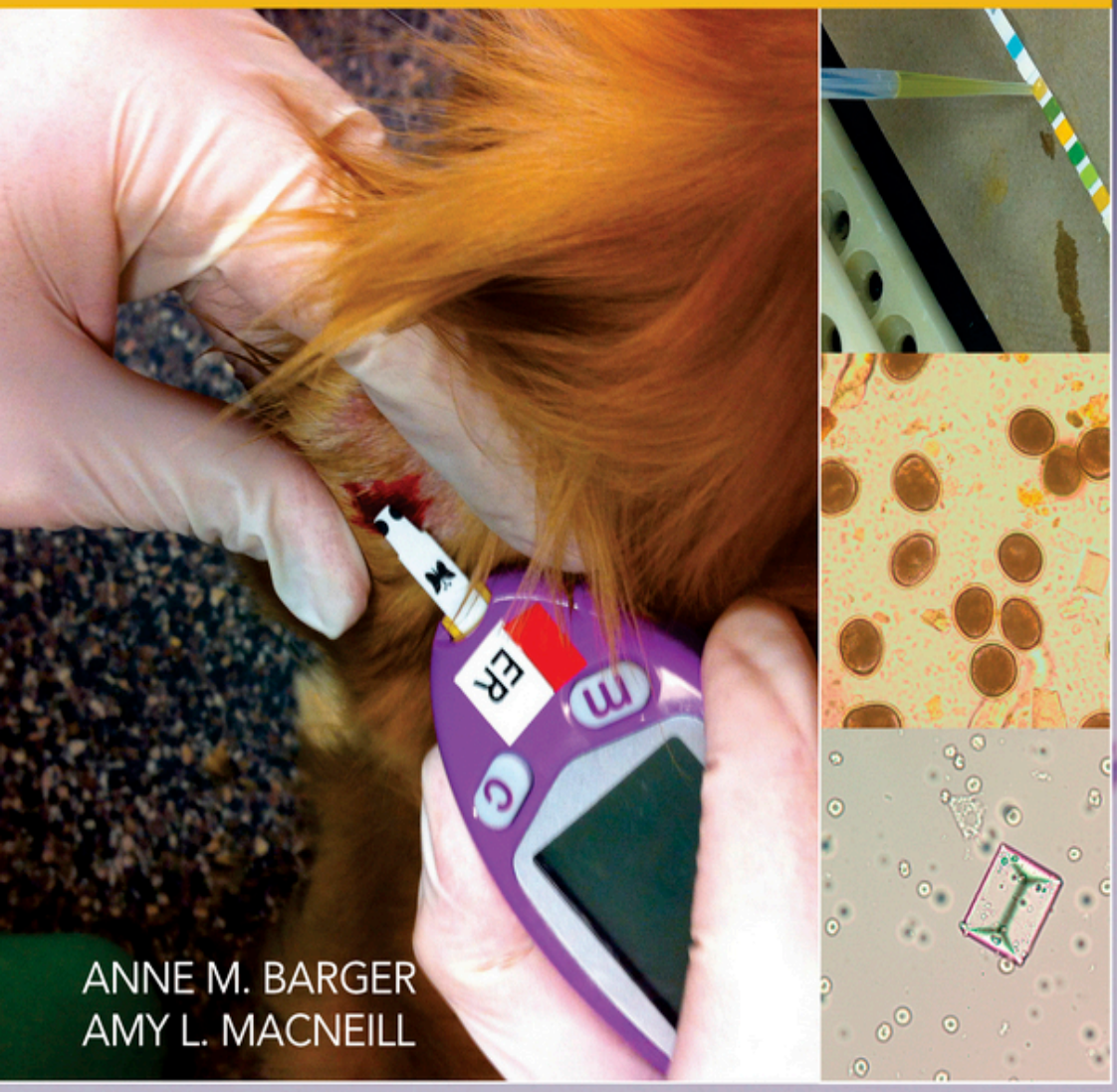
CLINICAL PATHOLOGY AND LABORATORY TECHNIQUES

FOR VETERINARY TECHNICIANS



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Clinical Pathology and Laboratory Techniques for Veterinary Technicians

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Amy MacNeill Dedication:

This book is dedicated to Dr. Bruce Ferguson, who taught me many of the techniques in this text and nourished my love of veterinary medicine.

Anne Barger Dedication:

I would to thank my partner in life Dr. Patty McElroy and my father Maurice Barger for their continued love and support.

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Preface

The objective of this text is to provide a thorough, practical guide to clinical pathology. It is directed toward veterinary technician students, veterinary technicians in practice, and veterinarians in general practice. Included in this text are learning objectives for students and educators, many high-quality images of techniques, instrumentation, microscopic cells, organisms, and patients. Each chapter contains cases meant to allow the student to understand the practical application of the material.

