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Introduction

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Introduction

Veterinary clinical pathology is a branch of laboratory medicine that focuses on the study of animal disease through the examination of blood, serum or plasma, urine, body fluids, and tissues. The discipline covers a wide range of laboratory assays and methods and is important for diagnoses, patient care, prevention of disease, and the quality and accuracy of the laboratory tests.

Obtaining the appropriate sample from exotic animals may be challenging because of widely varying physiology and anatomy. While appropriate references and excellent techniques and illustrations exist, it is difficult for the researcher, biologist, or clinician to find and access them from the literature. Hence, we review the biological, basic science, laboratory animal, and exotic animal references to create a single useful text which will serve as a quick and easy reference for the veterinarian, biologist, researcher, or technician in need of guidance regarding what is known and what is unknown in order to obtain, handle, and store diagnostic samples for exotic animals.

Interpretation of results from clinical pathologic testing of zoo, wildlife, and exotic animals may be just as challenging, if not more so, than obtaining the sample. In fact, some advocate foregoing many tests because of the challenge of interpretation. While physical examination should remain the bedrock of diagnosis in clinical exotic animal medicine, clinicopathologic testing is an extremely useful tool which should not be overlooked in the clinician's diagnostic arsenal. Additionally clinicopathologic testing may be more useful in these species than in others because of their instinctual stoicism. Many exotic animals are prey species, and occult disease is common; clinical signs may not be apparent until com-

plete health decompensation occurs. Clinical pathological testing is one of many possibilities that offer the hope of diagnosis earlier in the disease process and the chance of a better prognosis for the animal based upon early diagnosis.

Much of the challenge of clinicopathologic interpretation of exotic animals is based on lack of data. Another issue is the availability of data from the extant literature which, for exotic animals, is found scattered among many disciplines and stretches through time back to early anatomical drawings of the late 1800s. If knowing is indeed half the battle, then this text provides what is known and what is not known in a format accessible to the busy clinician. In addition, references have been curated to those of most use and importance to the researcher and clinician. Most chapters make the assumption that the reader has standard baseline veterinary clinicopathologic knowledge and continue into detailed specifics regarding sample collection and preservation, and result interpretation for the species. For more basic information regarding standard-specific clinical pathologic methods, we recommend consultation of the basic veterinary and exotic animal clinicopathology textbooks listed in the references of this section [1–15].

Reference Intervals

An additional challenge for those faced with interpretation of clinicopathologic data in exotic animals is that few true reference intervals are available for exotic, zoo, or wildlife animals. Reference intervals are an important part of all laboratory results. They are designed to be used as guidelines for interpretation and to determine if

a result is “normal” or “abnormal.” Reference intervals are often referred to as reference range, reference value, “normals,” normal range, or normal value; however, these terms are incorrect, and their use is strongly discouraged for several reasons. A reference interval represents a statistical calculation from a group of results obtained from a defined population of animals. In determining this population (often referred to as a reference population), defining what normal actually means is challenging. Furthermore, sick animals may have laboratory results that fall within the reference interval. Use of terms such as “normal range” or “normal values” is not recommended, and they should not be used because of the difficulty in defining “normal.” Reference intervals often vary from laboratory to laboratory depending on the instrumentation and methodology.

Reference intervals are influenced by many factors, such as the species, breed, age, and sex of the animal. Results can be affected by diet, exercise, excitement, medications, time of day, or season. Collection and processing of the sample can also influence the result. When establishing reference intervals, the criteria for inclusion of an individual needs to be defined before sample collection begins. Reference intervals are based on the measurement of an analyte in a population of clinically healthy animals that meet the inclusion criteria. Obtaining many samples from a single kennel or herd is not recommended as animals may lack sufficient variation needed for the reference interval to be representative, resulting in an overly narrow reference interval.

In general, samples from at least 120 individuals are recommended to establish a reference interval. However, this is usually quite difficult in veterinary medicine because of the lack of availability of appropriate individuals and the expense of obtaining and analyzing samples. Some veterinary guidelines suggest that a more realistic number is at least 60 qualified individuals that meet the selection criteria. Statistical guidance for the creation of reference intervals is available for researchers creating useful data sets for future reference [16]. Many of the data tables provided in this text fail to meet aforementioned criteria are therefore labeled as reference ranges, but the data may still serve as a starting point for evaluation of the patient and are meant to represent the best extant data available for the species group.

Reference intervals are typically derived from the mean ± 2 standard deviations of the mean of the values fitting a normal (Gaussian) distribution. This assumes that 95% of the healthy population will fall within the established reference interval. When data are not normally distributed, nonparametric analysis or data transformation is typically used to remove the top and bottom 2.5 percentiles, as 2.5% of “normal” animals have values outside the reference range on the basis of this

statistical model. Often, the ideal reference values are previous values, often called healthy or baseline values, obtained from the individual patient. Figure 1.1 illustrates the likely minimum number needed to create a reference interval for base excess in passerine birds. By convention, for a normally distributed data set, the reference interval for a particular test include 95% of all values from the general (presumed healthy) population. Because 5% of results fall outside this interval, values that may actually be unremarkable or acceptable can therefore sometimes be outside this range. Additionally, normal distributions may be less common for many analytes in exotic animal medicine, making statistical assessment of values for the creation of reference intervals more challenging.

In human medicine, reference intervals for a single analyte are created for a single instrument and/or laboratory, and values from a sample of hundreds of apparently healthy people may be stratified on the basis of gender, age, race, size, or other factors. The results are then further statistically evaluated to create a reference interval (Figures 1.1 and 1.2). Again, seldom in zoo, exotic, or wildlife species do we have the necessary numbers to create reference intervals meeting these stringent definitions, which creates a further barrier to publication of the data which we do have, but also creates a problem in interpretation. The publication and use of data for very small numbers of animals, which are of questionable health can lead to erroneous interpretation of clinical pathology, possibly to the animal’s detriment. *The wise clinician remembers that clinical pathology is used to confirm and further define a diagnosis, seldom to discover one, and this should be particularly true of nontraditional animal medicine.* This intent of this text is also to provide extant reference data and to provide guidelines for interpretation of these analytes on the basis of the species at hand. To accomplish these goals, we have asked authors to provide a relevant literature review combined with their experience in clinical medicine. Chapters are meant to provide a clinically useful overview with references available for additional in-depth consultation to the researcher and student.

