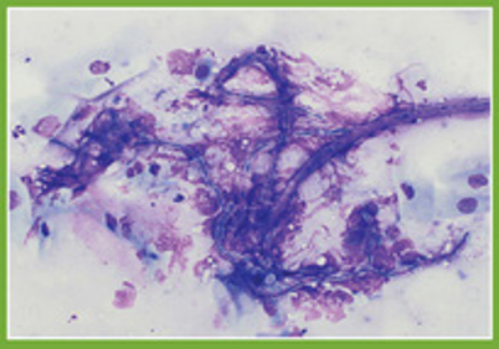
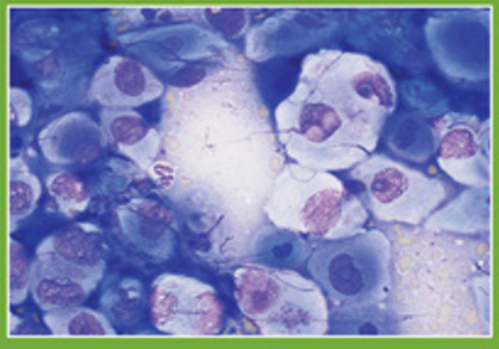
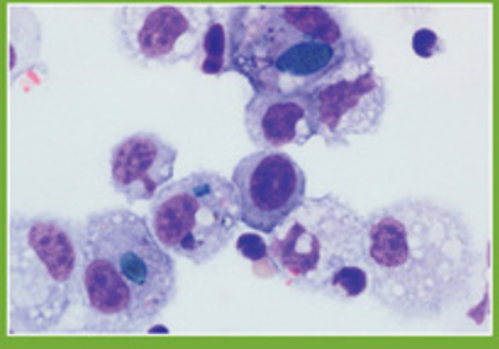
Second Edition

Equine Hematology, Cytology, and Clinical Chemistry

Edited by
Raquel M. Walton | Rick L. Cowell | Amy C. Valenciano







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I would like to thank my parents, Bryce and Elmira, for instilling in me a love of the biological world and all in it; my sisters, Judy and Laurel, for their guidance and love; and my friends, colleagues, and mentors who give life purpose and zest. I owe my colleagues and managers at IDEXX Laboratories a debt of gratitude and respect for their support, participation, and encouragement in the pursuit of knowledge and advancement in veterinary clinical pathology.

This book is dedicated to my fellow veterinary clinical pathologists and to veterinarians, veterinary students and technologists, who wander, never lost, pursuing answers but, more importantly, the questions preceding all answers.

Raquel

I dedicate this beautiful text to God and my family: my dear parents Norman Ross and Mary Ann, my twin sister Bonny, my husband Daniel, daughter Avery and son Ty. I thank my wonderful mentors especially Drs Dave Fisher, Sonjia Shelly, Carol Grindem, Jan Andrews, Mary Jo Burkhard, Gregg Dean, Christine Stanton, Lon Rich, and especially Rick Cowell. I also thank IDEXX Laboratories for supporting academic growth and for promoting excellence in veterinary pathology.

Amy

To my parents who taught me the value of honesty and instilled in me a work ethic that has served me well through the years. To my wife (Annette) and daughter (Anne) who have continually given support, meaning, and inspiration to my life. To my daughter (Rebecca) who showed me the face of true courage and taught me to laugh and love even in the worst of times. While she lost her battle with cancer at the age of 11, her memories and life lessons will forever be remembered. To the many outstanding veterinary clinical pathologists I have had the opportunity to learn from, especially Drs Ronald D. Tyler, James Meinkoth, Dennis DeNicola, and Amy Valenciano. To the many veterinary practitioners, residents, and students who taught me much more than I could ever have hoped to teach them and have become colleagues and friends.

Rick

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Preface

Veterinary clinical pathology is the study of disease in the living animal and encompasses hematology, clinical chemistry, cytopathology, endocrinology, urinalysis, coagulation, immunohematology, laboratory management, and general pathophysiology. The interpretation of clinical pathological data often leads to a disease diagnosis, from which treatment and prognosis are derived. Thus, as a discipline, clinical pathology is integral to the practice of veterinary medicine and is essential to the training of veterinary students, technicians, clinicians, and specialists.