About the Companion Website

This book is accompanied by a companion website:

www.wiley.com/go/barger/vettechclinpath

The website includes:

- Instructor questions
- Answers to the Multiple Choice Questions that are in the book
- Powerpoints of all figures from the book for downloading

The password for the site is the last word in the caption for Figure 2.11.

chapter 1

Getting Started with Clinical Pathology

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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.

KEY TERMS

Clinical pathology
Laboratory
Equipment
Supplies
Maintenance
Safety

Case example 1

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?

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Case example 2

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.
- 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

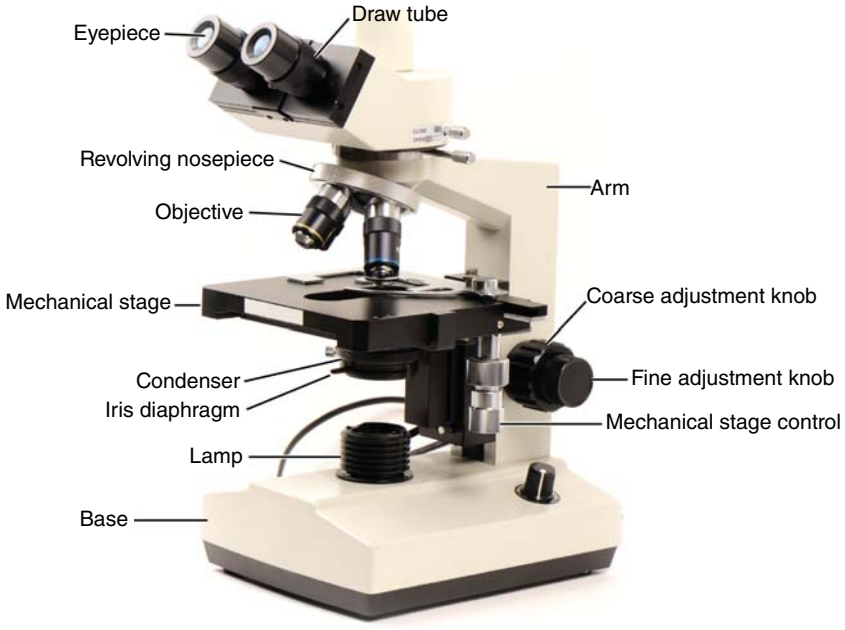


Figure 1.1 Microscope diagram. Components of an upright binocular light microscope are indicated. Common microscope components include two eyepieces, two draw tubes, a body tube, an arm, objective lenses, a revolving nosepiece, the microscope stage, stage clips, a mechanical stage control, a course adjustment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

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.

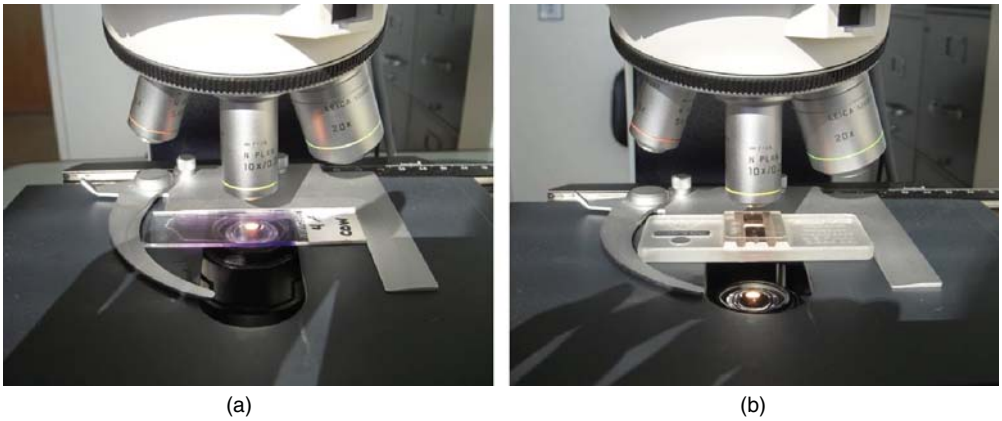
    - There is a condenser height adjustment knob in front of the coarse adjustment knob that changes the location of the condenser relative to the stage.
    - Condenser adjustment knobs are also located just below the condenser and are used to center the light source on the sample and align the light with 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.
  - 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 must 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.
  - 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.
  - 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.



(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.

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).

- 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.
      - 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. 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.

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. 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, lift the clear lid and fill the refractometer with the liquid (Figure 4.10d). Close the clear lid and angle the refractometer toward a light (Figure 4.10e). 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.

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).

