Chapter 1
Laboratory Equipment

Laboratory equipment

The variety of sophisticated laboratory equipment in a veterinary practice will depend largely on the size and scope of the practice itself. There are several pieces of core equipment that are standard in every practice that performs in-house testing and analysis.

Microscope

Purpose

The microscope is the most important piece of equipment in the veterinary clinic laboratory (Figure 1.1). The microscope is used to review fecal, urine, blood, and cytology samples on a daily basis. Understanding how the microscope functions, how it operates, and how to care for it will improve the reliability of your results and prolong the life of this valuable piece of equipment.

Parts and functions of a compound microscope

(A) **Arm:** Used to carry the microscope.
(B) **Base:** Supports the microscope and houses the light source.
(C) **Oculars (or eyepieces):** The lens of the microscope you look through. The ocular also magnifies the image. The total magnification can be calculated by multiplying the objective power by the ocular power. Oculars come in different magnifications, but 10× magnification is common.
(D) **Diopter adjustment:** The purpose of the diopter adjustment is to correct the differences in vision an individual may have between their left and right eyes.
(E) **Interpupillary adjustment:** This allows the oculars to move closer or further away from one another to match the width of the space between an individual's eyes. When looking through the microscope, one should see only a single field of view. When viewing a sample, always use both eyes. Using one eye can cause eye strain over time.
(F) **Nosepiece:** The nosepiece holds the objective lenses. The objectives are mounted on a rotating turret so they can be moved into place as needed. Most nosepieces can hold up to five objectives.

Veterinary Technician's Handbook of Laboratory Procedures, Second Edition. Brianne Bellwood and Melissa Andrasik-Catton.

Companion website: www.wiley.com/go/bellwoodhandbook2

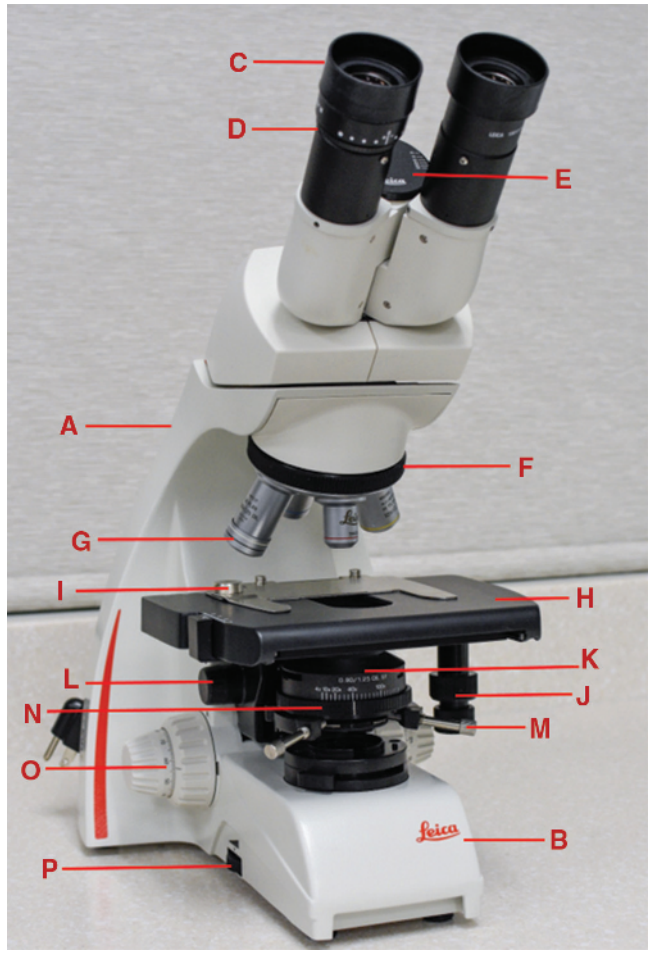

Figure 1.1 Parts of a compound microscope.

(G) **Objective lenses:** The objective lens is the lens closest to the object being viewed, and its function is to magnify the object. Objective lenses are available in many powers, but 4×, 10×, 40×, and 100× are standard. The 4× objective is used mainly for scanning. The 10× objective is considered "low power", 40× is "high power" and the 100× objective is referred to as "oil immersion." When the 10× and 40× objectives are used to view images, the terms "low-power field" (LPF) for 10× and "high-power field" (HPF) for 40× are often used. Once magnified by the objective lens, the image is viewed through the oculars, which magnify it further. Total magnification can be calculated by multiplying the objective power by the ocular lens power.

