Veterinary Technician's Handbook of Laboratory Procedures

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 expected 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. The microscope is used to review fecal, urine, blood, and cytology samples on a daily basis (see Figure 1.1). 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 (Figure 1.1)

(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 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 a period of 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.

(G) **Objective lenses:** The objective lens is the lens closest to the object being viewed, and its function is to magnify it. Objective lenses are available in many powers, but

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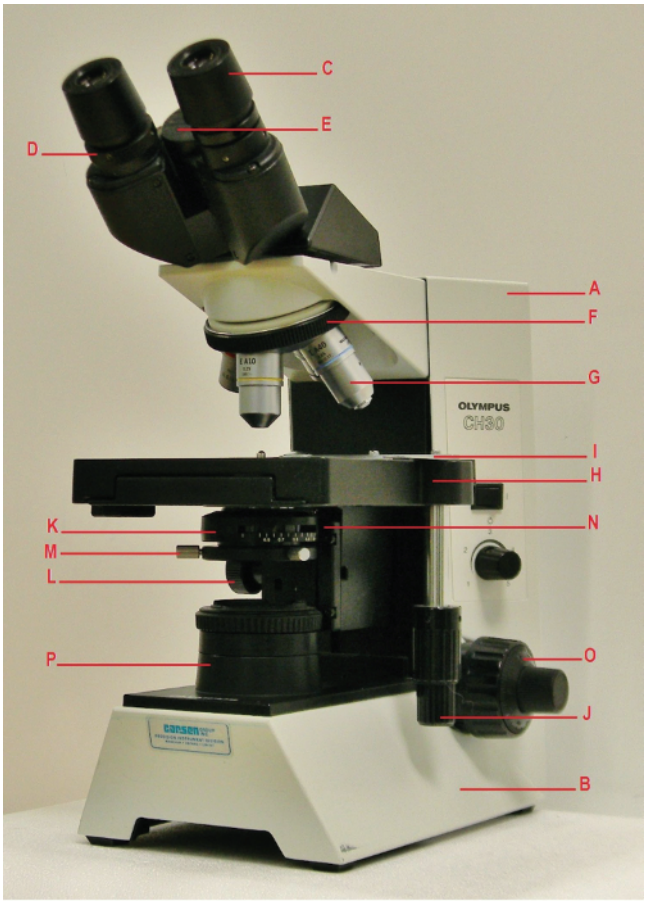

Figure 1.1 Compound microscope.

4×, 10×, 40×, and 100× are standard. 4× objective is used mainly for scanning. 10× objective is considered "low power," 40× is "high power" and 100× objective is referred to as "oil immersion."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. For example:

$$100\times \text{objective lens with } 10\times \text{oculars} = 1000\times \text{total magnification.}$$

(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 crews 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 adjustment that would require fine tuning.
(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 in Kohler, specimens will appear clearer, and in more detail.

Process of setting Kohler

Materials required

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

Kohler illumination

(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 becomes sharp (see Figure 1.2 and Figure 1.3).
(5) Open the iris diaphragm until the entire field is illuminated.

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.

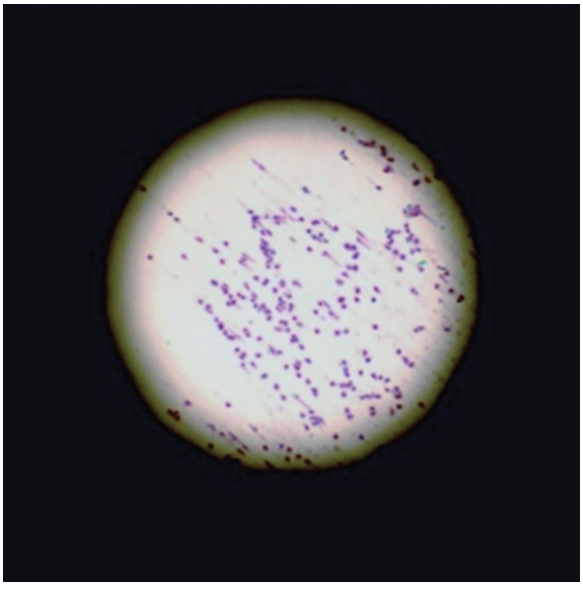

Figure 1.2 Note the blurry edges of the unfocused light.

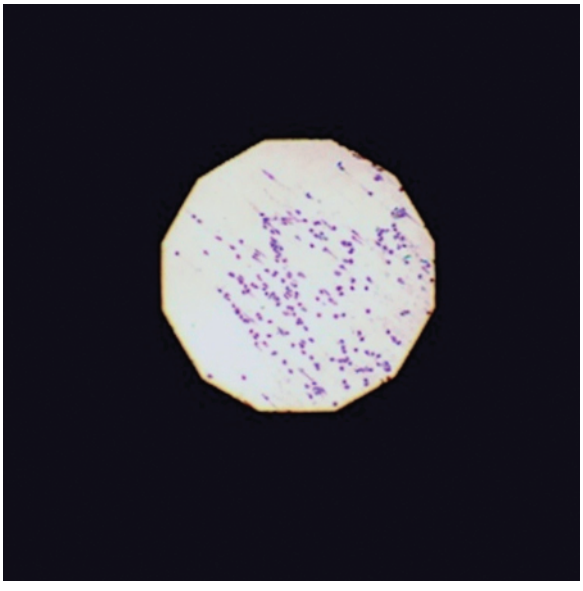

Figure 1.3 Adjusting the condenser height sharpens the edges of the "ball of light."