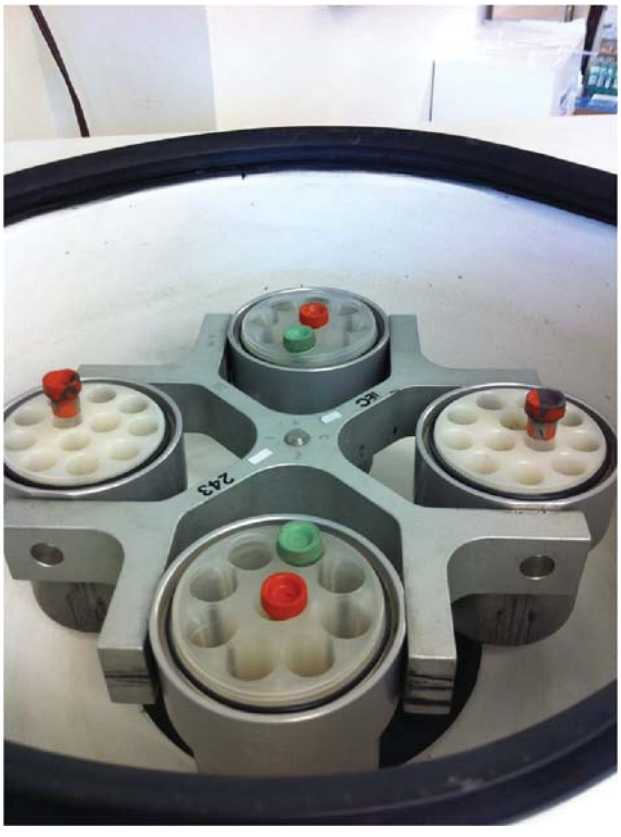


Figure 1.3 Balanced centrifuge. The same sample weight is placed on opposite sides of a centrifuge rotor to balance the sample load during centrifugation. This prevents damage to the rotor.

- 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).
- 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.

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.

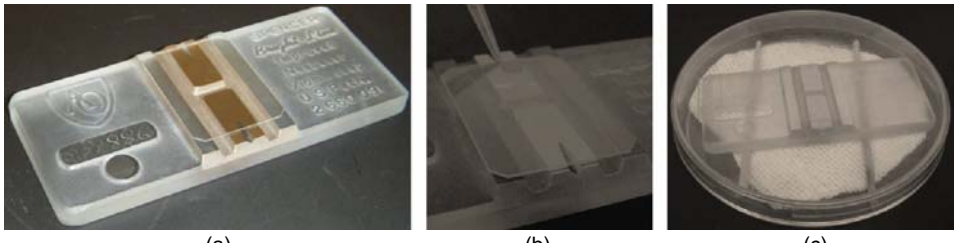


Figure 1.5 Hemocytometer. (a) Hemocytometer with two chambers for counting the number of cells in a fluid sample. (b) Ten microliters of fluid is loaded into each chamber of a hemocytometer. (c) Hemocytometer incubating in a petri dish over a damp gauze to prevent the sample from drying out and allow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

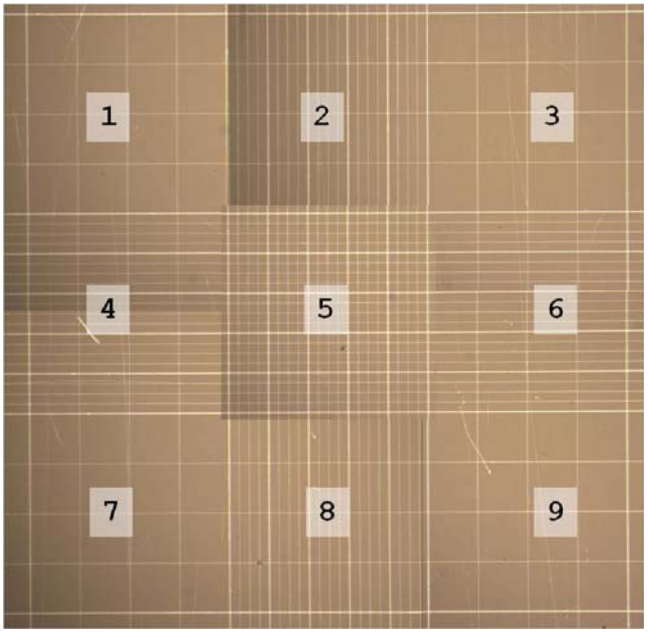
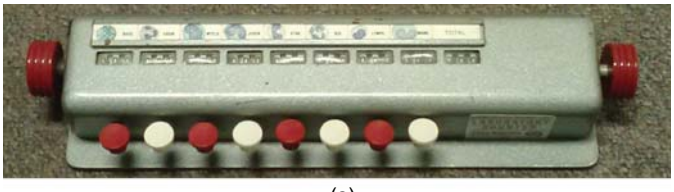


Figure 1.6 Hemocytometer grid. Each chamber of a hemocytometer contains nine 1-mm grid sections. Cells that fall within the grid section and on the upper and left edges of the grid section are counted. Cells that fall on the lower and right edges of the grid section are not counted.

- 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 as 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 one. 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-types 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 ten times.
    - This solution is red.
  - Repeatedly dip the sample in and out of the third staining solution eight to ten 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. 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 1–3: LABELING SLIDES Ink pen and marker will wash off of glass slides when samples are stained. When labeling slides, use a pencil, a marker specifically designated for sample labeling, 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