While there are general pathophysiological principles that carry across most genera, species-dependent deviations exist. Disease pathogenesis is a consequence of individual physiology and species differences produce unique disease characteristics. Significant differences between equids and other common domestic species exist and yet a comprehensive equine clinical pathology textbook has been lacking. The authors of this book present equine disease from a clinicopathological perspective, which is systems based rather than problem based. We hope that this book will fill an important need and serve as a valuable resource for all those engaged in the care of equids, from students to specialists.

1

General Laboratory Medicine

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Acronyms and abbreviations that appear in this chapter include: Hb, hemoglobin; MCH, mean cell Hb; MCHC, mean cell Hb concentration; MCV, mean cell volume; PCV, packed cell volume; POC, point of care; POCT, point-of-care testing; RBC, red blood cells; TP, total protein; TP_{Ref}, refractometer total protein; TS, total solids.

1.1 Introduction to Laboratory Medicine

Laboratory medicine, more commonly referred to as clinical pathology (or bioanalytical pathology), is a distinct specialty that overlaps other medicine specialties such as internal medicine and oncology in the area of diagnostics. In contrast to internists, clinical pathologists practice a systems-based rather than problem-based approach when interpreting hematological and biochemical results. However, in addition to recognizing disease-associated changes, two other phenomena contribute to test interpretation: how test results are generated and how "normal" is defined. Artifacts due to sample preparation, sample condition or disease processes need to be identified and distinguished from true disease-associated changes. Similarly, test interpretation is always performed in context – the context of health. The accuracy and sensitivity of tests and the use of appropriately established reference intervals are essential to the ability to diagnose disease.

This chapter will provide selected information on hematological and biochemical test methodologies and validation, and will discuss the basic knowledge needed for generating and/or using reference intervals. The remainder of the book will address test interpretation using a systems-based approach.

1.2 Preanalytical Factors

Preanalytical factors that may affect test results should be minimized in order to ensure result accuracy [1]. Specimens should be collected according to standard practices and transported to the laboratory in a timely manner under conditions appropriate for the type of specimen and its stability. The minimum information on a specimen label for laboratory evaluation should include the full name of the patient (animal and owner), the patient signalment, and the specimen type (e.g., whole blood, serum or plasma). Especially for hematological evaluation, it is important that the patient's signalment be correct as analyzer settings vary with respect to species.

Anticoagulated specimens for hematology that have visible macroclots in the tube will produce variably erroneous results. Because the degree of inaccuracy cannot be predicted, clotted specimens are unsuitable for analyzis and these specimens should not be analyzed or submitted for analysis.

Blood films and cytology smears should not be refrigerated and should be protected from condensation and freezing during transport to the laboratory to avoid condensation artifact (Figure 1.1). Failure to fully dry blood films or cytology preparations before placing them into slide holders can also result in moisture artefact.

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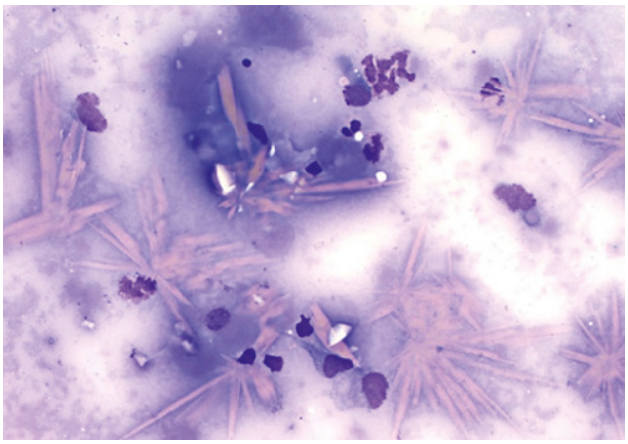

Figure 1.1 Condensation artifact caused by exposure of unfixed slides to moisture. The nucleated cells are lyzed and many hemoglobin crystals are present, formed from erythrocytes.

