1 Sample Handling and Laboratory Standardization—Developing Standard Operating Procedures

Over the last decade, the availability of hematology and clinical chemistry instrumentation for in-clinic use has increased exponentially. There is a wide choice of instruments being marketed for use in veterinary practices. While the convenience of rapid result turnaround is touted as a primary reason for purchase and use of in-clinic instrumentation, the full impact of their use on technical personnel time or training needs and the full cost of appropriate management of the in-clinic laboratory are usually not well addressed during the decision process. When a veterinary practice decides to provide in-clinic clinical pathology service, it must make the commitment to ensure that the data produced by its in-clinic laboratory is of the same quality as that obtained by reference laboratories. The management of the practice must understand the need for trained personnel, standard operating procedures, and appropriate quality control and quality assurance programs.

The In-House Clinical Laboratory

Once a veterinary practice decides to purchase in-clinic clinical pathology instrumentation, it is the veterinary technician who is given the responsibility to obtain accurate data from the diagnostic samples for clinical management of the patient. This chapter is an introduction to laboratory management. The first aspect of integrating an in-clinic laboratory into the general practice is to identify a location for the laboratory equipment with sufficient workspace that includes space for sample processing, sample/reagent storage, and instrumentation. The area should be convenient to the treatment/surgery areas and examination rooms but out of congested traffic areas. In addition to hematology and clinical chemistry instrumentation, a free-arm centrifuge for spinning down serum and urine samples, a microhematocrit centrifuge, a

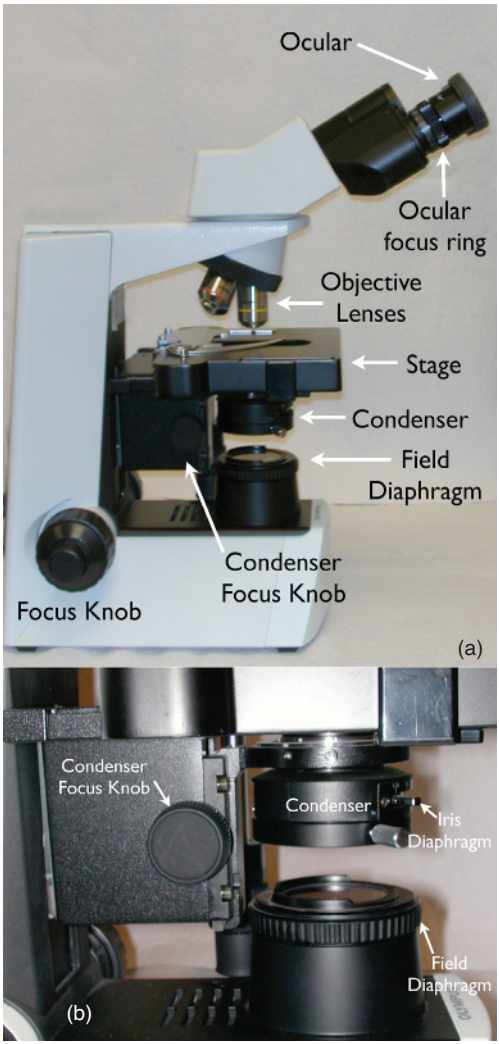

Figure 1.1. Image of a high-quality microscope as part of the in-hospital medical lab equipment.

quality microscope, and a refrigerator with a non–frost-free freezer compartment are needed.

The need for a quality microscope cannot be emphasized enough. The ability to critically examine hematology and cytology specimens depends upon the clear optics of the microscope. Figure 1.1 identifies the components of a compound bright field microscope. The objective lenses of the microscope should be the highest quality affordable. The standard lenses on most light microscopes are 10×, 40×, and 100× oil emersion. The addition of a 20× or a 50× oil emersion lens can facilitate the rapid examination of cytology and hematology samples. Each microscope manufacturer can provide options for magnification and optical correction and should be able to assist in the choice of objectives for the type of clinical use in each practice. Objective lenses differ considerably in cost. It is worth the additional cost for higher-quality lenses that provide a large, flat viewing field. The higher-quality objective will significantly reduce eyestrain experience by the microscopist and improve diagnostic accuracy by providing the best quality image for viewing.

One important note is that the 40× high dry objective requires a coverslipped slide. The optics of this objective are optimized for viewing histopathology slides that are always coverslipped. A simple way to use the 40× objective to view hematology and cytology slides is to use a drop of immersion oil as a coverslip mounting medium. Place a drop of oil on the microscope slide and place a coverslip in top of the oil. This will allow sharp focus of the slide with the 40× objective. It is imperative that the high dry objective does not get immersed in oil. If this inadvertently happens, immediate cleaning with a quality lens cleaner (not xylene) and lens paper is necessary. A good habit is to always rotate the microscope objectives in one direction, from lowest power to highest power. This will keep the microscopist from dragging the 40× objective through oil.

The microscope manufacturer and the individual who is chosen to clean and maintain the scope are great resources for choosing good immersion oil. Immersion oils have been standardized over the years and the most frequently asked question is concerning the viscosity of the oil. High-viscosity oils are often preferred by

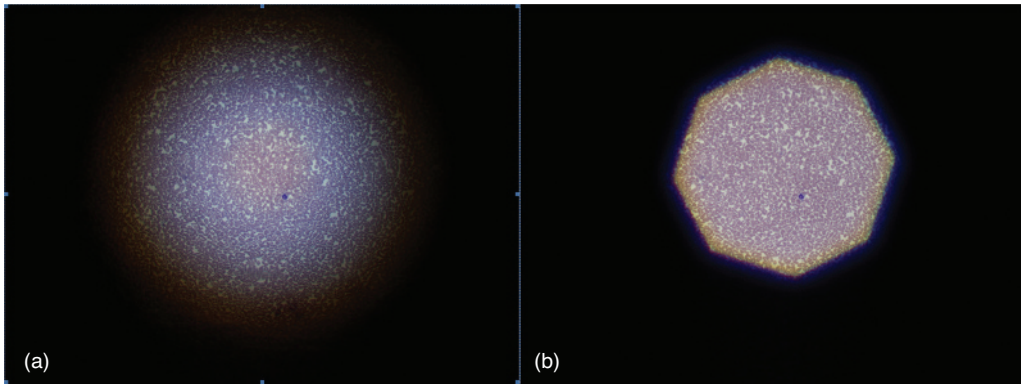

Figure 1.2. **(a)** An out-of-focus field diaphragm with fuzzy edges. **(b)** An in-focus field diaphragm with sharp edges.

microscopists because less oil is required to fill the gap between the slide and the objective lens (high-viscosity oil does not spread out as much as low-viscosity oil) and, when viewing multiple slides, less oil is needed on subsequent slides because the oil clings to the objective when the previous slide is removed. To prevent oil dropping on the next slide before it is needed, the microscopist should develop a habit of switching to the 10× objective before removing one slide and placing a new slide on the stage.