Example: 10× (ocular lens) × 100× (objective lens) = 1000× total magnification of the specimen

(H) **Stage:** The platform on which the slide or object is placed for viewing.

(I) **Stage brackets:** Spring-loaded brackets, or clips, hold the slide or specimen in place on the stage.

(J) **Stage control knobs:** Located just below the stage are the stage control knobs. These knobs move the slide or specimen either horizontally (***x*** axis) or vertically (***y*** axis) when it is being viewed.
(K) **Condenser:** The condenser is located under the stage. As light travels from the illuminator, it passes through the condenser, where it is focused and directed at the specimen.
(L) **Condenser control knob:** Allows the condenser to be raised or lowered.
(M) **Condenser centering screws:** These screws center the condenser and, therefore, the beam of light. Generally, they do not need much adjustment unless the microscope is moved or transported frequently.
(N) **Iris diaphragm:** This structure controls the amount of light that reaches the specimen. Opening and closing the iris diaphragm adjusts the diameter of the light beam.
(O) **Coarse and fine focus adjustment knobs:** These knobs bring the object into focus by raising and lowering the stage. Care should be taken when adjusting the stage height. When a higher-power objective is in place (100× objective, for example), there is a risk of raising the stage and slide and hitting the objective lens. This can break the slide and scratch the lens surface.

Coarse adjustment is used for finding focus under low power and adjusting the stage height. Fine adjustment is used for more delicate, high-power adjustments.

(P) **Illuminator:** The illuminator is the light source for the microscope, usually situated in the base. The brightness of the light from the illuminator can be adjusted to suit your preference and the object you are viewing.

Kohler illumination

What is Kohler illumination?

Kohler illumination is a method of adjusting a microscope in order to provide optimal illumination by focusing the light on the specimen. When a microscope is set up in Kohler illumination, specimens will appear clearer and in more detail (Procedure 1.1).

Procedure 1.1 Setting Kohler

Materials

- Specimen slide (need to focus under 10× power)
- Compound microscope.

Procedure

(1) Mount the specimen slide on the stage and focus under 10×.
(2) Close the iris diaphragm completely.
(3) If the ball of light is not in the center, use the condenser centering screws to move it so that it is centered.
(4) Using the condenser adjustment knobs, raise or lower the condenser until the edges of the field become sharp. (See Figures 1.2 and 1.3.)
(5) Open the iris diaphragm until the entire field is illuminated.

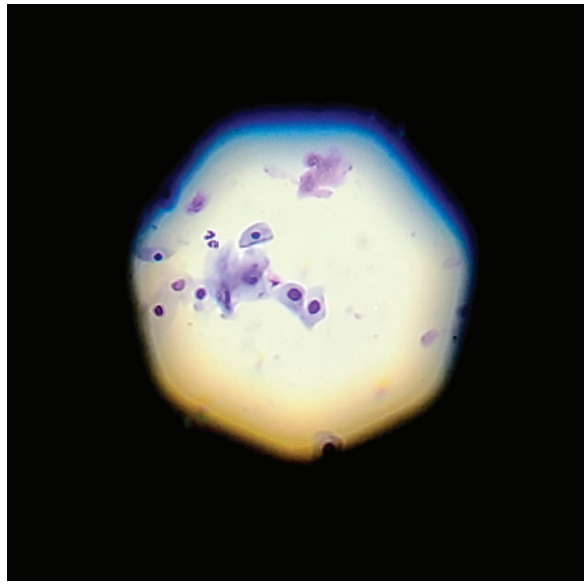

Figure 1.2 Appearance of image prior to setting the condenser. Note the softer edges of unfocused light.