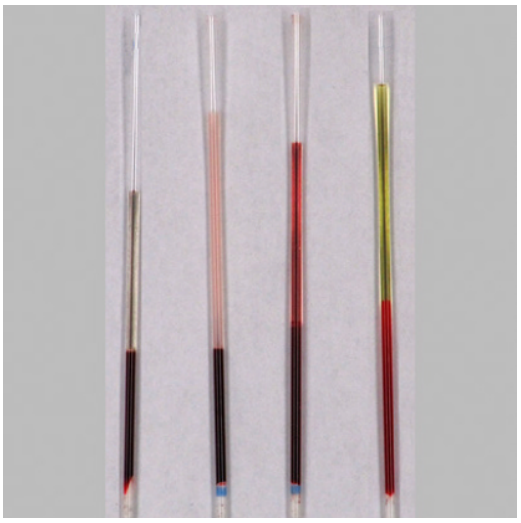

Figure 1.2 Evaluation of plasma. From left to right: normal plasma color and consistency; lipemic and slightly hemolyzed plasma; hemolyzed plasma; icteric plasma.

1.3 Basic Hematological Techniques

1.3.1 Packed Cell Volume and Plasma Evaluation: Disease and Artifacts

Measurement of the percentage of red blood cells in whole blood can provide more information than simply the packed cell volume (PCV). In addition to the packed erythrocytes at the bottom of a microhematocrit tube, there is the white buffy coat layer and a plasma layer. The size of the buffy coat is related to the white blood cell (WBC) (and platelet) count; a thick buffy coat would indicate a high leukocyte (and/or platelet) count, whereas a scant buffy coat suggests leukopenia. The character of the plasma can also yield valuable information pertaining to a disease process, as well as contributing to spurious results. The plasma can appear hemolyzed, icteric or lipemic (Figure 1.2).

Hemolysis in samples from horses usually indicates an *in vivo* phenomenon due to toxins or immune-mediated disease (see Chapter 4). However, hemolysis can also occur during blood collection if excessive force or too small needle gauge is used in phlebotomy. Whether *in vivo* or *in vitro*, hemolysis produces a color change that can make refractometer readings difficult or interfere with spectrophotometric tests.

Icterus indicates hyperbilirubinemia that usually exceeds 1.5 mg/dL (see Chapter 5). However, in herbivorous animals yellow-colored plasma is not a reliable indicator of hyperbilirubinemia due to the presence of diet-associated carotene pigments, which impart a yellow color to plasma. Icterus has not been demonstrated to interfere with refractometer readings [2]. Depending upon the chemistry analyzer, icterus can cause interference with some serum chemistry tests.

Lipemia is visible to the eye as increased turbidity in plasma or serum at triglyceride concentrations >300 mg/dL. Whether physiological (postprandial) or pathological (see Chapter 9), lipemia can cause spuriously high refractometer readings and will interfere with many chemistry tests.

1.3.2 Protein Measurement by Refractometer

Protein can be rapidly and accurately measured by handheld refractometers. Because refractometers measure protein via a total solids-based technique, the total dissolved solids in the sample affect light refraction. In addition to protein, total solids include electrolytes, glucose, urea, and lipids. The term "total solids" has caused much confusion in the reporting of refractometric protein results. Total protein (TP) and total solids (TS) are not synonymous. Currently, the vast majority of refractometers incorporate a conversion factor in their design so that the scales report TP and not TS. Contributing to the confusion is the fact that at least one refractometer is named the "TS meter" (AO Corporation) when it is in fact calibrated to report TP. While the altered refraction of plasma is mostly due to protein content, increases in lipid, glucose or urea content interfere with refractometric protein measurements. However, marked increases in urea or glucose (273 and 649 mg/dL, respectively) are needed to increase protein measurement by 0.4–0.5 g/dL. Increases in plasma cholesterol of 39 mg/dL are shown to increase the refractometer TP (TP_{Ref}) by 0.14 g/dL [2].