As with all laboratory equipment, maintenance is necessary to keep the microscope working appropriately. A yearly maintenance and cleaning schedule will result in a long and useful life for a good microscope. Microscopy training is essential for all personnel using the microscope. Tasks that should be understood by all microscopists include adjusting the microscope condenser to provide the best illumination (**Köhler illumination**), correct lens cleaning techniques, and bulb replacement. Köhler illumination is the process of adjusting the condenser to produce the best focus of the illumination source. The process is as follows:

1. Place a microscope slide on the stage and focus the image on the slide to 10×.
2. Close the field diaphragm completely.
3. Carefully move the condenser up or down to bring the edges of the field diaphragm into sharp focus (Figure 1.2).
4. Open the field diaphragm to allow complete illumination of the field.

Most good-quality microscopes sold today have an adjustable condenser and are parfocal. **Parfocal** means that if the image is in focus at 10×, there should be little need to focus at the higher magnification objectives. One way to optimize the parfocal lenses is to focus a microscope slide at the higher objective (preferably at 40×) and switch to the 10× objective. If the image is out of focus, use the focus rings of the eyepieces to bring the image back into focus. Once this is done, only fine focus should be needed as the microscopist moves from one objective to the next.

An excellent resource for microscopy tips is the individual that is contracted to maintain the microscope. In addition, there are excellent Internet resources for microscopy. Optimizing microscopy skills will allow veterinary technicians to fully utilize their microscopes and increase the efficiency of viewing hematology, urinalysis, and cytology slides.

A working understanding of the available clinical instrumentation and the procedures necessary to provide accurate

clinical laboratory values is necessary—in fact, required—to best serve the interests of the patient. Veterinarians will make better decisions based on their physical exam, history, and diagnostic skills without any clinical pathology data than they will if they are given erroneous data. No clinical pathology data is far better than trying to interpret erroneous data. The following are the primary responsibilities of the veterinary technician placed in charge of laboratory equipment:

1. Institute and monitor a quality control system.
2. Maintain maintenance protocols.
3. Develop standard operating procedures for all tests performed in the laboratory.
4. Provide consistent training for all individuals who will be using the equipment in the laboratory.

Currently, there are several hematology instruments available for the in-clinic laboratory. It is often the responsibility of the veterinary technician to review the instruments available prior to the investment of a large amount of money. An understanding of the types of instruments available and the differences in methods used by each instrument in obtaining data will greatly increase the potential for making an appropriate choice.

Hematology Instruments

There are basically three types of instruments available for the veterinary clinic: centrifugal, impedance, and laser-based. The centrifugal system uses a large-bore capillary tube with a special float that expands the buffy coat when the tube is spun. The tube is coated with an eosin stain that will stain DNA and RNA. The differential uptake of the stain by white blood cells and erythrocytes allows the instrument to separate the populations in the expanded buffy coat. In the erythrocyte population, immature erythrocytes (**reticulocytes**) will take up the stain for RNA and, therefore, an estimate of reticulocyte number is possible. Once the sample is spun, white blood cell and platelet counts are estimated based on the height of each portion of the buffy coat that corresponds to each cell type and the average size of the cell type. The packed cell volume (PCV) is determined by measuring the height of the red cell column as a percentage of the total blood column in the tube.

Studies evaluating the accuracy of this methodology have shown good correlation with standard impedance instruments when samples from healthy animals are analyzed. When samples from ill animals are compared, the expanded buffy coat has poor correlation with other methods and with blood film analysis. Many pathological changes in blood interfere with an accurate evaluation of blood parameters using this methodology because alterations in cells result in abnormal layering during centrifugation.

The impedance cell counters use the principle that cells are poor conductors of an electric current. In an impedance counter, individual cells are counted as they flow through an electric field. In addition, the size of cells can be measured because the magnitude of the change in current is proportional to the cell size. This methodology has been the mainstay of automated hematology for over 50 years. It is precise and accurate in counting leukocytes, erythrocytes, and platelets and can produce a reliable but limited automated differential.

Laser-based flow cytometry instruments depend on a cell's ability to scatter light as it passes through a sheath of diluent. The degree and direction of light scatter from the laser indicates the size and "complexity" of the cell. Based on these attributes, the laser-based instrument can provide up to a 5-part automated differential. The inherent variability in light stability of

individual lasers can affect the precision of the instrument.

Regardless of the methodology, blood film review is an essential part of the use of hematology instrumentation. It is an excellent internal control. When comparing what is seen on the blood film to what the instrument measures, well-trained veterinary technicians can and should trust their eyes over the instrument.

Clinical Chemistry Equipment

The currently available in-clinic chemistry instruments are primarily closed systems that utilize either "wet" or "dry" chemistry methods. The wet chemistry systems are based on traditional spectrophotometric methods with individual tests or test profiles contained in a cartridge with separate cells containing all the reagents needed for the individual test reaction. The serum (or, in some cases, plasma) is introduced into the cartridge and parsed to each cell. There is little possibility for user error other than storage of reagents, sample handling, and instrument maintenance. There are in-clinic wet chemistry units that are smaller versions of the large clinical chemistry instruments that require reconstitution of individual reagents for each test. In these instruments, the reagents and serum/plasma sample are pipetted into a reaction cell. These instruments require significant training to maintain adequate performance because there is more possibility for user (technician) error in managing the reagents, instrument, and sampling.

Dry chemistry instruments use the principle of reflected light rather than transmitted light (as used for spectrophotometric methods). This method is less affected by both hemolysis and lipemia than spectrophotometric methods. Dry chemistry instruments use slides or reagent "sticks" that contain a pad of dried reagent onto which the serum/plasma sample is placed. Once the appropriate reaction time has passed, the amount of a specific type of reflected light is measured.