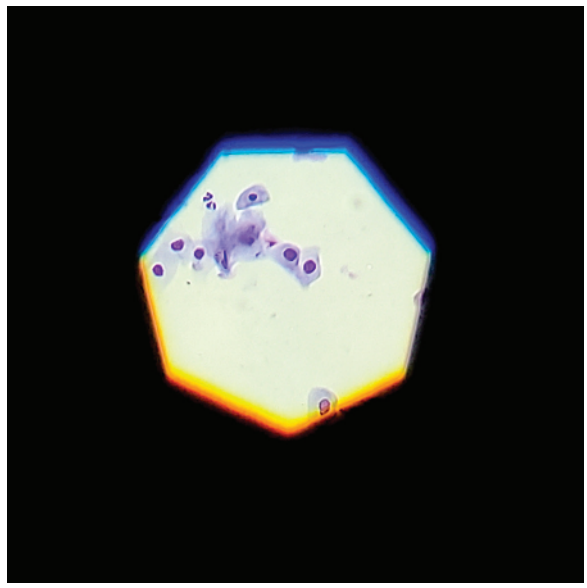

Figure 1.3 Sharpened edges following condenser adjustment.

When should you set/check Kohler?

- During regular microscope maintenance
- After the microscope is moved/transported
- Whenever you suspect objects do not appear as sharp as they could be

Microscope care and maintenance

Routine care and proper maintenance of the microscope will ensure good performance over the years. In addition to this, a properly maintained and clean microscope will always be ready for use at any time (Figure 1.4). Professional cleaning and maintenance should be considered when routine techniques fail to produce optimal performance of the microscope.

Cleaning and maintenance supplies

Dust cover: When not in use, a microscope should be covered to protect it from dust, hair, and any other possible sources of dirt. A dust cover should never be placed over a microscope while the illuminator is still on.

Lens tissue: Lint-free lens tissues are delicate wipes that will not scratch the surface of the oculars or objective. Always ensure that you are using these types of tissues. Never substitute facial tissues or paper towels, as they are too abrasive.

Lens cleaner: Lens cleaning solution assists in removing fingerprints and smudges from lenses and objectives. Apply the lens cleaner to the lens tissue paper and clean/polish the surface.

Compressed-air duster: Using compressed air to rid the microscope of dust particles is far superior to using your own breath and blowing onto the microscope. Compressed air is clean and avoids possible contamination from moisture.

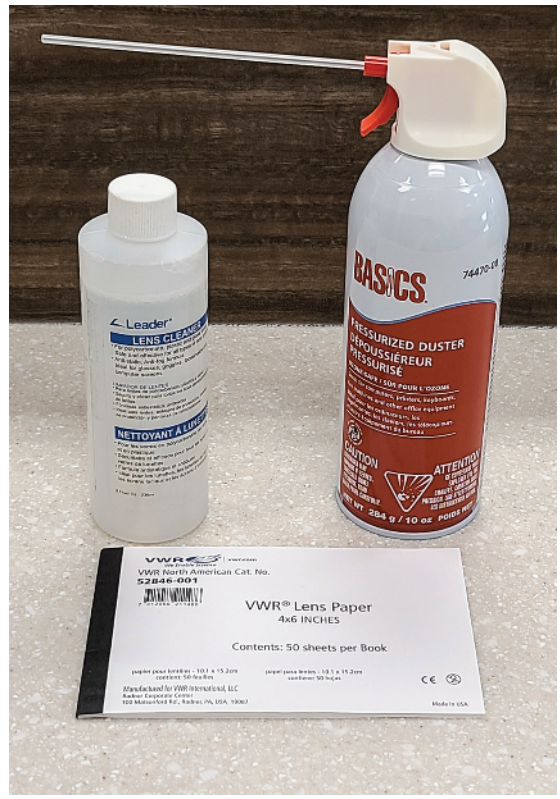

Figure 1.4 Examples of recommended cleaning supplies for the compound microscope.

Microscope cleanup procedure

When the use of the microscope is complete, following proper cleanup procedures will improve the quality of images that are viewed and extend the life of the microscope and its components.

(1) Remove the slide from the stage and dispose of it properly.
(2) Clean any oil residue or sample material that may have contaminated the stage surface.
(3) Lower the stage and move the smallest objective into place.
(4) Clean the objective lens and oculars after every use. The order in which they are cleaned is important. Cleaning the 100× objective first and then moving onto other parts will result in immersion oil being spread onto all other components. Using lens tissue and lens cleaner, begin with cleaning the oculars, then the 4× objective, the 10× objective, 40× objective, and finish with the 100× objective lens.