Laboratory Choices: The Use of a Reference or an In-House Laboratory

The ability to perform or request various diagnostic assays is available to the clinician in a variety of settings. Choices include sending samples to diagnostic reference veterinary laboratories or laboratories at teaching universities, establishing an in-house laboratory, or utilizing a combination of both; there are advantages and disadvantages to each of these scenarios. Exotic animal

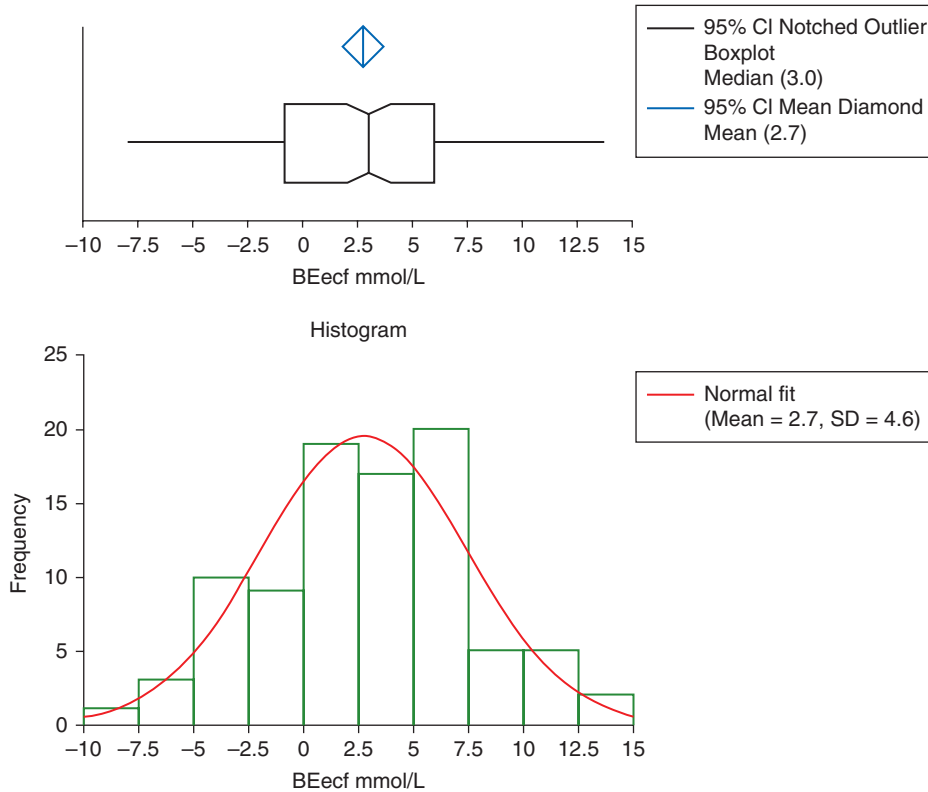


Figure 1.1 Normal distribution of base excess for passerine birds. Note that the tails (edges) of the histogram are the minimum number needed to create the shaper of a normal distribution (1–5), but the total number (because the top bars to create the parabola must be incrementally higher) for this study was 91.

practitioners face specific challenges regarding this choice, including analyte determination, sample volume, costs, client expectations, and patient health.

Diagnostic reference laboratories and laboratories at teaching universities typically process high sample numbers daily. They have trained personnel who perform laboratory tests and maintain the instrumentation by performing the required instrument maintenance and routine quality control protocols on a regular basis. These activities are extremely important to ensure that results obtained are valid. Many larger laboratories have established reference intervals for the common species (canine, feline, equine, and bovine). Some may have reference intervals for additional species or resources about some of the more uncommon species. An additional advantage of using a larger laboratory is the availability for consultations with clinical pathologists, internists, radiologists, or other specialists. Some laboratories have courier services, but many require that samples be mailed or shipped by the user. The turnaround time for routine laboratory results can be variable, but next day reporting is typical. Larger laboratories commonly offer specialized or advanced testing such as flow cytometry, specialized chemistry tests (e.g.,

hormone assays), molecular diagnostics for certain diseases and infectious agents, as well as histology, serology, toxicology, parasitology, and microbial culture and susceptibility testing.

Many private practices choose to invest in an in-house laboratory. Some tests such as urinalysis or cytology require minimal equipment and can be performed in the clinic at relatively low costs. In addition, smaller, relatively less expensive benchtop analyzers for complete blood counts (CBCs), clinical chemistry, blood gas analysis, and basic coagulation assays are available and are validated for certain veterinary species.

A major advantage of having an in-house laboratory is the shorter turnaround time for results, which can be crucial for critically ill patients and convenient for patient management and owners. However, there are many aspects to consider when thinking about having an in-house laboratory. One of the most important is the establishment of a quality control program and the willingness to dedicate the time and expense that is required. Additional considerations include the cost-effectiveness of not only purchasing an instrument, but the associated costs of maintenance and upkeep, upgrades and replacements, as well as disposable products and ancillary

Box 1.1 Considerations for Establishing an In-House Laboratory

- How often will the instrument be used?
 - 1) What is the anticipated or expected number of patient samples and corresponding tests: How many patients are there and how often will tests be run?
- Financial aspects
 - 2) Acquisition costs: How much does the instrument cost, and what are the costs of the accessories such as software, and disposables such as calibrators, controls and reagents, tubes, pipettes, and paper?
 - 3) Operating costs: What does it cost to run the instrument? This includes controls, reagents, disposables, service contracts, repair costs, and instrument and/or software upgrades. What kinds of ancillary equipment, such as computers and printers, are needed? How much does a yearly maintenance agreement cost?
 - 4) Revenue generated from having the instrument: Is it sufficient to cover the cost of the instrument throughout its life span and possibly use toward an upgrade later?
- How easy is a test to perform and how easy is it to maintain the instrument?
 - 5) Technical expertise required: How user-friendly is the instrument, is the instrument easy to run and maintain, and what training is required?
- 6) Who will be responsible for routine and preventative maintenance? Who is responsible for running and recording the tests?
- 7) Customer technical service: Does the manufacturer provide technical support when there are problems with the instrument? Who will troubleshoot problems?
- What else is there to think about?
 - 8) Establishing reference intervals, test validation, quality assurance and quality control protocols, preventative maintenance protocols and schedules, record keeping: Who will be responsible?
 - 9) Turnaround time: How fast are you going to generate results?
 - 10) How long has the instrument been on the market? What is the company's reputation? Will they be around for the next several years?
 - 11) Who else uses the instrument and what is their opinion and experiences? Find out!
 - 12) What is the average life span of the instrument? When does it become obsolete?
- Will you be committed to a quality control program?
- Should the practice just send samples to a reference laboratory? This is a viable option.

equipment that are needed. Although many instruments are relatively user-friendly, time is needed to train personnel to use the instrument, perform routine maintenance, and troubleshoot when problems arise or the instrument is not working properly. As with any piece of equipment used in a private practice, the question of how often it will be used and how many samples will be run are important to know because these factors directly impact the actual costs to the practice. Box 1.1 summarizes some of the questions to be asked when deciding whether to establish an in-house laboratory.

The Quality Control Program: What Is It and Is It Really Important?

It is imperative that laboratory testing yield correct and reliable results. Valid data are essential for making medical decisions. Inaccurate results are misleading and can prove disastrous. Every laboratory or clinical practice that analyzes samples should have a program that is designed to prevent and detect unacceptable errors in its assays and ensure that only valid results are generated and reported.

Quality control (QC) measures should be in place to minimize laboratory errors and ensure that the instrument is working properly. A QC program will help identify problems with the equipment, test methodology, operator, or potentially, multiple factors. The manufacturer should provide recommendations for the care and QC of the instrument. A QC program should not rely solely on internal, electronic quality control capabilities that are programmed into an instrument and should incorporate the use of commercially available liquid controls and calibrators specific for the instrument (Box 1.2). A summary of a basic QC program is provided in Box 1.3.

Quality Control: Record Keeping

A record of control results should be kept and reviewed regularly. The most common record keeping method is the use of Levey–Jennings plots (also referred to as QC charts) (Figures 1.2–1.5). These are graphs used to plot daily results obtained from each of the controls. Controls are typically designed to test upper limits (“high”), normal limits, and low limits. A predetermined, acceptable range for each control, usually within ± 2 standard deviations, for the expected results of that particular control is

Box 1.2 Definitions: Reagents, Controls, and Calibrators

Calibrator: These are solutions designed to adjust or establish instrument settings for a particular assay.