Considerations in Purchasing an Instrument

In most cases, the purchase of a clinical hematology or chemistry instrument is a major decision. There are several aspects to consider as this decision is made. One of the most important questions is how many samples will be run on the instrument each day. In most cases, greater than 5 CBC or clinical chemistry panels a day are necessary to warrant in-house instrumentation. The more often the instrument is used the more familiar the staff will be with the procedures needed to provide accurate data. Other considerations include the following:

1. Cost analysis for tests performed
    a. Cost of reagents (include 10% additional reagent costs for rerunning samples if needed)
    b. Cost of consumables (pipette tips, cuvettes)
    c. Cost of technician time
    d. Cost of overhead (electricity, building expenses)
    e. Cost of quality control product
2. Available workspace for the instrument
3. Technical skill of the individuals performing the tests
4. Sample size required (the smaller the better for many small animals)
5. Test performance time
6. Monitoring requirements for the instrument
    a. Quality control needs
    b. Maintenance time and cost
7. Technical support and cost of service contracts

Evaluation of Instrumentation

Prior to purchase, the clinical staff should be allowed to evaluate the precision and accuracy of an instrument. This is done by performing a series of evaluations, usually by serial testing of a control product or products. In most cases, 20 consecutive samplings of the control product are run and their **standard deviation** and **coefficient of variance** is determined.

To calculate a standard deviation and coefficient of variance:

1. Run a selected control product on the new instrument. Add all 20 sample values of the variable together and divide by 20. This gives you the average or mean of the data set.
2. Calculate the deviance of the data from the mean. This is done by subtracting the total mean from each individual number.
3. Square each of the individual deviations and add these squared differences.
4. Divide that sum by 19.
5. Calculate the square root of the result of the previous step. The result of this calculation is the standard deviation.
6. Calculate the **coefficient of variation** (**CV**) by dividing the standard deviation by the mean. It can be expressed either as a fraction or a percent.

For Example: In Chart 1.1, your hospital team measures the following 20 control samples and notes the following values with a mean of 2.95.

Step 1 Calculate the average of these numbers or the mean.
   a. Sum of all these numbers is 59
   b. Average of these numbers is $59/20 = 2.95$

Step 2 Calculate the deviance from each value by subtracting 2.95 from each value (see above).

Step 3 Square each of the individual deviations and add these squared differences. The sum of the squared differences is 5.21

Step 4 Divide the number you got from adding the squared differences together by 19. In this case:

$$5.21/19 = 0.274211$$

Step 5 Calculate the square root of the result of the previous step. The result of this calculation is the standard deviation. In this case:

$$0.274211^2 = 0.075191$$

Step 6 Calculate the coefficient of variance by dividing the standard deviation by the mean. In this case:

$$0.075191/2.95 = 0.0255 \text{ or } 2.55\%$$

A low coefficient of variance indicates good instrument precision (<5%). In addition, running 20 samples of the control product over a period of 10 to 20 days will measure the long-term stability of the instrument. Once the 20 samples are obtained over 20 days, the coefficient of variance should be evaluated again. This will allow the hospital team to determine whether the coefficient varies over time, affecting overall precision.

Once precision is determined, the results of a specific set of tests on 20 patients can be compared to a reference laboratory to determine the instrument's correlation to standard methods. It is very important to make sure that the reference laboratory to which the comparison samples are sent uses the same methodology as the in-clinic instrument. If the methods differ, the values may not be similar, especially the liver and pancreatic enzyme values. Together these two evaluation methods will help determine the accuracy of the instrument's readings. The importance of determining both

Chart 1.1

Sample #	Value	Step 2 Calculate the deviance from the data (sample value: 2.95).	Step 3 Square each of the differences.
1	3.2	0.25	0.0625
2	3.0	0.05	0.0025
3	2.8	−0.15	0.0225
4	2.4	−0.55	0.3025
5	2.6	−0.35	0.1225
6	2.4	−0.55	0.3025
7	2.9	−0.05	0.0025
8	3.6	0.65	0.4225
9	3.8	0.85	0.7225
10	2.5	−0.45	0.2025
11	3.1	0.15	0.0225
12	3.0	0.05	0.0025
13	3.7	0.75	0.5625
14	2.5	−0.45	0.2025
15	2.2	−0.75	0.5625
16	4.2	1.25	1.5625
17	2.8	−0.15	0.0225
18	2.7	−0.25	0.0625
19	2.8	−0.15	0.0225
20	2.8	−0.15	0.0225
Sum	59		5.1

precision and correlation is illustrated in Figure 1.3.

An instrument's precision can be evaluated as one of three types: precise but not correlated with a target method, imprecise but correlated with the target method, or both precise and well correlated to the target method (the optimal scenario). When purchasing an instrument, the instrument company's technical staff should assist in performing these important evaluations. It is in their best interest to prove the utility of their instruments in providing accurate and reliable data.

Quality Control—Minimizing Analytic Error

Once an instrument has demonstrated good precision and correlation, it is the ongoing responsibility of the veterinary technician

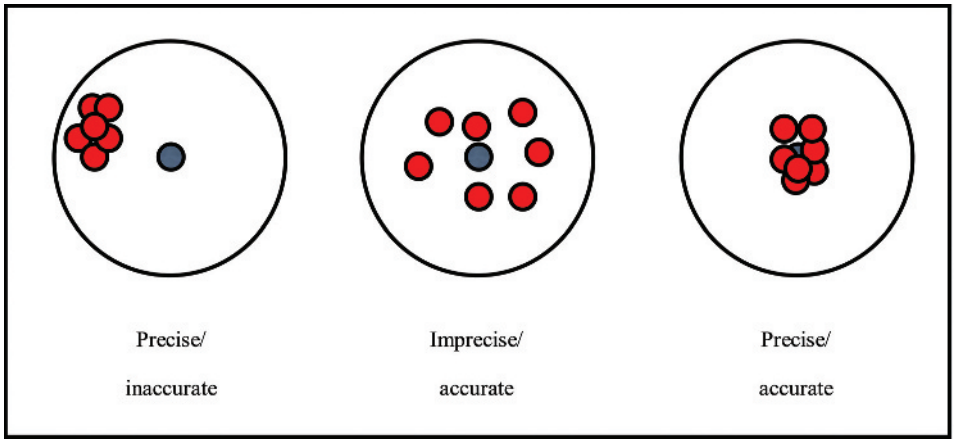

Figure 1.3. Target illustrations of test values (red) and target value (gray) relationships. Shown are an instrument with good precision but poor correlation with the target test value, an instrument that shows correlation but poor precision, and an instrument that is both precise and has good correlation with the target value.