Maintenance tips

(1) Whenever the microscope is not in use, turn off the illuminator. This will greatly extend the life of the bulb, as well as keeping the temperature down during extended periods of laboratory work.
(2) When cleaning the microscope, use distilled water or lens cleaner. Avoid using other chemicals or solvents, as they may be corrosive to the rubber or lens mounts.
(3) After using immersion oil, clean off any residue immediately. Avoid rotating the 40× objective through immersion oil. If this should occur, immediately clean the 40× objective with lens cleaner before the oil has a chance to dry.
(4) Do not be afraid to use many sheets of lens tissue when cleaning. Use a fresh piece when moving to a different part of the microscope. This avoids tracking dirt/oil/residue to other areas of the microscope.
(5) Store the microscope safely with the stage lowered and the smallest objective in position (4× or 10×). This placement allows the greatest distance between the stage and the objective. If the microscope is bumped, the likelihood of an objective becoming damaged by the stage surface will be greatly minimized.

Centrifuge

Another key component of the veterinary laboratory is the centrifuge. Numerous centrifuge types exist for different purposes, such as those for microhematocrit, fecal, urine, and blood samples. It is not uncommon to use a multifunction centrifuge that can be set to spin at a speed appropriate for the biological sample, with specialized holding devices for each type of sample. The manufacturer's guide should be used for operation, maintenance, and cleaning instructions.

Microhematocrit centrifuge

This centrifuge is used exclusively for spinning down microhematocrit tubes (Figure 1.5). This process is used for determining a patient's packed cell volume (PCV) and can also provide a plasma sample for protein analysis.

Figure 1.5 Microhematocrit centrifuge.

Clinical centrifuge

Clinical centrifuges are available in two main types: variable-angle centrifuges and fixed-angle centrifuges (Figures 1.6 and 1.7).

The variable-angle centrifuge (also called a horizontal centrifuge) has swinging buckets that hold the specimen tubes. As the centrifugation begins, these buckets swing out horizontally, and the particles within the specimen are pushed to the base of the tube to form the sediment. Once the rotation stops, the buckets return to their upright position. This change of position from horizontal to vertical can result in a slight remixing of the sample. This effect should be taken into consideration when preparing a sample.

The fixed-angle centrifuge has buckets that are in a fixed position, typically about 50°. The specimen tubes are held in this position for the entire centrifugation process.

Refractometer

The purpose of a refractometer is to measure the refractive index of a solution. When a solution (e.g., urine) is measured, light passes through the sample and bends. The angle of this refraction is visualized as a shadow and correlates with the concentration of the solution. Veterinary-specific refractometers are now on the market, allowing minor differences between dog and cat urine specific gravity and total protein values to be discerned. Most are temperature compensated and are intended to be used between 60°F to 100°F. This should be taken into consideration when analyzing samples which have been stored in the refrigerator. They should be allowed to warm to room temperature prior to measuring to maintain accuracy.

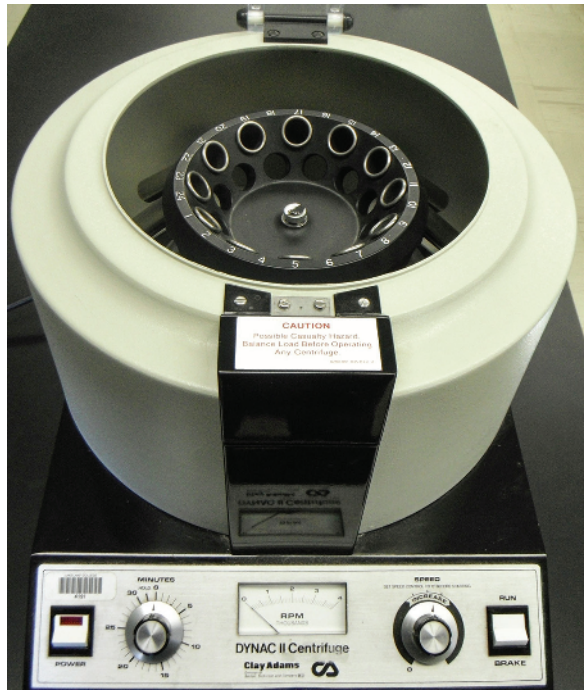

Figure 1.6 Fixed-angle centrifuge.