Microscope care and maintenance

Routine care and proper maintenance of 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. 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. It is important to note that 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 would not scratch the surface of the oculars or objective. Always ensure that you are using these types of tissues. Never substitute facial tissue or paper towel, as they are too abrasive.

Lens cleaner: Lens cleaning solution assists in removing fingerprints and smudges on 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 of saliva particles.

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 keep 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 (or a clean area of the same 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 for 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.

Troubleshooting

"I can focus my slide under 10×, but not under 40×."

A common reason for this is that the slide is upside down. Double check which side the smear is on (may not be the same side as the label!) and try focusing again.

Another cause could be dried immersion oil on the 40× objective that is obstructing your view. When switching from oil immersion (100×) to 40×, there is a good chance that the tip of the 40× objective could be dragged through some immersion oil. If it is not immediately cleaned off, it will dry, producing a thick haze.

To fix: Use lens paper and lens cleaner to clean the end of the 40× objective. This may need to be repeated several times depending on how thick the dried oil is. After cleaning, use a dry piece of lens paper to polish the objective.

To avoid the problem: Clean up oil immediately after use. Clean the end of the 100× objective and any heavy oil present on the slide before moving back down to 40× objective.

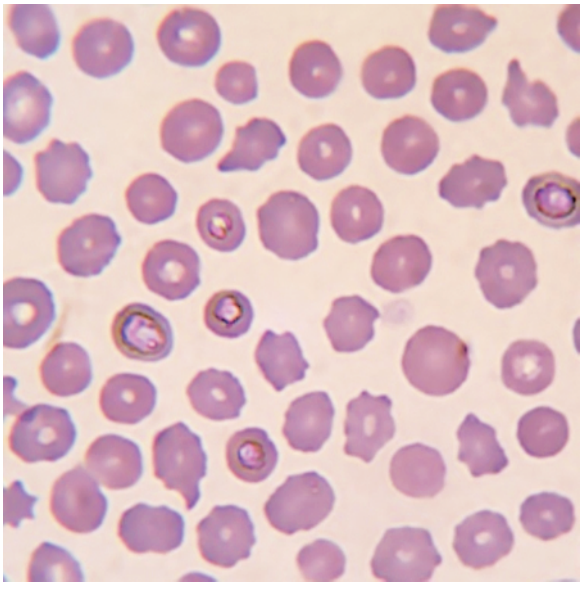

Figure 1.4 Water artifact seen on the surface of the red blood cells (1000× magnification).

"In hematology, when I focus under 40×, my red blood cells appear shiny."

This is most likely due to water artifact during the staining and drying process (see Figure 1.4). To make visualization of the cells easier, add a small drop of immersion oil to your slide. *Gently* spread the drop of oil over the area you will be examining. Wipe of excess oil using the side of your finger. *Be very gentle when doing this, and use a clean finger each time you wipe. Wiping too hard or rough will cause your smear to rub off.* This technique will leave a very thin layer of oil on your smear. The film is thin enough that you can use the 40× objective without running the risk of the lens becoming contaminated with oil.

Try focusing under 40× again, and the shininess should have been resolved.

"There's no light coming from the illuminator."

The first assumption is always that the bulb is burnt out, but it is a good idea to check a couple of other possibilities as well.

If the iris diaphragm is closed and the brightness of the illuminator is at its lowest, the light may be so small that it appears as if there is no light present.

Check to make sure the cord is fully plugged into the back of the microscope. This plug can become dislodged slightly during transport and microscope set up.

If your microscope is the type that uses fuses, it may be the fuse—not the bulb—that needs replacing.

When the microscope is not in use, be sure to turn it off. This will help prolong the life of the bulb.

Clean up

When the use of the microscope is complete, following proper clean up 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.

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 multiuse 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 (see Figure 1.5). This process is used for determining a patient's PCV (packed cell volume) or can also provide a plasma sample for protein analysis.

Clinical centrifuge

Clinical centrifuges are available in two main types: a variable angle centrifuge or a fixed angle centrifuge.

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 (see Figure 1.6). 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° (see Figure 1.7). The specimen tubes are held in this position for the entire centrifugation process.

Figure 1.5 Microhematocrit centrifuge. This centrifuge is used for spinning microhematocrit tubes only. It is preset to 10,000 rpm.

Figure 1.6 Variable angle centrifuge. Buckets swing out horizontally during the centrifugation process.