who supervises the laboratory to insure the continuance of accurate data. This is done by close monitoring of a well-defined in-house quality control system. All reference laboratories must employ a quality control system to monitor the overall performance of the instruments in the laboratory. A quality control system is much more than simply running a control product through an instrument at a standard interval to check the performance of the individual instrument. Quality control systems include the following:

1. A set of standard operating procedures (SOPs) for all tests performed by the veterinary technician, for evaluation of daily quality control data and actions to determine the cause of failed control data, and for appropriate training of all personnel that use the instruments
2. Maintenance records for all instruments
3. Documentation of all samples run, including name of operator, date, patient identity, results, lot number of controls, and calibrators
4. Quality control log to track daily quality control values and all actions taken when the controls are not within the established control ranges
5. Guidelines for maintenance of the laboratory environment

Standard Operating Procedures

Standard operating procedures are often neglected in clinical practices. An SOP can be an active document if written well. The document should be written with the goal of providing anyone with the basic background in clinical pathology all the information needed to perform a specific test. The SOP is usually written in an outline form to include the following:

- Title (specific procedure)
- Scope (what is the goal of the procedure)
- Definitions pertinent to understanding the procedure
- Training level required of person performing the test
- Necessary equipment and reagents (and location)
- Procedure (step-by-step instructions)

- Quality control procedures specific to the test
- Any logs associated with procedure or instruments used in the procedure

Instrument procedure manuals can be used to supplement procedures performed by each instrument rather than duplicating the information.

Daily Quality Control

All clinical pathology results can be affected by three types of errors: preanalytical (sample handling), analytical (instrument errors), and postanalytical (reporting errors, interpretive errors). Sample handling and results reporting will be discussed later in this chapter.

All hematology instrumentation requires daily quality control measurements. The need for daily clinical chemistry quality control measurements vary between instruments. Some dry chemistry instrument manufacturers suggest that their instruments need only weekly, rather than daily, QC checks. However, if the instrument is in control limits on a given day but out of control limits 1 week later, there is no way to determine when the instrument shifted. Therefore, daily quality control is the best insurance that the clinical instruments are performing in a consistent manner.

The quality control product used should be similar to the sample being tested. For hematology systems, commercial quality control products consisting of stabilized whole blood are used. For chemistry instruments, the control product is usually a lyophilized serum–based product. In both cases, extensive analysis of each lot of control product is performed by the manufacturer to provide expected ranges for each parameter with stability over a set time once the product is opened for use. It is important to remember that control products are different than calibrators.

Calibrators are products used to set the electronics of the instrument and "tell" it what signal corresponds to a given value. In-clinic instruments are usually calibrated by the manufacturer with periodic calibrations as needed if the instrument fails to perform correctly. Control products are sample equivalents with the same physical properties as the patient's sample. The manufacturer of the clinical instrument should be able to assist in identifying appropriate commercial quality control products for use in their instrument. There are references in the literature that discuss the use of "pooled" serum samples that have been analyzed in the clinic as "control" products. Although this is a much less expensive method compared to buying commercial control products, the stability and validity of this type of quality control is questionable. **The commitment of the practice to providing quality clinical pathology data should include the use of good commercial control products with well-documented reference values and stability.**

Large reference laboratories use quality control "rules" such as the Westgard rules that employ multiple control values for each run, depending on the number of samples being run at any one time. In-house laboratories rely on quality control usually run at the beginning of each day to determine whether the instruments are working appropriately. Optimally, two to three control products are measured, one with values within the reference range for each parameter and the others with values above or below the reference range. The use of multiple control levels allows better use of rules in evaluation of quality control values. A simplified set of Westgard rules can be used when multiple control levels are evaluated with each run. When evaluating control values, it is not enough to just look at the individual value. Each new control value should be inspected in reference to the previous values. The following set of rules will assist in determining

whether the instrument measurement is not stable:

1. One control value is outside 2 SD (warning rule). Repeat control measurement. If the second control value is within control reference range and none of the following rules apply, the instrument is stable. Instrument measurement is not stable if one value is outside 2 SD (warning rule) and one of the following also applies.
2. Two consecutive values are outside 2 SD on the same side of the mean.
3. The range between two consecutive values is greater than 4 SD.
4. Four consecutive values exceed 1 SD on the same side of the mean.
5. Ten consecutive values are above or below the mean.

If, using the above rules, the instrument measurement is determined to be unstable, measures should be taken to determine the cause. In most cases, the steps involved in determining the cause of erroneous control data include the following:

1. Running a new set of controls from the same lot as the original control.
2. Running a new set of controls from a new lot.
3. Performing any maintenance procedures as needed and rerunning controls after the maintenance is performed. Most instruments have a troubleshooting guide that will assist in determining the cause of instrument error.
4. Contacting the instrument's technical service if the procedures above do not reestablish the appropriate performance of the instrument.

No patient sample can be run on the instrument until it is performing appropriately and providing control data values within the expected range.

Documenting quality control can be done by daily recording the results of each quality control test performed in a quality control log. Alternatively, a graphic representation of the control data can be used. This is usually done with a Levy-Jennings graph. Figure 1.4 illustrates the use of this type of graph to recognize two distinct changes

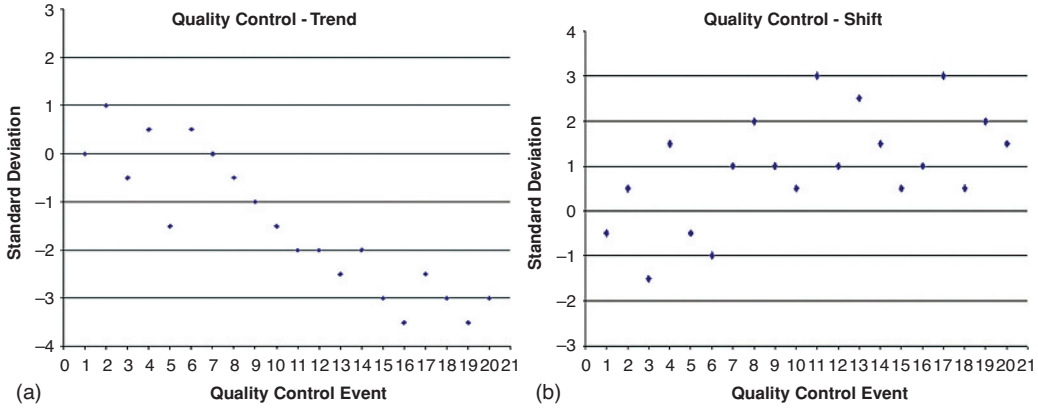

Figure 1.4. **(a)** Levy-Jennings plot showing a trend in control values. A trend suggests a gradual change in the instruments reading for a parameter in a control product. A common cause of this change is deterioration of the control product. Opening a new control product in the same lot or using a new lot may be needed to bring the values back toward the mean. **(b)** Levy-Jennings plot showing a shift in control values. A shift suggests an abrupt change in the instruments reading for a parameter in a control product. A common cause of this change is loss of calibration for that parameter. Recalibrating the instrument would be warranted.