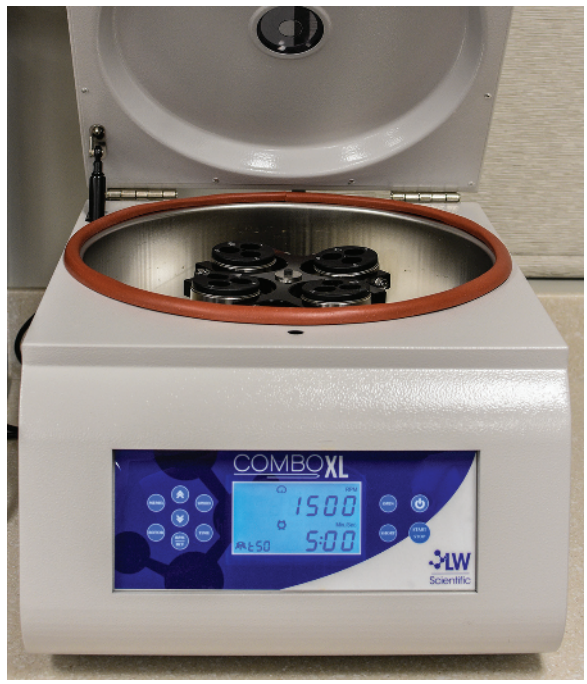

Figure 1.7 Variable-angle centrifuge.

The most common use of a refractometer in veterinary laboratories is to measure urine specific gravity and plasma total protein. Refractometers have built-in scales to measure both, and some brands of refractometers will also possess a refractive index scale. This scale, with the use of an appropriate conversion chart, can be used to measure the concentration of many other solutions (Figures 1.8 and 1.9).

Figure 1.8 Refractometer.

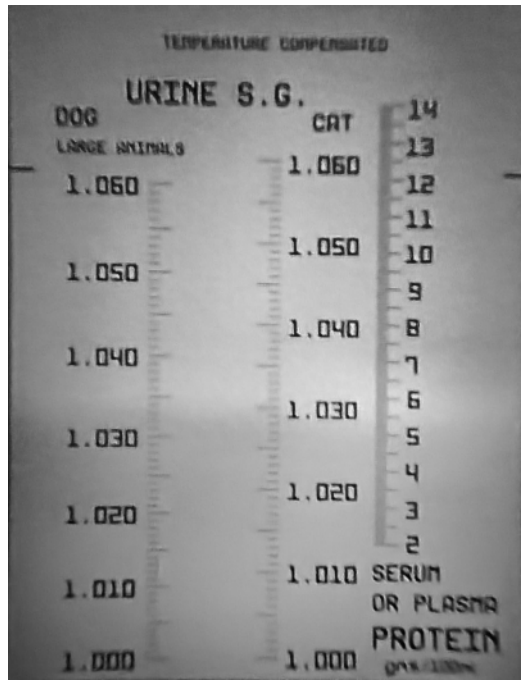

Figure 1.9 Refractometer scales.

Calibration

It is good practice to calibrate the refractometer on a regular basis (daily or weekly, depending on use). This is achieved by applying a large drop of distilled water on the prism and adjusting the blue/white line to read exactly 1.000 on the scale (see Chapter 4, Figure 4.4). Calibration should be done using distilled water at a temperature of 60°F to 100°F. The adjustment knob or screw is variously located on different refractometers; therefore, the manufacturer's guide will need to be consulted.

Incubator

An incubator provides the ability to artificially control the environmental temperature (and humidity, to some extent) for many microbiological procedures. Common in-clinic incubators have a temperature setting and require the placement of a container of water to maintain humidity (Figure 1.10). More expensive incubators can also control the level of humidity as well as the oxygen and carbon dioxide levels; however, these types are not normally seen in general veterinary practices.

In-house analyzers

Chemistry analyzers

There are a wide variety of chemistry analyzers available for veterinary use (Figure 1.11). Most use the principles of photometry to quantify analytes, such as enzymes, proteins, and other constituents in the blood. Electrochemical methods are used to analyze ionic compounds such as electrolytes. These two methods may require the use of two separate analyzers, or they may be combined into one.

Figure 1.10 Incubator.

Figure 1.11 IDEXX Catalyst One in-house chemistry analyzer. (Photo provided courtesy of IDEXX Laboratories, Inc.) *Source:* Courtesy of IDEXX Laboratories, Inc.

Another variation among analyzers is the way they facilitate the photometry testing procedure. A sample needs to be added to a substrate to initialize the test. Examples include slides, rotors, and cartridges (Figure 1.12).