Instruments should be recalibrated for every new reagent lot as needed, when the instrument malfunctions, or if the control results are no longer precise and accurate.

Control: Solutions designed to determine the precision and accuracy of an assay (and the instrument) on an hourly, daily, or weekly basis. These are generally available in low, normal, and high concentrations for the analyte of interest. The chemical and physical characteristics (i.e., the "matrix") of control solutions must approximate those qualities of the unknown sample.

Reagent: Reagents contain chemicals, dyes, enzyme cofactors, or other substances necessary to measure the amount, concentration, or activity of a particular substance. A reagent can be in the form of a liquid or a dry slide, depending on the instrument requirements.

Box 1.3 Basic Constituents of a Quality Control Program

- 1) Regular scheduled use of liquid controls to ensure that the instrument, test reagents, and operator are working properly.
- 2) Performance of routine maintenance to include cleaning, replacement of components that wear out or expire on the basis of the manufacturer's recommendations.
- 3) Routine scheduled use of calibrators to calibrate the instrument to ensure proper working order and valid test results.
- 4) Keep daily records, and review them regularly.



Figure 1.2 A Levey-Jennings plot (or quality control chart). The mean and standard deviations (SD) are provided for each control lot and are used to create the values for the Y axis. The X axis is typically plotted on a daily basis.

recommended. When results fall outside this acceptable range, this should alert the operator to a potential problem. There are several steps that can be taken when this occurs (Box 1.4).

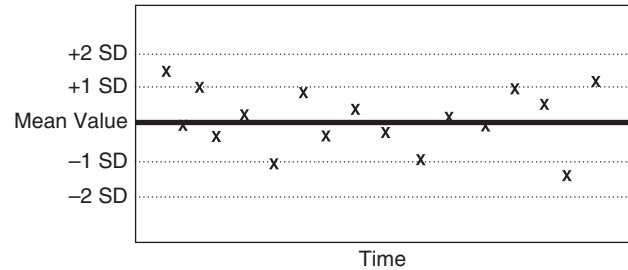


Figure 1.3 Levey-Jennings plot, acceptable. In this example the control data are within the acceptable ± 2 SD of the mean for the control.

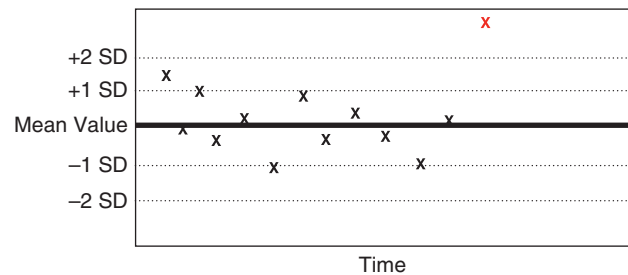


Figure 1.4 Levey-Jennings plot, error. In this example, a control result (red X) falls outside of the acceptable ± 2 SD range. This error requires correction, and no patient results are reported until the problem is resolved.

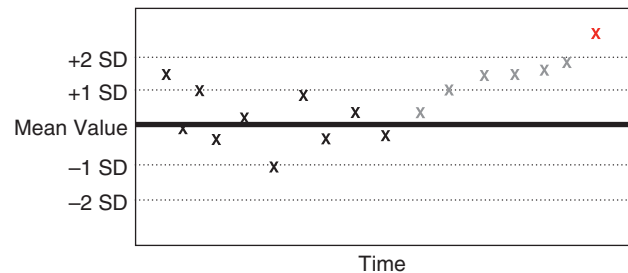


Figure 1.5 Levey-Jennings plot, drift. In this example, the control result is "drifting" upward (blue X's) and eventually is (red X) within the acceptable range. This may indicate a need to calibrate the instrument.

Box 1.4 What to Do When a Control is Out of the Acceptable Range

- 1) Repeat the test. If result falls within the acceptable range, patient results can be reported.
- 2) If result falls out of acceptable range, proceed with the following as appropriate:
- 3) Make sure correct control was used and the correct test was performed.
- 4) Check expiration dates of reagents and control.
- 5) Make up new reagent and repeat the test.
- 6) Make up new control and repeat the test.
- 7) Consult the troubleshooting section of the instrument operation manual.
- 8) Call for technical assistance.

Laboratory-Associated Error

Error can occur due to many causes. Make it a policy to call the laboratory if any results you receive do not make sense. Conversations with the clinical pathologist or laboratory technician allow you to develop an appropriate relationship with the laboratory and will improve diagnostic satisfaction as well as clinician education regarding laboratory needs to provide the best patient diagnosis. If you are unfamiliar or unsure of the appropriate samples for a desired test, always call the laboratory before obtaining samples. This policy conserves fiscal resources and time and reduces the patient stress of multiple sampling episodes. Particularly in exotic animal medicine, you may only have one chance to get the right sample. Preparation gives you the best chance of getting the correct sample. While many blame laboratory error when apparently erroneous results are obtained, *many* potential reasons may cause questionable clinical pathology analyte results. Valid test results depend on *preanalytical*, *analytical*, and *postanalytical* factors.

Preanalytical factors that can lead to an error typically occur before the sample reaches the laboratory and include factors endogenous to the patient, the sample, or a combination. Examples of common patient factors that can affect results include lipemia, hemolysis, icterus, agglutination, and drugs, which may interfere with test method.

Examples of preanalytical error associated with the sample include collection methods, anticoagulant used, sample container, and sample handling. Results of testing may be altered depending if a sample is collected from an atraumatic versus a traumatic venipuncture, forced through a needle into the collection tube, obtained from a catheter, or from different sites, which is especially important in some of our exotic species. The anticoagulant used may affect results. For example ethylenediaminetetraacetic acid (EDTA) should not be used for clinical chemistry panels because it prevents coagulation by binding divalent ions (calcium, magnesium) and contains either sodium or potassium. Furthermore, EDTA should not be used for CBCs in some species, such as corvids, because it causes increased hemolysis. Heparin samples can be used for CBCs and clinical chemistry, but it affects blood cell morphology and can cause clumping of leukocytes and thrombocytes. The sample container (plastic versus glass; colorless versus dark) may affect results. Sample handling and processing are especially critical. Storage temperature (room, ice, refrigerated, or frozen), period of time between centrifugation and separation of serum or plasma, and period of time between collection and actually performing the testing can influence results. In addition, correct sample identification with appropriate and clear labeling, completing the submission form

correctly, and requesting the appropriate test are other factors that can lead to preanalytical error if not done properly. Many studies have shown that the highest incidence of laboratory-related errors occur in the preanalytical phase of laboratory testing.

Factors that can result in analytical error are those that involve the laboratory and running the test. If a test method has not been validated, questionable results may be obtained. Unfortunately validation of many tests is seldom done in less common species that exotic animal veterinarians see often because it is cost prohibitive. Quality of instrumentation and equipment as well as routine maintenance and use of in-date reagents and materials are of particular concern for in-house laboratories and can be a significant source of error in the exotic animal veterinary practice. The lack of or a poor QC program in the laboratory is especially concerning. If you or your practice or laboratory cannot provide adequate time or personnel to ensure that all of the aspects of the laboratory and instrumentation is properly maintained, this may present serious sources of error which could lead to a wrong treatment plan or diagnosis. In this case, use of a reference laboratory is highly recommended.