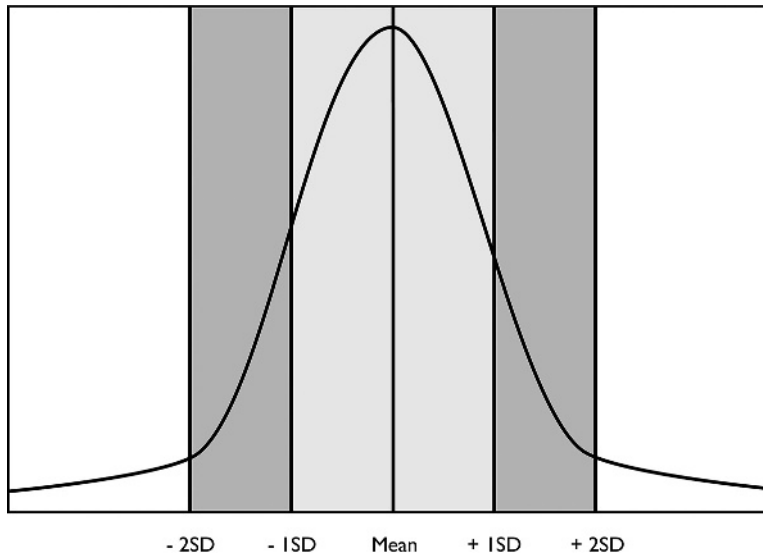

Figure 1.5. A distribution curve from a normal population. Shown are the mean (heavy black line) and limits for 1, 2, and 3 standard deviations from the mean of the population. Reference values for a control product are generated from this type of distribution curve by measuring the control product many times and determining the values representing the limits that include all values falling between two standard deviations from the mean. For example: If the mean for hemoglobin concentration for a control product 12.3 and the mean ±2 std is 12.9 and 11.7, respectively, then the expected value for hemoglobin in that control product should be between 11.7 to 12.9 gm/dL.

in control data, shifts, and trends. The advantage of the Levy-Jennings graph is the ability to visualize change over time that can warn the technician that intervention may be needed soon. As such, the technician will be able to take actions to keep the instruments on track before the controls indicate failure. Levy-Jennings graphs can be made using standard graph paper and the expected results and standard deviations provided with the control products used. A chart for each analyte or parameter is needed.

The expected range for a control product is determined by the manufacturer by repeated measurement of the control with calculation of the mean and standard deviation. The reference range is usually the mean value plus or minus two standard deviations (Figure 1.5). Setting the range to include all values within these limits will include 95% of the results obtained from that control product. This means that 5% of the time, when measuring this control product, the results will be either above or below the expected range. As a result, measurements outside the range should be expected periodically.

Reference Ranges

Large laboratories usually develop their own reference ranges for each specific test using a large number of samples from healthy animals of each species for which they offer tests. This is a costly, time-consuming procedure that is essential for appropriate interpretation of laboratory data. For most small in-clinic laboratories, this process is not realistic. In most cases, the instruments in the clinic laboratory use standardized reagents and come with

well-established reference ranges for all analytes tested.

However, it is still appropriate for the clinician and/or technician to review the veterinary literature for published studies that support the instrument manufacturer's claim of validation for its methods using veterinary samples. This independent validation for an instrument may point out individual parameters that are not as reliable as others measured by the instrument. For example, most in-clinic hematology instruments provide reliable erythrocyte measurements but vary significantly in the reliability of their leukocyte differential, especially when counting monocytes and eosinophils. A clinic can use wellness exams and presurgical blood work from patients undergoing elective surgeries, such as spays and neuters, to collect data over time to compare to the reference ranges provided by the instrument manufacturer. Forty to sixty samples per species can be used to generate reference ranges for the population of patients seen at a specific clinic.

Sample Handling—Minimizing Preanalytic Error

The first step in collecting any sample for clinical evaluation is adequate labeling of all containers and slides with the patient's name and the date. This is an extremely important step in sample handling that is often neglected in veterinary medicine. Close attention to sample labeling will ensure that the correct results are reported for each patient. Many clinic management software programs include label-making programs for the in-house pharmacy that can easily be used for clinical samples. The use of glass slides with a frosted end will allow labeling with a pencil. Placing labels on glass slides prior to staining can result in loss of the information during the staining procedure.

The adage "garbage in, garbage out" is very appropriate in the clinical pathology laboratory. Samples for evaluation will provide useful information only when obtained and processed correctly. Blood samples should be collected using the needle with the largest bore available to minimize the possibility of hemolysis, especially in ill animals that may have fragile erythrocytes.

The age-old controversy over the use of syringes versus vacutainer systems is not expected to end any time soon. The best compromise is to try both and use the method that is most comfortable for each individual and provides the best sample. Figure 1.6 illustrates venipuncture using a syringe.

Butterfly catheters with a connector for vacutainer tubes have nicely addressed the problem of vein collapse because of excess vacuum in patients with compromised blood pressure (Figure 1.7) and of trauma to the vein if the animal struggles. The primary advantage to using the vacutainer system is the standard volume that is drawn into the collection tube. This is extremely important when drawing blood for coagulation studies that require a precise ratio of coagulant to blood.

Whole blood for a complete blood count (CBC) must be collected in an anticoagulant. EDTA is the most common anticoagulant used for this purpose. It is essential that the blood be promptly mixed to prevent the formation of clots that will interfere with cell counting. Heparin can be used in certain cases, but it interferes with staining of the blood cells for blood film analysis. The ratio of blood to anticoagulant is important, as mentioned above. If too little blood is drawn into the collection tube, the anticoagulant, if in liquid form, will cause dilution of the blood resulting in artifactual low values.