Another potential cause of erroneous refractometer readings is the addition of EDTA from K_3EDTA anticoagulant tubes. At the standard concentration of EDTA (5 µmol/mL), K_3EDTA by itself has minimal effect on the plasma's refraction (≤0.1 g/dL increase). This is not true for peritoneal fluid, however, where overestimation of TP_{Ref} by 0.7 +/− 0.1 g/dL was reported in one study (see Chapter 18). At higher concentrations of EDTA (10 and 20 µmol/mL), EDTA can increase TP_{Ref} by 0.9–1.0 g/dL. Underfilling of EDTA tubes has the effect of increasing the EDTA concentration and will cause spurious increases in the TP_{Ref} [3]. Some commercial tubes with K_3EDTA anticoagulant may also contain additives to prevent crystallization of the EDTA. Tubes that contain the additive may increase TP_{Ref} readings by up to 0.9 g/dL, even when properly filled. In general, polypropylene (plastic) tubes are more likely to include additives to prevent evaporation than glass tubes [4]. While sodium heparin anticoagulant has no effect on TP_{Ref}, heparin has deleterious effects on cellular morphology and is not recommended for samples that will be evaluated cytologically.

1.4 Point-of-Care Testing

Point-of-care testing (POCT) is defined as testing done at or near the patient with the expectation that results will be available quickly to facilitate immediate diagnosis and/or clinical intervention [5]. Whilst POCT provides quick, relatively inexpensive results with small volumes of blood, it also comes with its own set of risks. The major sources of error associated with POCT were categorized in one study as most often due to operator incompetence, nonadherence to test procedures, and the use of uncontrolled reagents and testing equipment [6]. Instrument calibrations and quality control measures may be omitted due to ignorance or the need for fast results. And, in veterinary medicine, analyzers may be used with species for which the instrument has not been validated. It should also be noted that diagnostic instruments for veterinary use are not subject to government regulations as they are for human use, which means that devices may not have been independently evaluated or tested [7]. Finally, poorly maintained instruments that are carried from one area to another may be a source of nosocomial infection or may transmit antibiotic-resistant bacterial strains [5].

As part of the process of ensuring accuracy in an analytical method, calibrators and controls are used. A *calibrator* is a material of known or assigned characteristics that is used to correlate instrument readings with the expected results from the calibrator (or standard). A *control* is a preparation of human or animal origin intended for use in assuring the quality control of the measurement procedure, not for calibration. Controls usually represent abnormal and normal concentrations of the measured analyte. Currently, there are some POC analyzers marketed as "maintenance free" that do not come with controls and some that do not have calibrators. These instruments should be used with caution as there is no way to verify assay accuracy.

1.4.1 Hematology Analyzers

1.4.1.1 Impedance Technology

Many POC hematology analyzers are based upon impedance methodology. Examples include the HM series (Abaxis, Union City, CA), the HemaVet 950 (Drew Scientific, Oxford, CT), the HemaTrue® (Heska, Loveland, CO), and the scil Vet abc™ (Scil, Gurnee, IL). Impedance technology employs an electric current that flows through a conductive liquid. When cells, which are nonconductive, pass through an aperture containing this fluid, there is an electrical impedance created for each cell that is proportional to the size of the cell. The impedance method facilitates measurement of the mean RBC and platelet volumes, as well as enumeration of WBCs, RBCs, and platelets. The WBCs (and any nucleated red blood cells) are counted separately from RBCs and platelets after cell lysis. Hemoglobin (Hb) concentration is also measured after RBC lysis. In the isotonic solution, nucleated cells are prevented from being counted along with RBCs and platelets because they are too big to pass through the aperture (Figure 1.3).

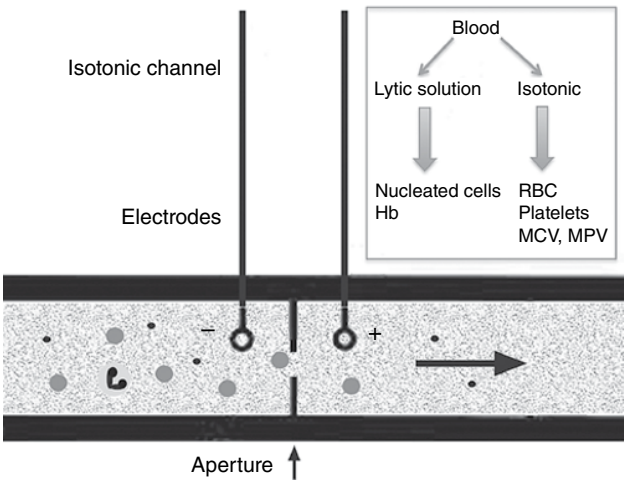

Figure 1.3 Schematic representing standard impedance methodology. Blood is directed into two chambers. In one chamber, a lytic solution is used to obtain the WBC count by evaluating bare nuclei and measuring the hemoglobin released from erythrocytes. The second chamber contains isotonic solution and an aperture of limited size through which erythrocytes and platelets are enumerated.