Post analytical error occurs after the testing, once a result is obtained as well as when the result is reported. Often, this involves human error such as reporting incorrect results due to inaccurate or incorrect manual entry, or reporting results from the wrong patient. The result delivery to the clinician may have an unreasonable turn-around-time, which can delay treatment. Data should also be reported in a manner that is clear, easy to read, and not prone to misinterpretation by the clinician.

In summary laboratory-related errors may be attributed to many sources. To avert preanalytical error, the clinician, biologist, or researcher should strive to avoid being the cause of the error and have knowledge of how to obtain, label, handle, process, store, and ship samples. A full understanding about how reference intervals are generated and the lack of appropriate reference intervals for many species as well as the potential limitations will allow the clinician to better interpret results. Finally, if an unusual or unexpected result is obtained and potential preanalytical factors have been eliminated as a cause, the clinician should contact the laboratory.

Basic Clinicopathologic Concepts

Some basic methodologies are necessary to understand more advanced concepts and to appreciate why certain tests, and their inherent bias and foibles, are more appropriate for certain species. To avoid revisiting these concepts ad infinitum throughout the text, we have outlined

the major methods below from a clinician's standpoint. More in-depth methodological reviews can be found in many of the references at the end of this chapter. These selected concepts are limited to major examples which are either under the clinician's control or those that may affect clinical interpretation of results and include choice of cellular stain solutions, cell counting techniques, and last but not least, the creation of the blood smear.

Cell Staining Solutions

Options for cell staining are many and include Phloxine B (PB), Natt and Herrick (N&H), methanol-based quick stains (Diff Quick), and modified Wright's stains. In general practice, methanol-based stains are commonly used because of ease of use. These stains do not require a slide stainer, and should they become overly stained, they can be de-stained using methanol and then restained. Modified Wright's stains are commonly preferred by reference laboratories. Most of the images in this book are taken from slides stained with modified Wright's stain, but, sometimes, slides stained with Diff-Quik or other quick-based stains are included for comparison.

Phloxin B and N&H stains (Table 1.1) are preferred for counting cells in the hemocytometer. Manual cell counts remain necessary in avian, reptile, amphibian, and fish species because automated cell counts using current instrumentation are not possible due to nucleated thrombocytes and erythrocytes. However, depending on the character of each stain, inherent bias exists in cell counts performed with them. Phloxine B or an eosin-based stain is preferred in species in which the heterophil predominates in the total white blood cell count (TWBC) count because only the granulocytes (heterophils and eosinophils) are stained for counts. The TWBC is then obtained from fractions determined from the leukocyte differential (aka indirect WBC). This method is likely less accurate and less precise when used in species in which lymphocytes are the predominant leukocyte. Conversely, N&H stains stain all cells for differentiation and counting

Table 1.1 Natt and Herrick solution and stain.

| | | |
|--|---------|--|
| Sodium chloride (NaCl) | 3.88 g | |
| Sodium sulfate (NaSO ₄) | 2.50 g | |
| Sodium phosphate (NaHPO ₄) | 1.74 g | Bring to a final volume of 1000 ml with distilled water, and then filter through Whatman #10 medium filter paper |
| Potassium phosphate (KH ₂ PO ₄) | 0.25 g | |
| Formalin (37%) | 7.50 ml | |
| Methyl violet | 0.10 g | |

in the hemocytometer. However, depending on the increased number of cells to count and differentiate and the difficulties in doing so, this method provides vastly differing results compared to the previous method. Both methods are dependent upon adequate laboratory skills and proficiency. Most reference diagnostic laboratories choose to use only one technique depending on the expense, training, expertise, and time necessary to properly and reproducibly complete these rather cumbersome tasks in a cost-effective manner.

Cell Counting Techniques

Leukocyte counting techniques include the direct total white blood cell count (TWBC) and the indirect total white blood cell count (iTWBC), which is dependent upon the cell-differential (Boxes 1.5 and 1.6). Advantages of the TWBC method include the following: (1) total erythrocyte and thrombocyte counts can also be obtained from the same charged hemocytometer (Figure 1.6), (2) it is less differential dependent, and (3) it may be more accurate for species which have a granulocyte-poor differential. In the direct TWBC method, blood is diluted 1:200 using the N&H solution (Box 1.5) and red blood cell diluting pipettes. After mixing, the diluted blood is placed into a Neubauer-ruled hemacytometer counting

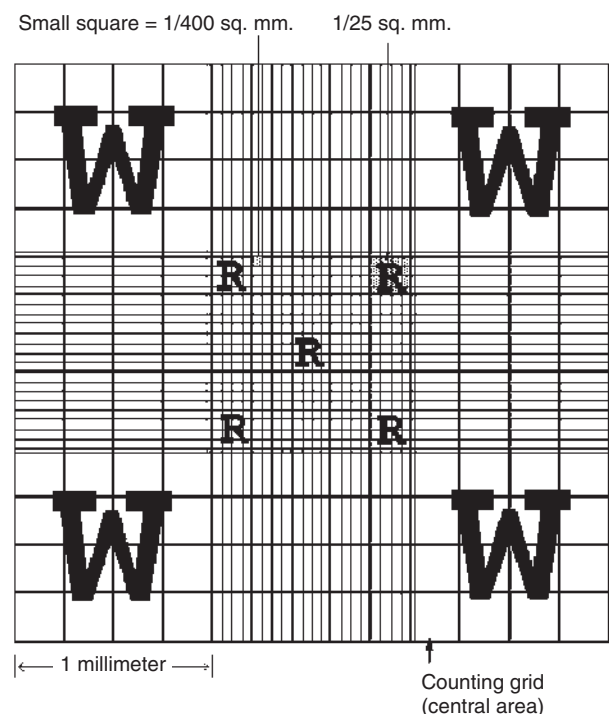


Figure 1.6 Neubauer-improved hemocytometer grid. Erythrocytes are counted based on the small cell grid in the squares denoted R, while granulocytes are counted in the larger grid within the four squares denoted W.

Box 1.5 Method for Indirect White Blood Cell Count

- 1) 0.01 ml (10 µl) blood (pipettor).
- 2) Add 0.31 ml eosinophil stain (syringe).
- 3) Swirl to mix.
- 4) Place 0.01 ml in one side of hemocytometer (1 drop fills chamber).
- 5) Wait 5 min for cells to settle.
- 6) Count total cells in 4 (W) large corner squares = X.
- 7) (X)(80) = total granulocyte count = TG.