In addition, erythrocytes will shrink due to the high osmolality (solute concentration) of the anticoagulant fluid. This can result in artifactual changes in red cell shape when the blood film is reviewed, in an

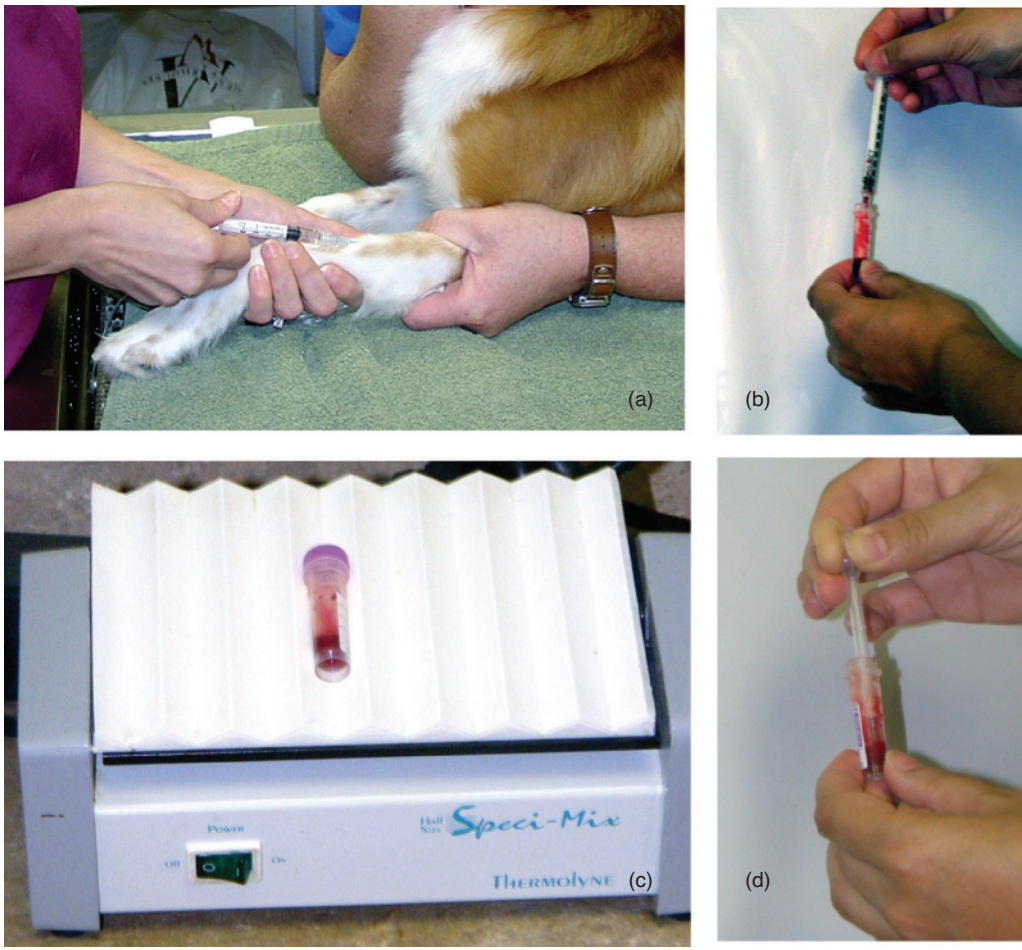

Figure 1.6. The steps for venipuncture using a syringe method. **(a)** The patient's forearm is held off and the technical team member draws blood from the cephalic artery. The needle should be at least 22 gauge or larger to prevent hemolysis. **(b)** Once the blood is drawn, it is placed into the EDTA blood tube; care should be taken to remove the top of the tube and expel the blood directly into the tube. This helps to reduce hemolysis. The EDTA tube should be inverted 7–8 times so that the blood and EDTA can properly mix. **(c)** If possible, place the sample on a sample rocker to allow the blood samples to continue to mix properly with the EDTA. **(d)** Finally, before making the blood smear, a small section of the sample should be examined with a pipette or wooden applicator sticks to make sure there is no obvious clot formation. If clotting is observed, this sample should be disposed of and a new sample obtained.

artifactual decrease in the packed cell volume (PCV), and in discordance between the PCV and the hematocrit (Hct) value in the automated CBC. In this case, the instrument Hct will be the more accurate value because the erythrocytes will "rehydrate" when the instrument performs the necessary dilution of the blood sample for counting.

If too much blood is placed into the collection tube, clots will form. The clots in this case may be too small to visualize but can affect the results and may cause

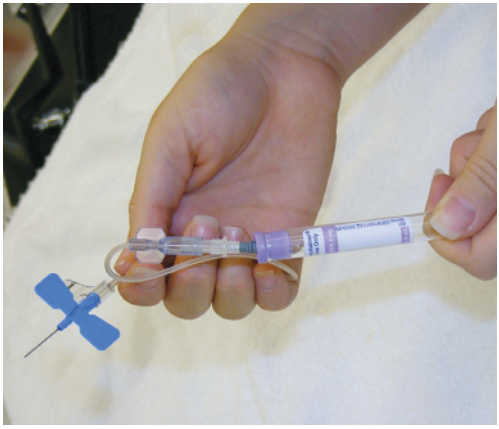

Figure 1.7. The use of a butterfly catheter with a vacutainer adapter often makes the collection of blood easier than using a needle and syringe. After the catheter is placed it can be secured to the patient, diminishing the chances of damaging the vessel if the patient struggles. An additional advantage is that this method allows the collection of a small amount of blood for a quick PCV, total protein, and blood film while clearing the catheter and tubing of blood that may contain procoagulant factors that would promote clot formation in the EDTA tube.

obstruction of the tubing within the instrument causing instrument failure. Gross inspection of the blood for clots is essential prior to introducing a sample into an instrument. In addition to checking for clots, the presence of agglutination should be noted if present. Agglutination will artifactually alter most erythrocyte indices reported by the instrument. Since a total protein and a PCV are important aspects of a complete blood count and should be performed on all whole blood samples, the appearance of the buffy coat can be recorded as well. A thick buffy coat supports the presence of a **leukocytosis** or **thrombocytosis;** a thin buffy coat indicates a **leukopenia** or **thrombocytopenia;** and reddish discoloration suggests possible **reticulocytosis.** Young erythrocytes are less dense than mature erythrocytes and can sediment out in the buffy coat when the blood is spun.