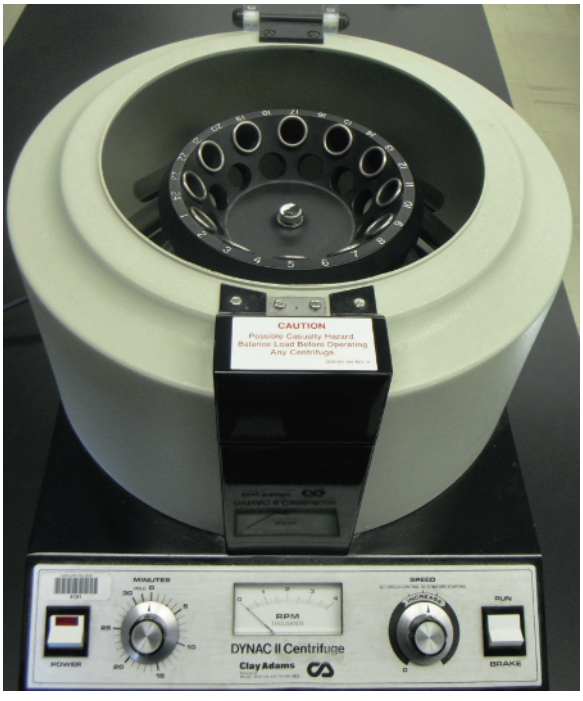

Figure 1.7 Fixed angle centrifuge. Buckets are at a fixed angle (typically about 50°) and do not move during the centrifugation process.

StatSpin

Another option is the StatSpin (see Figure 1.8). This centrifuge is a fixed angle centrifuge designed for small samples. It has a faster spinning speed, and as a result, a shorter processing time. These qualities could be beneficial in a small animal or exotic veterinary practice.

Refractometer

The purpose of a refractometer is to measure the refractive index of a solution. When measuring a solution (i.e., urine), light passes through the sample and bends. The angle of this refraction is visualized as a shadow and correlates to the concentration of the solution. Veterinary specific refractometers are now on the market allowing for minor differences between dog and cat urine specific gravity and total protein values (see Figure 1.9).

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 of these, 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.

***Tips*: Cover the entire prism with the liquid (urine or plasma) being examined. Reading urine and plasma at room temperature will provide the most accurate results.**

Figure 1.8 StatSpin. Another type of fixed angle centrifuge.

Calibration

It is good practice to calibrate the refractometer at the beginning of each day. 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. The adjustment knob or screw is located on various places of a refractometer; therefore, the manufacturer's guide will need to be consulted.

Figure 1.9 Refractometer.

Chemistry analyzers

There are a wide variety of chemistry analyzers available for veterinary use. 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.

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, or cartridges (see Figure 1.10).

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

Regardless of the analyzer type chosen, it is important to maintain the equipment according to the manufacturer's recommendations. With such a wide variety of analyzers available, this book cannot describe the operation, maintenance, and cleaning of each brand. The manufacturer's guide should be consulted for this information. Regular maintenance is essential for ensuring the precision and accuracy of the analyzer. It may also be necessary for complying with the manufacturer's warranty.

Hematology analyzers

As with chemistry analyzers, there are many types of hematology analyzers to choose from. There are several different types of technology used to quantify cell types, and

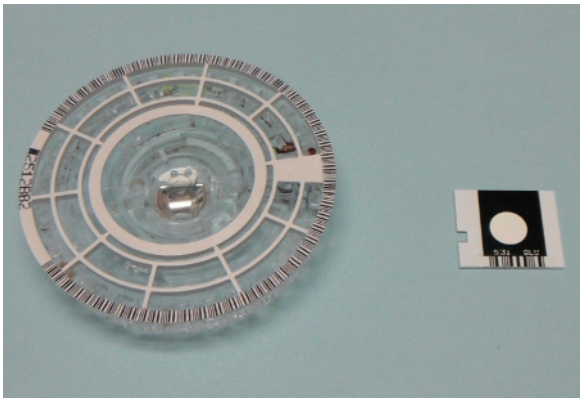

Figure 1.10 Testing supplies for various types of chemistry analyzers.

each has its own advantages and disadvantages. Examples of the technologies used are impedance, laser-based, and optical fluorescence. An analyzer may use one, or a combination of these technologies to detect and enumerate the cells present in the sample.

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

Coagulation analyzers

In-house coagulation testing is available to screen for coagulation disorders and measure fibrinogenlevels. Tests such as prothrombin time (PT), activated partial thromboplastin time (aPTT), and fibrinogen 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.

Quality control of all laboratory equipment

Because diagnoses and treatment plans are made based on laboratory findings, it is imperative that the equipment utilized in the lab be in excellent working order, serviced at regular intervals, calibrated and cleaned as recommended by the manufacturer, and used properly. In addition to properly functioning equipment, there are things the technician can do to improve the accuracy of their test results:

(1) Follow manufacturer directions precisely.
(2) Become familiar with normal and abnormal findings.
(3) Log all activity of equipment, including daily, weekly, and monthly servicing.

(4) Standardize test methods within the hospital.
   (a) Perform urinalysis, fecal analysis, packed cell volume and total protein analysis, and cytology in the same manner no matter who is conducting the test.
(5) Save enough sample to perform tests more than once to verify accuracy of findings.

Remember, all laboratory equipment and its results are only as reliable as the human operating the equipment!