Box 1.6 Method for White Blood Cell Differential Count

- 1) Create two direct fresh blood smears.
- 2) 20 DIPS in each quick stain.
- 3) Count 100 WBC, get percentages.
- 4) % Heterophils + % Eosinophils = TG.
- 5) Recalculate remaining cells from ratios.

chamber (Figure 1.7), and the cells are permitted to settle for 5 min. The TWBC is obtained by counting all the dark-blue staining leukocytes in the nine large squares in the ruled area of the hemacytometer chamber using the formula:

$$\text{TWBC} = \left[\left(\# \text{ cells in 9 large squares} \right) \times 1.1 \times 0.2 \right] \times 10^9 / \text{l} \left(\text{or} \times 10^3 / \mu\text{l} \right)$$

The iTWBC procedure was simplified by using the eosinophil Unopette 5877 system (Becton Dickinson); however, this is no longer commercially available. Advantages of the indirect method are that it is easier to perform and has been shown in birds to be more precise for hemacytometer counting than the N&H method [17]. However, in cases where the heterophil/eosinophil count is low, greater inaccuracy is expected. Blood is diluted 1:32 with 0.1 Phloxine B solution. After loading, the hemacytometer counting chamber is permitted to sit for 5 min in high humidity. The eosin-stained heterophils and eosinophils are then counted in both sides of the chamber (18 large squares). A leukocyte differential is also required from a stained smear (see below) in order to calculate the TWBC indirectly using the following formula:

$$\text{iTWBC} = \frac{\left(\# \text{ cells in 18 large squares} \times 1.111 \times 16 \times 0.1 \right)}{\left(\text{differential \% of heterophils and eosinophils} \right) \times 10^9 / \text{l} \left(\times 10^3 / \mu\text{l} \right)}$$

A leukocyte differential, morphological evaluation of erythrocytes and leukocytes, and presence of any extra-cellular or intracellular or inclusions parasites require

examination of a blood smear stained using a Romanowsky stain (e.g., Wright–Giemsa). While rapid stains (e.g., Diff-Quik, American Scientific Products, McGraw Park, Illinois) may be preferred in practice, they can produce inferior results, causing heterophils to be less distinct due to granule coalescence [18, 19]. Furthermore, if the stain is old or not changed on a regular basis, increased stain precipitate or bacterial contamination may occur.

The standard technique of using a microhematocrit capillary tube and centrifugation at 12,000g for 5 min can be used to obtain a packed cell volume (PCV). To run a PCV for a for an exotic animal patient, blood is placed in a microhematocrit tube filled approximately to 3/4 of the tube. After a clay plug is placed at the bottom of the tube, the tube is centrifuged and then placed against a chart to determine the PCV. The PCV with the TRBC is used to determine the mean corpuscular volume (MCV). The calculation for MCV (fl) is (PCV × 10)/TRBC and expresses the average volume of individual erythrocytes [2].

The total red blood cell count (TRBC) can be determined using automated or manual methods established for mammals. Using either of the manual methods outlined below, erythrocytes located in the four corner and central squares are counted, and the TRBC is calculated as follows:

$$\text{TRBC} = \left[\left(\# \text{ erythrocytes counted in 5 squares} \right) \times 10 \right] \times 10^9 / \text{l} \left(\text{or} \times 10^3 / \mu\text{l} \right)$$

Hemoglobin concentration (Hb) is determined by the standard cyanmethemoglobin method, except that the free nuclei must be removed by centrifugation of the cyanmethemoglobin–blood mixture before obtaining the optical density value, to avoid overestimation.

The mean corpuscular hemoglobin concentration (MCHC) and the mean corpuscular hemoglobin (MCH) can be calculated using the PCV and Hb and the Hb and TRBC, respectively. The MCHC represents the average Hb concentration per average erythrocyte. The MCH represents the amount of Hb in an average erythrocyte. The formula for MCHC (g/dL) is (Hb × 100)/PCV. The formula for MCH (pg) is (Hb × 10)/TRBC. Generally, MCHC is considered more accurate than MCH because it does not use the TRBC in the calculation.

Preparation of the Blood Smear

Best preparation of the blood smear is critical for accurate evaluation of the total white blood cell counts and the leukocyte differential in nonmammalian vertebrates. If a delay is expected between sample collection, submission, and processing, for example, in the case of samples shipped to the laboratory or collected after hours, always make two or three peripheral blood