Making a good blood film is essential to blood film analysis. There are several techniques used to make good blood films. There is no absolute correct method. Two methods are illustrated in Figure 1.8. The best method to use is the one that allows the individual to produce the best blood film. Try as many methods as possible, and review the blood films for even distribution of **leukocytes** and the presence of an adequate counting area. Adopt the one that works best for each individual. The only method that is discouraged is the used of the "pull" preparation normally used for cytology preparation (see Chapter 15).

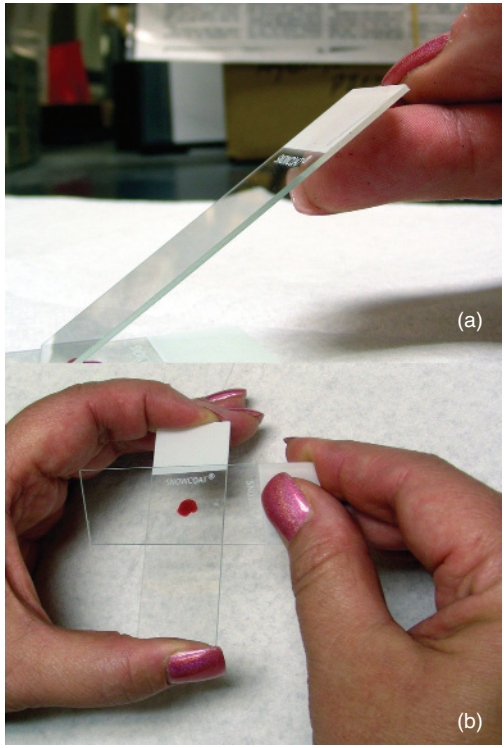

Figure 1.8. Two methods for preparing a blood film. **(a)** The push slide is rested on one finger rather than being held between the thumb and index finger. The method prevents excessive pressure on the push slide. Excessive pressure results in uneven distribution of the leukocytes to the feathered edge. **(b)** Both the push slide and blood film slide are in the hands. This technique allows some individuals better control of the slides. There are additional techniques for blood film preparation. Try as many methods as possible and choose the one that produces the best blood film.

The methods of blood film evaluation and estimates for leukocyte and platelet numbers were developed for the distribution of these cells in the traditionally prepared blood film. One important note on preparing blood films is to note that the angle of the "push" slide needed to provide a good blood film depends on the viscosity of the blood. Thin (anemic patient) blood requires a steep angle or the blood film may run off the end of the slide. Thick blood (dehydrated patient) requires a shallow angle or the blood film will be too short and there will be an inadequate counting area (Figure 1.9).

Practice is the only way to develop good blood film—making skills. Figure 1.10 illustrates common problems in blood film preparation.

The most common stain used in practice is a modified quick **Romanowsky stain** (**Diff-Quik**). Romanowsky-type stains differentially stain the cytoplasm of the leukocytes. The azure or blue dye stains the acid components of the cells (nuclei and proteins), the eosin or red dye stains the basic components of the cell (carbohydrates, some granules). The pH of the stains is critical. Therefore, keeping the stains fresh and limiting evaporation are important in maintaining the pH of the solutions.

Stains should be changed frequently (at least every 2 weeks if used often). There are a number of Romanowsky-type stains available to the practice. The most commonly used stain in reference laboratories is the Wright-Giemsa or Modified Wright-Giemsa stain. These have the advantage of providing a truer staining quality to reticulocytes on a blood film and are preferred for staining cytological preparation of lymph nodes and other lymphoid organs. Spending the time to evaluate different stains is recommended.

Samples collected for clinical chemistry tests are usually collected in collection tubes without anticoagulant and allowed to clot for at least 15–20 minutes. Following clotting the sample is centrifuged and the serum is pipetted off, or, if a serum separator tube is used, poured off and placed in a clean tube and sealed tightly. Occasionally,

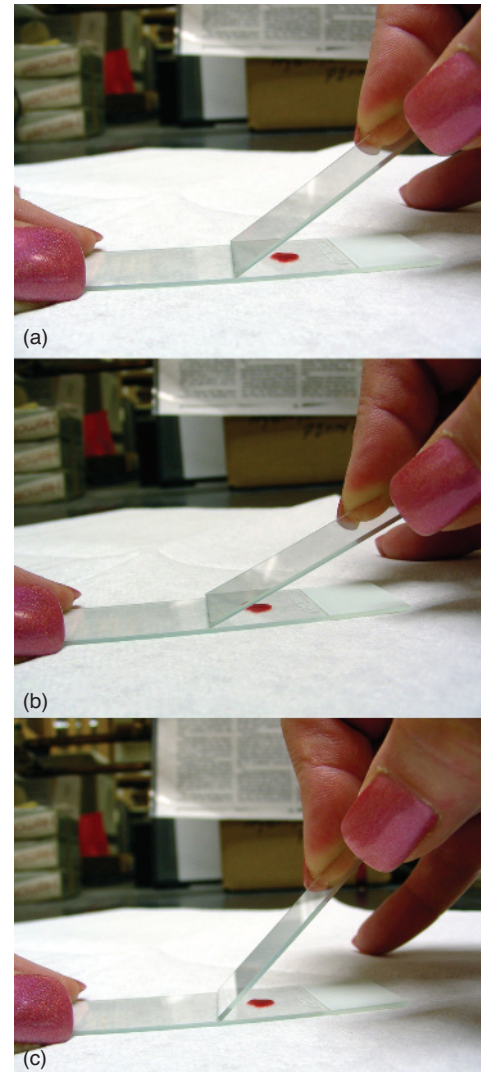

Figure 1.9. The angle of the push slide and the viscosity of the blood determine the length of the blood film. **(a)** For blood with a normal PCV, a 45° angle will produce a blood film with a good feathered edge and counting area. **(b)** For blood that is thick (dehydration), a shallower angle is needed or the blood film will be too short. **(c)** For blood that is thin (anemia), a steeper angle is needed or the blood film will be too long.

