed in a separate tube to avoid alterations in serum chemistry values.
- Purple-topped tubes
  - Used to collect whole blood
  - Contains the anticoagulant ethylenediaminetetraacetic acid (EDTA)
    - The final concentration of EDTA should be 1.8 mg/mL of blood.
  - Preferred collection tube for complete blood counts (CBCs) in mammals

- Green-topped tubes
  - Used to collect plasma
  - Contains the anticoagulant lithium heparin
    - The final concentration of heparin should be ~16 international units per milliliter of blood.
  - The sample can be centrifuged immediately (as the sample will not clot). After centrifugation, the plasma should be removed from the RBCs with a pipette and placed into another tube.
  - When submitting heparinized samples to an outside laboratory, label the sample as heparinized plasma, so the laboratory knows what they are dealing with.
- Blue-topped tubes
  - Contain 3.2% sodium citrate
  - Dilutes the blood sample by 10%
  - Preferred for most platelet function tests

Sample Types

There are several sample types that may be submitted for analysis using clinical pathology tests. Below is a short list of the most common samples processed. These are the sample types discussed in this book.

A) Whole blood includes the unclotted cells and the fluid portion of the blood.
B) Serum is the fluid portion of the blood that remains after the formation of a blood clot.
C) Plasma is the fluid portion of the blood that remains after unclotted cells have been removed from the sample.
D) Urine is the fluid waste product removed from the blood by the kidneys.
E) Feces are solid waste products produced by the digestive tract.

Sample Storage and Preparation

The way that samples are manipulated in the laboratory has a direct effect on the accuracy of test results. The chapters in this book provide guidelines for storage and preparation of samples for several types of clinical pathology tests. If you are asked to process a sample for a test you are not familiar with, it is best to research the proper way to handle the sample before sample collection. If the sample is being shipped to another laboratory, speak with a customer service representative to ensure that the sample is collected, stored, and shipped properly. This will provide veterinarians with more reliable data, which should be a priority whenever a sample is processed for clinical pathology testing.

Basic Laboratory Safety

Every laboratory should designate a person to oversee the safety of the laboratory. Individuals that work in the laboratory must follow the safety guidelines that are in place. Some basic laboratory safety guidelines are listed in the following text:

A) Sharps.
- Needles are encountered commonly in a laboratory setting. Sharps containers for proper disposal of needles should be easily accessible at all times. These containers must be closed, sealed, and removed from the laboratory before they become filled to the brim. Most containers have a fill line that should be observed.
- Glass slides and tubes may break in the laboratory. If samples contain biologic materials, the glass should be cleaned up using a broom and a dust pan and then disposed of in a sharps container. If the glass does not contain sample material, it may be cleaned up using a broom and a dust pan and then disposed of in a trash container. Just be sure that the glass cannot break through the trash container and injure other workers.

B) Chemicals.
Laboratories often store chemicals and solutions that are used in various tests. Material safety data sheets should be posted for each chemical. Familiarize yourself with this information so that spills or inadvertent contact with the material can be cleaned up and treated appropriately.

C) Biological materials.
Clinical pathology laboratories process several types of potentially biohazardous materials including blood and feces. These samples must be handled properly to prevent alteration of the sample with environmental contaminants and to prevent contamination of the environment with infectious organisms from the samples. If samples are spilled, be sure to wear gloves when cleaning them up.
- To clean a contaminated surface, soak the area in 10% bleach solution for at least 10 minutes and then wipe the area dry with disposable towels. Dispose of the towels into a garbage can.
- To clean contaminated clothing, remove soiled articles of clothing and wash them on site in 10% bleach solution. Do NOT bring contaminated clothing home to be laundered.

  Once processed, samples should be stored or disposed of. Typically, body fluids and feces are discarded into biohazard bags that are clearly marked with a biohazard symbol. These bags are then autoclaved before being placed into a proper garbage container.

D) Personal protective equipment (PPE).
Personal protective equipment (PPE) includes gloves, laboratory coats, eye protection, masks, surgical caps, closed-toed shoes, and booties. Laboratories often require personnel to wear one or more of these items, particularly if potentially biohazardous samples are being processed in the laboratory. Gloves, laboratory coats, and closed-toed shoes are particularly important to minimize contamination of skin and clothing. Remember that gloves and laboratory coats should be removed as you are leaving the laboratory to avoid spreading any contaminants throughout the hospital. Always follow the PPE guidelines that are recommended by the laboratory you are working in.

Interpretation and comments for Case 1

If a dried cytology sample is visible using a 10× objective lens but not a 40× objective lens, the first thing to do is to ensure that the slide is right-side-up with the sample facing toward the objective lens. Samples placed on the microscope stage upside down will not be able to be viewed with high-power lenses. If the sample is placed on the microscope correctly, it is likely that the 40× objective lens is simply dirty (e.g., covered in immersion oil). Use lens cleaning solution and lens paper to clean the objective lens. Placing a coverslip on the slide also might improve the performance of the 40× lens. If none of these troubleshooting techniques work, perform routine daily microscope maintenance protocols and then try to view the sample again.

Interpretation and comments for Case 2

- To convert gravitational force to rpm, determine the radius of the centrifuge rotor in cm and calculate rpm using the formula: rpm = square root $\{g \div [r \times (1.118 \times 10^{-5})]\}$.
- Example calculation if the centrifuge rotor radius = 14.5 cm.
  - rpm = square root $\{450\,g \div [14.5\,\text{cm}\ (1.118 \times 10^{-5})]\}$
  - rpm = square root {450 ÷ 0.00016211}
  - rpm = square root {2,775,893}
  - rpm = 1666
- Centrifuges are typically calibrated to the nearest 100 rpm, so a centrifuge with a 14.5 cm rotor should be run for 5 minutes at 1600 or 1700 rpm to collect urine sediment for cytologic analysis.

Interpretation and comments for Case 3

Refractometers can be difficult to read if there is not enough sample loaded onto the instrument, if the lid of the refractometer is held slightly ajar, or if a weak light source is used. Check the refractometer to see if the sample fluid extends across the entire width of the refractometer. If not, load additional sample fluid onto the refractometer. If there is enough sample but the lid is ajar, remove any debris and press the clear lid against the sample. If a reading still cannot be obtained, adjust the angle of the refractometer and the light source to increase the amount of light focused on the sample.

Interpretation and comments for Case 4

It is important because the findings of anemia and thrombocytopenia may be inaccurate. The cell counts were likely decreased due to dilution of the sample by the EDTA in the tube.

Interpretation and comments for Case 5

Ask the caretaker to stop and wait until you get a broom and dustpan to clean up the glass. The glass can then be placed into a trash container. Be sure that the shards of glass do not protrude from the trash bag.