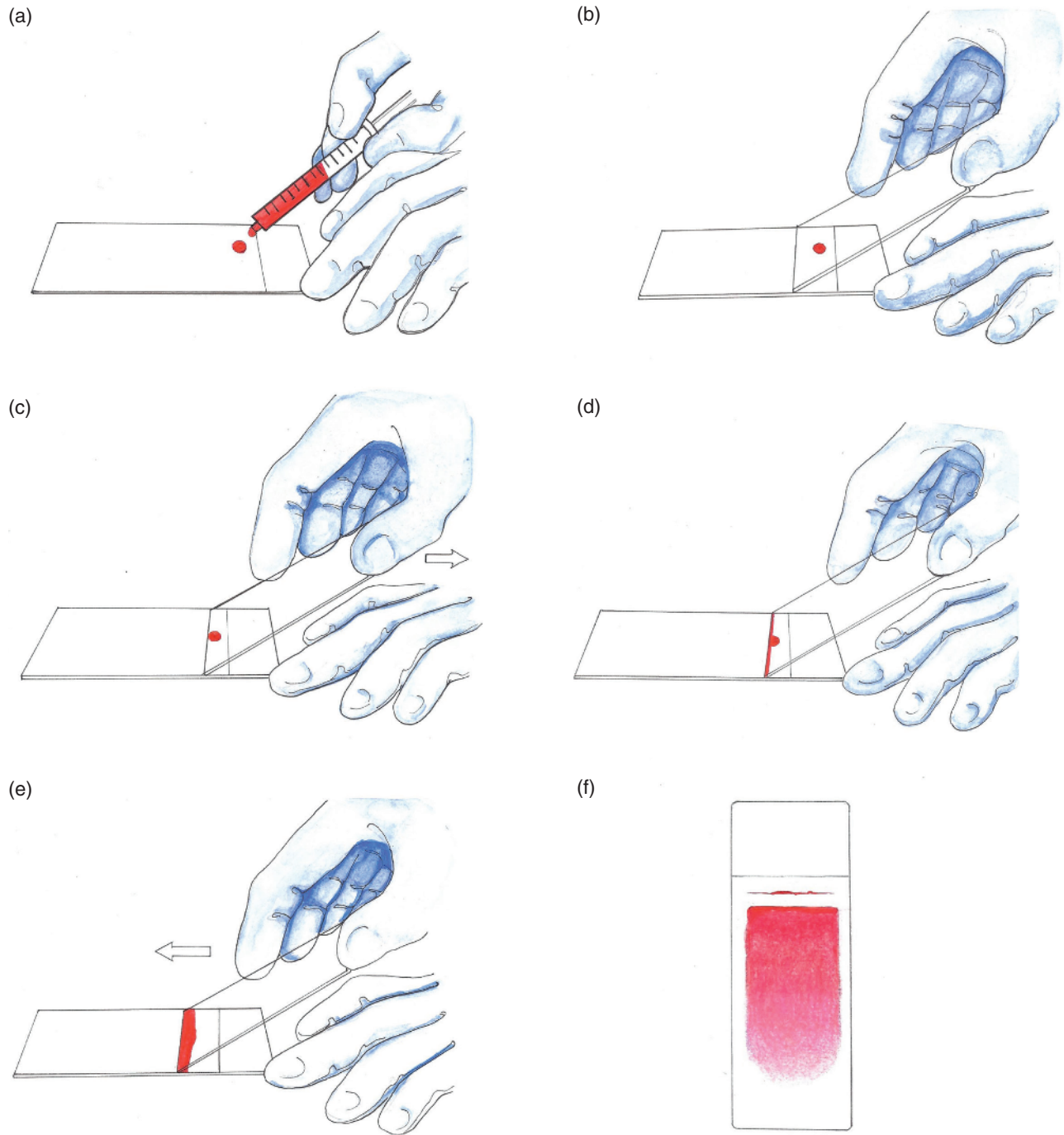


Figure 1.7 Blood smear technique: Wedge smear or slide-to-slide technique of making a peripheral blood smear. Use two clean, preferably precleaned, high-quality slides with an epoxy coated end, labeled with patient identification. (a) Place a drop of blood on the slide by touching (not dropping) the end of the venipuncture sample collection syringe. One drop of blood ($2\ \mu\text{l}$) is placed at one end. Allowing blood to drop from the syringe often creates an overly large amount of blood to properly spread. Instead create a slight eversion of blood from the syringe end and by touch allow this microdrop to adhere to the slide. This provides a blood smear free from anticoagulant artifact should the smear be completed prior to clot formation. The drop of blood should measure approximately 4 mm in diameter and be placed approximately 0.5 cm from the labeled area. (b) Correct angle to hold spreader slide. Hold the spreader slide edges with your first two or three fingers and your thumb. Do not touch the spreading edge (short non-frosted end) with your hands. Use complete and even contact to the spreading end of the spreader slide at a 30° – 45° angle to the blood drop slide in front of the blood droplet. Hold the blood drop slide to prevent movement during the smear process. Blood spreads across width of slide. (c) In one smooth motion, draw the spreader slide back through the entire drop of blood. (d) and (e) Once the blood spreads along the edge of the spreader slide (this occurs quickly), gently and steadily drive the spreader slide forward and push the blood forward along the length of the lower slide. Maintain a constant smooth motion and the same angle for the spreader slide when spreading the drop of blood as well as a consistently even contact (with very slight downward pressure) between the two slides. (f) Completed wedge smear: if the drop size, speed, contact, pressure, and angle of the spreader slide are correct, you will exhaust the blood before reaching the slide's end of the slide, create a feathered edge, and the smear will not extend more than three-quarters of the slide's length.

smears. Many factors may compromise the quality of the blood smear. Clean slides must be used. Ground glass dirt, oil or other materials imparted by touching either the slide surface or the spreader slide edge will result in a poor-quality smear. Inappropriate drop size is a common problem. An overly large blood drop or too much blood picked up by the spreader slider may result in a smear which extends to the slide edge, is too thick to evaluate microscopically, lacks a feathered edge, and may extend beyond the area that can be stained if an automated stainer is used. A small drop may produce a smear which is poorly representative of patient blood, and may be too thick to adequately evaluate cell morphology because of no monolayer. The speed and angle of the spreader slide across and from the smear slide determine the length and thickness of the smear. In general, 30°–45° is optimal. A short smear with most of the cells at the feathered edge may result from an overly quick motion of the spreader slider at a >40° angle, and most cells may be ruptured. A long smear which, lacks a feathered edge, may result from a lower (more acute angle) or a slow movement of the spreader slide. Continued even contact between the two slides must be maintained during smear preparation, but avoid applying excessive pressure to the spreader slider. See Figures 1.8–1.11 and Table 1.2 for examples of acceptable and unacceptable blood smears as well as methods of preparation and a brief overview of the approach to slide examination. Blood films should be actively air-dried and fixed immediately after preparation through immersion in absolute

methanol for 5 minutes for fixation. Unfixed blood films should not be exposed to the elements or volatile compounds (including formalin fumes), to ensure retention of staining integrity and cellular morphology. Once fixed, blood films can be stained at a later time with Wright–Giemsa stain. Use of clean fresh materials and equipment to fix and stain blood films will reduce the incidence of artifacts.

Organization of This Book

This book is specifically designed to help the reader access information quickly. The text is organized into taxonomically based sections of commonly presented animals in private veterinary practice. Each major section heading has a unique color code on the side table to facilitate quick reference: Herbivore Mammals, Carnivore Mammals, Marsupials and Insectivores, Terrestrial Invertebrates, Primate, Reptile, Avian, and Aquatic. Author guidelines discouraged speaking in generalities and required that useful analyte values be clearly referenced *for the species determined* and that analytes of unknown value in certain species also be clearly stated. Each section's general outline is similar and includes the following topics, in order: introduction and species definition, obtaining the sample, sample handling and storage, hematology, biochemistries (to include blood gases and acid base balance), vitamins, minerals, metals and toxins, and urinalysis and

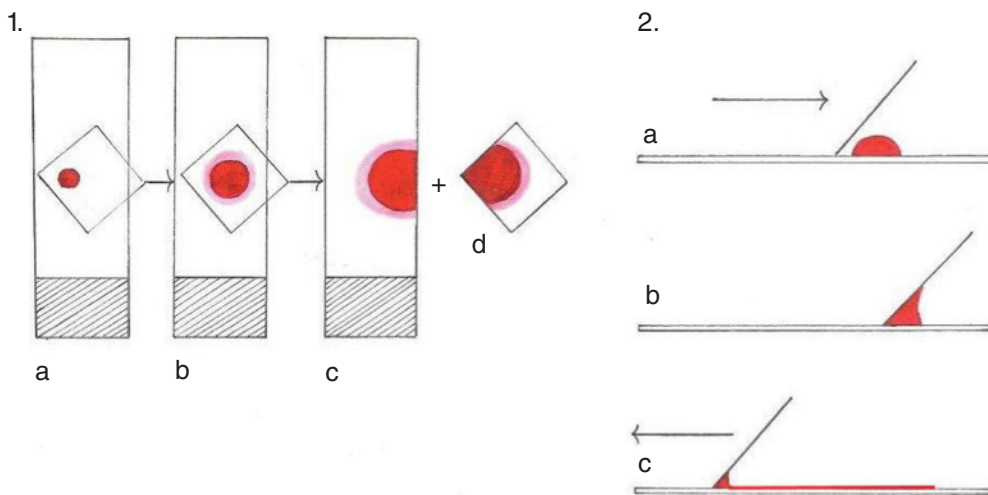


Figure 1.8 Blood smear technique: Coverslip to slide. This method is best used should you experience numerous smudge cells upon slide review (you are heavy-handed) or should you have concerns that a particular blood sample may have fragile cells on the basis of the species or suspected condition. 1. After the sample spreads between the slide and coverslip, the coverslip is slid smoothly from the glass slide in a horizontal plane at a right angle to the glass slide, and both coverslip and glass slide are air-dried. 2. the cover slip is used as a lighter version of the spreader slide in the wedge or slide to slide technique. a. approach to the blood drop b. blood drop adhesion c. blood smear creation via cover slip advancement.