Depending upon the analyzer or the analyte being tested, a serum or plasma sample is required. Some analyzers can process whole-blood samples as well. The type of anticoagulant recommended should be confirmed by reviewing the manufacturer recommendations.

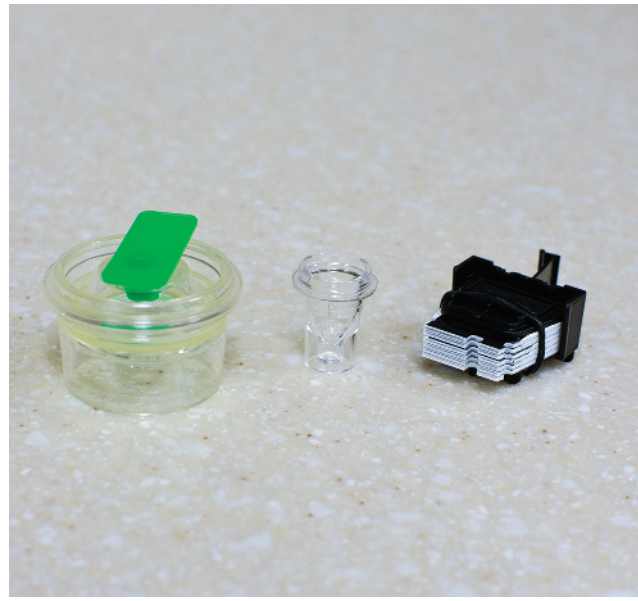

Figure 1.12 Common chemistry testing supplies. Supplies required will depend on the analyzer.

Regardless of the analyzer type chosen, it is important to maintain the equipment according to the manufacturer's recommendations. Regular maintenance and quality control monitoring are essential for ensuring the precision and accuracy of the analyzer. It may also be necessary for complying with the manufacturer's warranty.

Cell counters

As with chemistry analyzers, there are many types of cell counters to choose from (Figure 1.13). There are several different technologies used to quantify cell types, and each has its own advantages and disadvantages. Examples of the technologies used are impedance, laser-based technology, and optical fluorescence. An analyzer may use one or a combination of these technologies to detect and enumerate the cells present in the sample. Most commonly, these analyzers are used for hematology tests (complete blood counts); however, many types can also assess fluids such as peritoneal, thoracic, and synovial fluid samples.

While the analyzers can provide a large amount of information about the patient, it is recommended to follow up with a manual examination of the sample to confirm readings and review morphology of cells.

Coagulation analyzers

In-house coagulation testing (Figure 1.14) is available to screen for coagulation disorders and measure fibrinogen levels. Tests such as prothrombin time (PT), activated partial thromboplastin time (aPTT), and fibrinogen tests can be performed using fresh or citrated whole blood.

As with all analyzers, it is recommended to follow appropriate maintenance and quality control procedures to ensure the accuracy and precision of the equipment.

Figure 1.13 IDEXX ProCyte One in-house hematology analyzer. (Photo provided courtesy of IDEXX Laboratories, Inc.) *Source:* Courtesy of IDEXX Laboratories, Inc.

Figure 1.14 IDEXX Coag Dx in-house coagulation analyzer. (Photo provided courtesy of IDEXX Laboratories, Inc.) *Source:* Courtesy of IDEXX Laboratories, Inc.

Quality control and quality assurance

Quality control (QC) consists of actions taken which monitor the performance of the equipment. Internal QC are processes which are "built into" the equipment and are performed automatically (e.g., the quality control on an ELISA [enzyme-linked immunosorbent assay] test kit). External QC processes are not built into the piece of equipment and are required to be performed by veterinary personnel, typically the veterinary technician (e.g., controls used to assess the accuracy of a blood analyzer by comparing generated results to known values). External QC results must be assessed by trained personnel to determine if they are acceptable, and if not, troubleshooting should follow to determine the reason for the failure.

Quality assurance (QA) is defined as procedures which are performed with the goal of minimizing errors and achieving reliable results of diagnostic quality. Examples of QA procedures include proper patient handling, proper sample handling, and following manufacturer's recommendation regarding equipment usage and maintenance. These QA procedures are best to be documented into standard operating procedures (SOPs) to ensure that all veterinary personnel are consistent in their methods. SOPs should be reviewed on a regular basis and updated as needed.