Failure of RBCs to lyze may result in their being counted as WBCs, thereby falsely increasing the WBC count. Similarly, large platelet aggregates may be erroneously counted as WBCs, resulting in spuriously low platelet and high WBC counts. Very large platelets may be miscounted as erythrocytes.

1.4.1.2 Centrifugal Hematology Analyzers

Centrifugal analyzers operate by taking quantitative measurements on the cell layers below and within the buffy coat. The quantitative buffy coat (QBC) VetAutoread™ (IDEXX Laboratories Inc., Westbrook, ME) is an example of a centrifugal hematology analyzer. Granulocytes, mononuclear cells (monocytes and lymphocytes), erythrocytes, and platelets are separated into layers in an enlarged microhematocrit-like tube using a cylindrical float to further expand the buffy coat layer. Cells separate into layers upon centrifugation according to relative density and fluorescent staining differentiates layers. Centrifugal analyzers can also provide fibrinogen concentrations by rereading the sample after incubating in a precipitator.

Only the spun hematocrit is measured with centrifugal analyzers. Since erythrocyte counts are not determined, the MCV cannot be calculated. The Hb can be estimated assuming a constant relationship between hematocrit and Hb. From Hb and hematocrit, MCHC can be calculated. Estimated WBC counts are obtained from the thickness of layers by assuming an average cell size.

1.4.1.3 Laser Technology

Laser hematology analyzers generate both cell counts and differentials using light scatter. Single cells pass through a laser beam and scatter light at forward and side angles from the cell, which is picked up by photoreceptors (Figure 1.4). Forward, right-angle, and side light scatter represent cell size and complexity.

While this technology affords the opportunity to generate leukocyte differentials, in general there is not good precision with differential leukocyte counts [7, 8]. The presence of band neutrophils, toxic change or reactive lymphocytes can result in poor separation between leukocyte groups, adversely affecting the instrument differential (Figure 1.5). A manual differential from a blood film is still recommended to verify instrument differentials. Examples of POC hematology analyzers using light scatter are the ProCyte® and LaserCyte® (IDEXX) and ElementHT5® (Heska).

1.4.2 Clinical Chemistry Analyzers

1.4.2.1 Dry Reagent Analyzers

The majority of in-clinic chemistry analyzers are based upon dry reagent technology, which uses reflectance

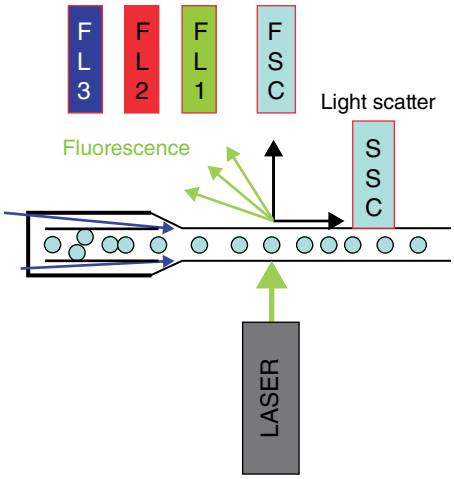

Figure 1.4 Schematic representing the principle of hematological analysis using laser methodology. Light passing directly through the cells (forward scatter; FSC) and light deflected 90° (side scatter; SSC) is captured by detectors. FSC and SSC correspond to cell size and complexity, respectively. Complexity refers to the character of the cytoplasm (e.g., presence or absence of granules). Fluorescence detectors capture fluorescence from dyes that stain RNA, myeloperoxidase or reticulum to differentiate leukocytes or to count reticulocytes.