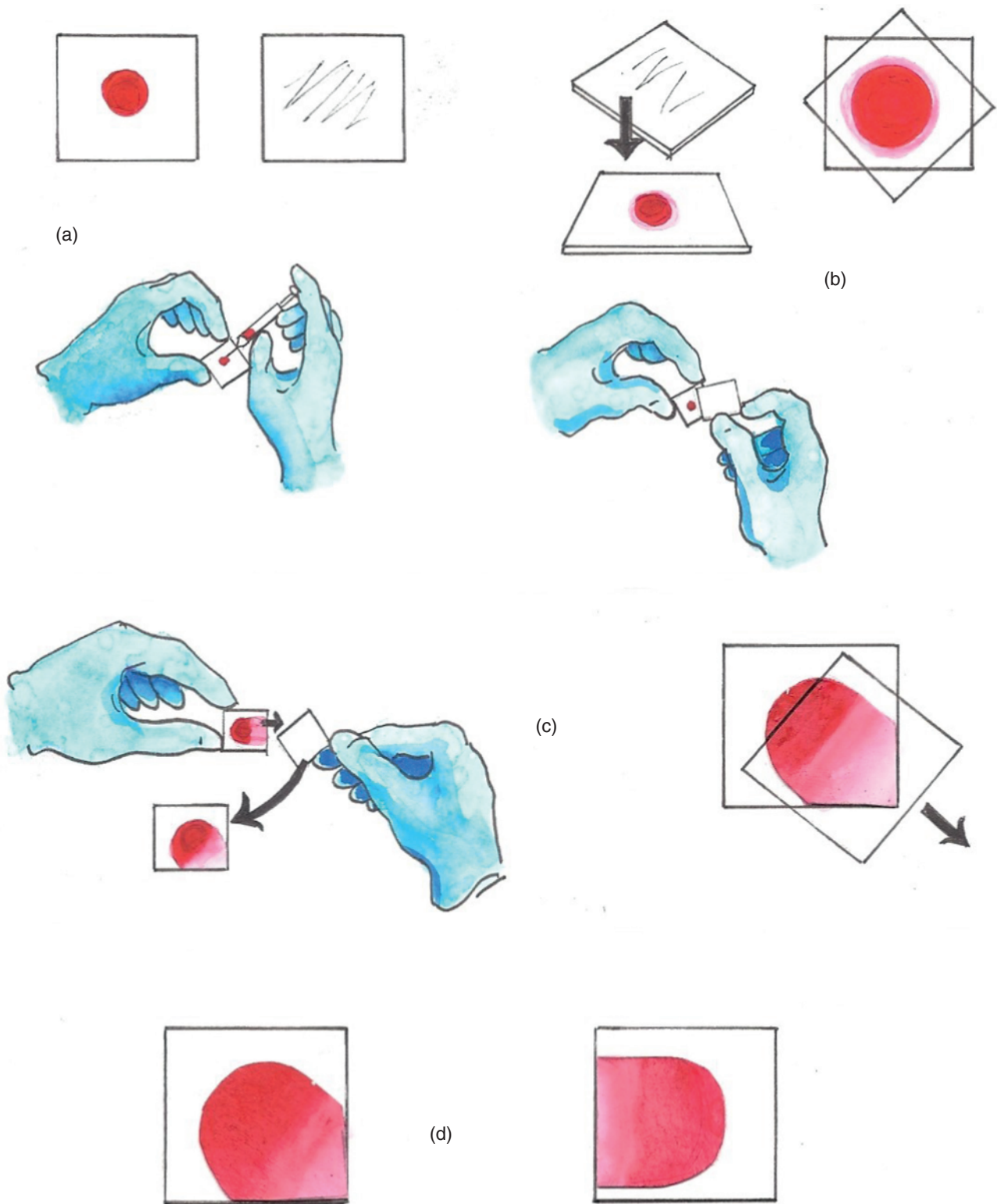


Figure 1.9 Blood smear technique: Coverslip to coverslip. Coverslip smears are made on 22×22 mm coverslips. The correct method will result in two quality smears that will appear similar to a thumbprint (d). To create a coverslip smear a coverslip is picked up by the corner, and the point is held between the thumb and forefinger in one hand. A small drop of blood is placed on to the center of the coverslip (a). A second coverslip is then placed over the first. The two entwined overlapping offset squares then form an octagram star shape (b). When the blood has spread almost to an edge, the coverslips are slid apart in a parallel fashion, without rotation, using the points of the star for manipulation (c). Challenging aspects of the coverslip blood smear include the excellent manual dexterity and vision necessary for manipulation and handling of the small fragile coverslip to avoid breakage. Coverslip smears are not compatible with standard automated slide stainers, and method modification is necessary. One may store many coverslips in the space required for only a few slides; however, their small size makes continuous handling, storage, and labeling problematic.