Accuracy, precision, and reliability

"Accuracy," "precision," and "liability" are common terms encountered when assessing a quality control program. Accuracy refers to how close the reading is to the correct value. Precision refers to the reproducibility of a result, and reliability incorporates both the accuracy and precision of a test procedure. The images below (Figures 1.15 to 1.18) are examples of how reliability of a test procedure can be described.

Sources of error

If a quality control procedure fails, troubleshooting should be done to determine the cause. A number of factors can influence the reliability of a test method, many of which are human error; however, the possibility of equipment malfunction should not be overlooked.

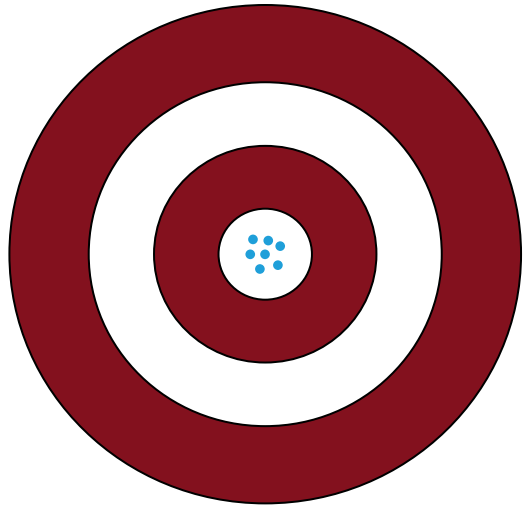

Figure 1.15 High accuracy, high precision.

Figure 1.16 Low accuracy, high precision.

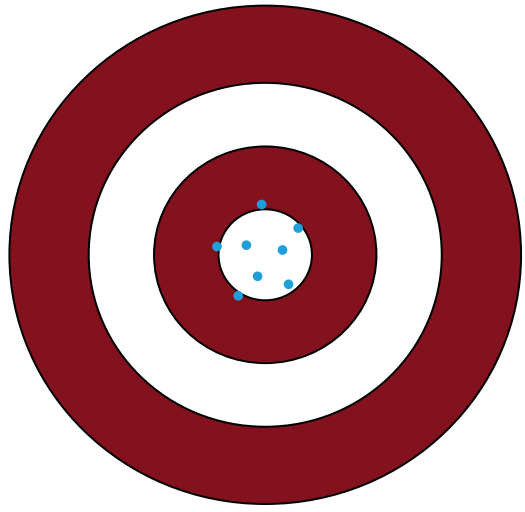

Figure 1.17 High accuracy, low precision.

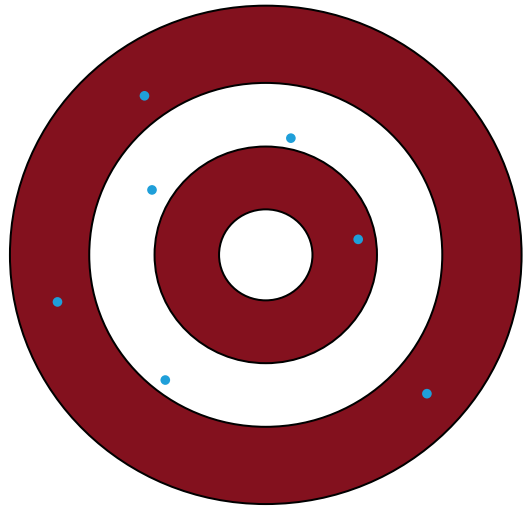

Figure 1.18 Low accuracy, low precision.

Preanalytic variables

Preanalytic variables include any variations from the standard which occur prior to testing. Biologic variables are related to the patient; examples include breed and age. These variables cannot be controlled; however, if comprehensive patient information is included with the test results, the veterinarian can more accurately interpret the results. Other biologic variables can be controlled and should be minimized whenever possible. An example would be having a patient fast prior to sampling. Nonbiologic variables are not related to the patient and could include mislabeling of a sample or sample collection and handling errors. Efforts should be made to avoid these by following clinic SOPs and educating the veterinary personnel on the importance of these methods.

Analytic variables

Analytic variables are related to the instrument measurement itself. These errors can arise from inadequate maintenance of equipment or using outdated reagents. Such errors tend to reveal themselves gradually, and a proper QC program should highlight any trends and identify issues early.

Postanalytic variables

Postanalytic variables occur following sample processing and include the incorrect reporting or recording of results.