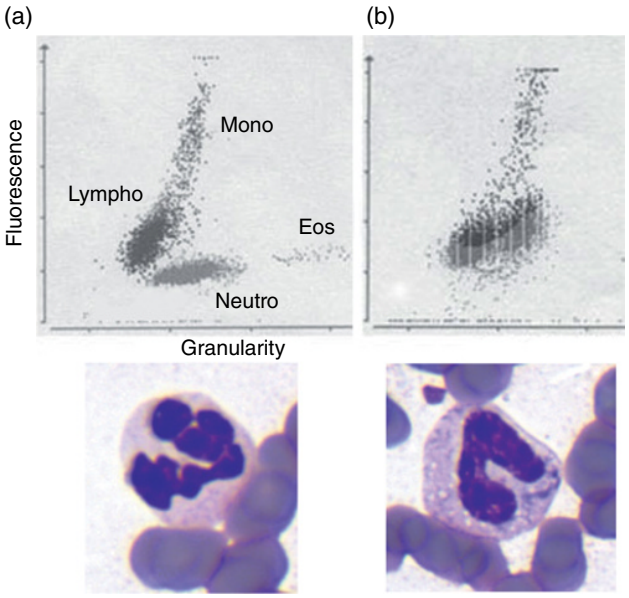

Figure 1.5 Laser-generated leukocyte differentials from the ProCyte Dx POC hematology analyzer (Idexx). The scatterplot is based upon side scatter (granularity) and fluorescence from a fluorescent polymethine dye that stains nucleic acids. (a) Scatterplot from a healthy horse. Neutrophils have the least amount of cytoplasmic RNA, thus are located at the base of the y-axis. (b) Scatterplot from a horse with toxic change in neutrophils and a left shift to band neutrophils. Neutrophils with toxic change and bands both have increased RNA content relative to normal mature neutrophils. Note how the increased RNA staining causes the neutrophil plot area to move upwards on the y-axis, blending into the lymphocyte region.

photometry. Similar to absorbance photometry, a chemical reaction (occurring within a dry fiber pad or multilayer film) results in a product that absorbs a portion of the light that illuminates it. The remaining reflected light reaches a photodetector that measures its intensity relative to the original illuminating light or a reference surface. There is an inverse relationship between reflected light (transmittance) and absorbance, where T is the percent transmittance (Equation 1.1). Analyzers will convert transmittance into absorbance because of the linear relationship between concentration and absorbance. Thus, concentration can be directly calculated from the absorbance.

$$\text{Absorbance} = 2 \quad \log \%T \tag{1.1}$$

Dry reagent technology has the advantage of minimal interference from hemolysis, lipemia, and icterus relative to wet chemistry analyzers. While most of the common chemistry analytes can be measured with dry chemistry systems, electrolytes cannot. Common in-clinic analyzers using this methodology include the Spotchem® (Heska), VetTest® (IDEXX), and RefloVet® Plus (Scil Animal Care Company, Grayslake, IL).

1.4.2.2 Reconstituted Liquid Chemistry Analyzers

Liquid chemistry analyzers operate via absorbance photometry. Reconstituted liquid systems use lyophilized rather than liquid reagents in cuvettes attached to rotors so that centrifugation mixes the sample with the reagent. Similar to reflectance photometry, when the sample is added to the reagents a chemical reaction occurs, manifesting as a color change in the liquid. Light of a specific wavelength is then passed through the liquid; the wavelength used is usually the one at which maximum absorbance for the substance being measured occurs. The light transmitted through the fluid post reaction is measured and converted into absorbance. Liquid chemistry systems are affected by hemolysis, lipemia, and bilirubinemia more than dry reagent systems. If not already known, determining the effect of substances such as these on the measurement of specific analytes should be part of the validation of a methodology.

Examples of this type of chemistry analyzer include VetScan® (Abaxis) and Hemagen Analyzt® (Hemagen Diagnostics, Columbia, MD). Just as with dry reagent systems, most common chemistry analytes, with the exception of electrolytes, can be measured.