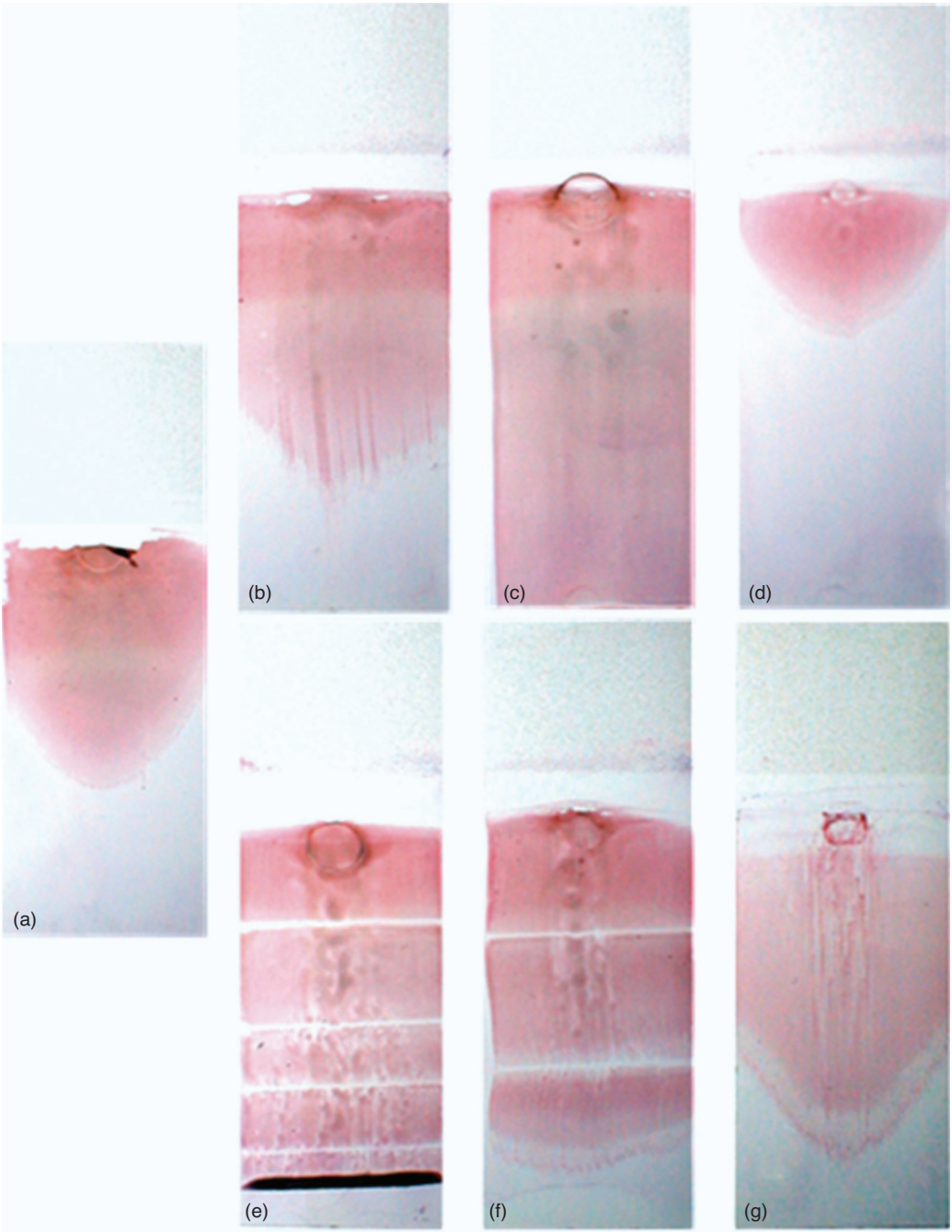

Figure 1.10. Blood film examples. **(a)** An example of a well-made blood film. **(b)** Streaks in the blood film due to platelet clumps or dust on the slide. **(c)** Too much blood dropped on the slide or the angle of the push slide is too low. **(d)** Too little blood dropped on the slide or the angle of the push slide is too high. **(e)** Hesitation with abrupt lifting of the push slide at the end. **(f)** Hesitation. **(g)** Drops of blood allowed to sit too long before making the slide.

samples are collected in heparin and plasma is collected. This is usually done when the volume of the blood sample is expected to be small, such as with puppies, kittens, or pocket pets and in severely dehydrated patients. The volume of plasma obtained from a blood sample is often greater than for serum.

In addition, the sample can be centrifuged immediately for testing rather than waiting for clot formation. It is important to remember that not all chemistry tests can be performed on heparinized plasma and that the correct type of heparin must be used (lithium heparin). It is essential that the serum or plasma portion of the blood is separated from the cellular portion as soon as possible. Delayed separation of the blood sample can cause significant artifactual changes in several chemistry parameters. The tests most consistently affected by prolonged exposure of the serum or plasma to cells are glucose and phosphorus. Potassium concentration can also be significantly increased over time in patients with increased platelet numbers and in certain breeds such as the Akita due to active transport of potassium out the cells into the serum.

As with whole blood samples for hematology, serum samples should be examined and changes in color and clarity recorded. The presence of **lipemia**, **hemolysis**, and **icterus** should be noted in the record when present. Most clinical chemistry instruments will provide information concerning what individual clinical chemistry tests are affected by lipemia, hemolysis, and icterus. It is important to note these effects when reporting results for a sample with these changes.

For urine samples, the method of collection should always be recorded. Interpretation of the result of the urinalysis will be greatly affected by the method used. Optimally, cystocentesis and catheterization are the preferred methods of urine collection. Collection and storage of the urine sample in a clean, sterile container will allow culture of the sample if the urinalysis results suggest a possible septic process. Urine samples can be stored in the refrigerated for 24 hours if the sample cannot be processed within 1–4 hours. If the sample is refrigerated, it must be allowed to reach room temperature prior to analysis. The artifactual effects of urine sample handling will be further outlined in Chapter 9.

The veterinary technician plays a pivotal role in the provision of clinical laboratory data in the veterinary practice. To do this, an understanding of how laboratory data is produced (instrumentation), an understanding of the basic physiology behind disease processes, and experience and training in blood film evaluation will allow the technician to provide excellent data, recognize incongruent laboratory values, and anticipate further testing by the veterinarian.

Although the veterinarian is the individual responsible for interpretation of data during the process of diagnosis and treatment follow-up, the technician is the individual that is responsible for overseeing the provision of accurate data and troubleshooting the process when problems arise. The following chapters focus on providing an overview of hematology, clinical chemistry, urinalysis, and cytology that will enable the veterinary technician to be a proactive part in practical clinical pathology.