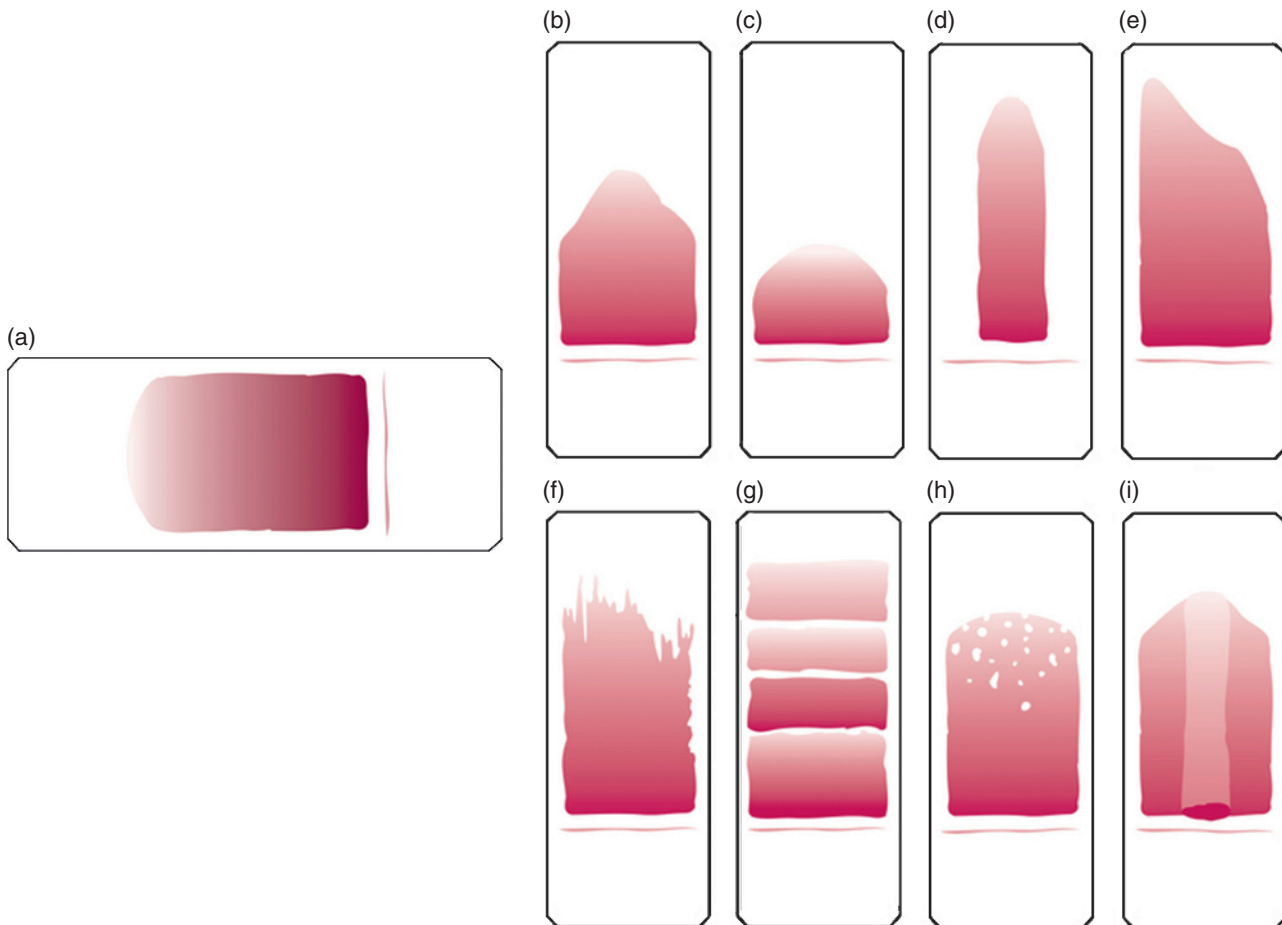


Figure 1.10 The good, the bad, and the ugly: Preferred, acceptable, and unacceptable blood smears. Appearances of optimum blood smears and those associated with the most common errors are shown. However, multiple causes may combine to result in unacceptable blood smears. In the author's experience: (a) shows the optimal shape of a peripheral blood smear. Characteristics of the best blood smear include: from two-thirds to three-fourths of the length of the slide is occupied by the smear; the thin feathered edge is slightly rounded; the entire drop is spread and the lateral edges of the smear are visible (using beveled cornered slides may help); the smear lacks irregularities, streaks, or holes, creating a smooth appearance; and the feathered edge diffracts light (has a rainbow appearance) when light is viewed through it. However, images (b–d) (passing grade) may also produce a reasonable result for cell differentials. Images (e–i) illustrate blood smears that should be remade if possible. (b) Spreader slide pushed too quickly; generally, the spreader slide should be moved through the same time as the blood takes to spread across the short edge of the spreader upon contact with the blood drop. (c) Blood drop overly small. (d) Impatience: Spreader slide moved before the blood drop spread across the width of the slide. (e) Uneven pressure applied to the spreader slide. (f) Rough, chipped, or dirty edge of spreader slide. (g) Hesitation or variable forward motion of spreader slide. (h) Dirt, grease on the slide; or increased blood lipid content. (i) Drop of blood began to dry or clot depending on time delay. *Source:* From Ref. [1].

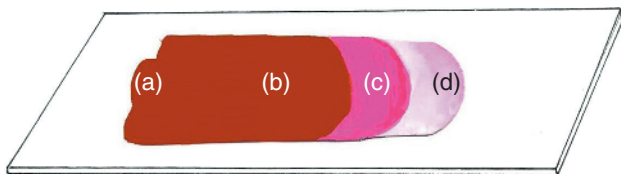


Figure 1.11 Zones of an ideal blood smear as a guide for cytological assessment. The good blood smear progresses from thick (a) to thin (d). The head application point of the blood drop is generally a nondiagnostic area. The thick region of smear is generally too thick for most things like cell morphology, but one may find some things like microfilaria. In the intermediate zone of the smear the goal is to create a **monolayer** of cells. This is the ideal or area to assess cell morphology, perform a cell differential, and estimate platelet counts. The **feathered edge**, also called the **tail** is the thinnest area of the blood smear. This is a good zone to observe large platelet clumps, very large cells and sometimes microfilaria.

serology and PCR use for the species. Tables include at least hematology and biochemistries, but may also include a variety of other diagnostics useful in the species such as blood gases, clotting times, urinalysis, laboratories familiar with diagnostic testing of the species, and so on.

Organization of Each Section

Each section will begin with an introduction and overview of the blood collection sites, techniques, appropriate sample preparation and collection techniques, and recommended anticoagulants and storage options.

Table 1.2 Common blood smear problems and suggested corrective actions.

| Problem | Corrective action(s) |
|---|--|
| Small, short smear | Increase blood drop size Decrease angle and or speed of spreader slide |
| Long smear, no feathered edge | Decrease blood drop size Increase angle and or speed of spreader slide |
| Waves, ridges, or uneven smear | Decrease downward force of spreader slide Increase speed of spreader slide Maintain even contact between the two slides, and push forward with smooth motion |
| Drop incompletely moved by spreader slide Lopsided blood pick up | Draw spreader slide completely back through drop or wait for blood to spread the width of the slide via contact. Make complete contact of edge of the spreader slide with the stationary slide |
| Overly thick smear | Decrease blood drop size, decrease angle of spreader slide Increase the speed of the spreader slide |
| Overly thin smear | Use a larger drop of blood Increase the angle, and/or decrease the speed of the spreader slide |

Sample analysis methodology will be discussed when multiple techniques are available that may affect results and interpretation. The effects of common sample abnormalities which may result in sample degradation will include the effects of hemolysis, lipemia, and increased time on sample degradation and expected changes in certain factors. Each chapter will progress in a similar format to include clinical enzymology, electrolytes, blood gases, urinalysis, coagulation testing, endocrine testing, toxicology, and finally serologic immunologic testing. Lipid analysis and appropriate gastrointestinal testing may also be included in select species where applicable. Diagrams for appropriate testing and diagnosis of certain systems may be included in each section.

Common clinical enzymes useful in each species will be reviewed in the next section on the basis of system utility. Liver, kidney, muscle, cardiac, gastrointestinal enzymes, and lipid biomarkers useful in each species will be covered. Expected changes in these enzymes in disease processes of the species will also be detailed. Electrolyte normals and abnormalities will briefly be reviewed for each species; similarly, blood gases and their possible application in this species will be covered. End-tidal CO_2 as a diagnostic modality may also be included in this section. A section on urinalysis will include expected normals for the species as well as known abnormalities which do and do not affect the urinary system of this species. Additionally, appropriate approaches for collection of urine for the species will be reviewed. Coagulation testing will be reviewed as to the coagulation factors known for each species, and the current state of testing, and the necessity of cross-matching and known blood types will also be included.

Endocrine testing will be reviewed from the perspective of the normal endocrine physiology of the species, known effects of epinephrine and other endogenous hormone on clinical hematology, and the utility of hormonal testing for diagnosis of endocrine disease. Reference ranges for common toxicants will be detailed when diagnostic testing is available. Rodenticide toxicities and the resultant necessity of coagulation testing, expected heavy metal levels for most species, and acetylcholinesterase testing will be reviewed in applicable species. Expected results for certain toxicants – example: elevation of certain enzymes in lead toxicity – will also be reviewed when these are known for each species.

Serologic, PCR, and immunologic tests are overviewed for common diseases in each species. Included within these sections are diagnostics applicable for each species and a table of available laboratories for tests. Available tests for each common disease (PCR, Elisa, or other serology), and comments on sample types, handling and shipment, and result interpretation are also provided. The individual clinician, researcher, or biologist is responsible for assessment of diagnostic laboratories with respect to experience with exotic species and test validity.

Discussion of clinical hematology in this text is limited to expected findings for healthy species, interpretation of reference ranges, and common cellular and cell count abnormalities associated with disease or dysfunction. Reference ranges for common species will be included for each species in both standard international (SI) and American (US) units. For each of the commonly seen species wherein a full hematologic profile of white and red blood cells is not given in previous

texts, a color plate of cells is included. Images such as urinalysis findings, illustration of cellular inclusions or toxic change, cell staining artifacts or cells comparatively different from other species are included for each species.

Reference ranges for hematology, biochemistries, and other common analytes are provided in tabular format at the end of each species-based section. Both SI and US units are used in the text on the basis of the original source material, and both units SI and US units are provided in all tables. A conversion table and commonly used abbreviations for most analytes provided in the text are located on the back inside cover. Inside the front cover is a key for many of the tables of this book, in which animal-specific and sample-specific characteristics have been indicated by symbols to allow ease of referencing the numeric values. Listings at the

ends of each section will allow the reader to flip to the end of each tabbed section to determine abnormalities and then read the significance of these abnormalities within the same section. The reader can also quickly ascertain which values currently have no reference ranges and therefore may not be as useful in diagnostic testing or may require the sampling of additional apparently healthy animals at that time for test interpretation.

We certainly hope this brief introduction provides you with an overview of the goals and design of the text and the options for incorporation of clinical pathology into your daily diagnostics in the practice of exotic animal veterinary medicine. Below please find additional recommended texts for basic and advanced in-depth clinical pathologic reference.

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