1.4.2.3 Electrochemistry

In order to measure ion concentration, electrochemistry (also known as ion selective electrode [ISE] methodology) is employed in POC analyzers. Examples include the VitalPath™ (Heska), VetLyte® and VetStat® (IDEXX), and EasyLyte® Plus (Hemagen). ISE technology relies upon development of a membrane potential for the ion being measured. This is achieved by using an electrode with a membrane selective for the ion being measured. The membrane potential that develops when the membrane is in contact with the sample is then proportional to the activity of the ion of interest (Figure 1.6). This is compared to the reference electrode to calculate the ion concentration using the Nernst equation. Unlike flame photometry methods to measure electrolytes, ISE is not affected by lipemia or hyperproteinemia.

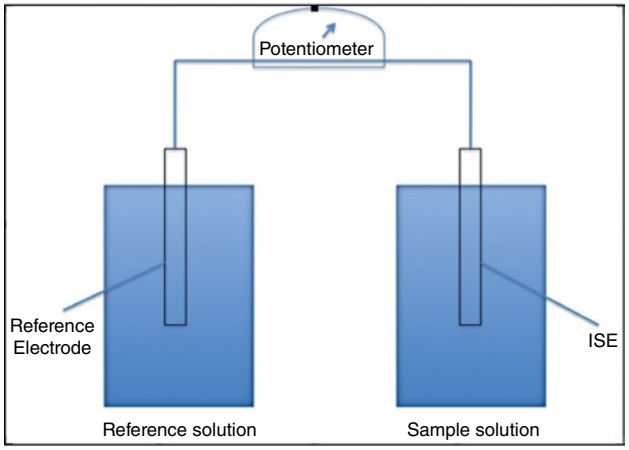

Figure 1.6 Ion selective electrode (ISE) methodology. When a sample is in contact with the membrane selective for the ion to be measured, a membrane potential proportional to the activity of the ion develops. The ion concentration is calculated using the Nernst equation by comparing the sample potential to the potential generated from a reference electrode in a reference solution.

1.5 Test Validation and Reference Values

1.5.1 Test Validation

Laboratory test method validation refers to the multitiered process of evaluating the performance of a new instrument or test methodology, often in relation to an instrument or methodology that is currently in use. In its broadest sense, method validation comprises the evaluation of test performance following a change in reagents, instruments, methodology, or – unique to veterinary clinical laboratories – introduction of a new species. The importance of test validation for different species cannot be overstated. As a result of the interspecies structural differences in any given analyte, a methodology that is adequate for one species may be inappropriate for another. Differences in expected reference values may affect whether a test has an appropriate

detection limit and analytical range. Species differences exist also in how lipid, hemoglobin or bilirubin interfere with analyte measurements [9]. Certainly, drug interferences could also be species specific. Thus, in the age of POC instrumentation, it is essential that the instrument be validated for the species in which it is used.

Before evaluating a test for a novel species, it is important to know whether the analyte to be measured is clinically relevant. For example, in equids there is little need to validate an alanine aminotransferase (ALT) assay for clinical purposes (see Chapters 5 and 10). The ultimate goal of method validation is to provide objective evidence that the evaluated method will show acceptable reproducibility and accuracy so as to be clinically applicable.

The major steps in test validation consist of estimating the following.

1) Precision
2) Accuracy
3) Sensitivity
4) Specificity
5) Reference intervals

Reproducibility of results is referred to as *precision*. Precision is measured as a coefficient of variation and reflects the amount of variation inherent in the method and is estimated by repeating measurements of the same sample at least 20 times (intraassay precision). Estimating day-to-day precision (interassay precision) requires running aliquots of the same sample over 20 days [10].

Accuracy or *bias* measures the amount of closeness in agreement between the measured value of an analyte and its "true" value. Accuracy is estimated by comparing the performance of the candidate method with that of a definitive or reference method (gold standard), by performing a recovery experiment, or by comparing the candidate method with the established method that is being replaced. Recovery experiments estimate the ability of an analytical method to correctly measure an analyte when a known amount of the analyte is added to authentic biological samples.

Sensitivity is related to precision and refers to a test's ability to detect both small quantities of the analyte and small differences between samples. A "sensitive" methodology has a high level of analytical sensitivity and a low detection limit. The detection limit and analytical sensitivity are related but not synonymous. The detection limit is defined by the International Union of Pure and Applied Chemistry (IUPAC) as the smallest quantity or concentration that can be detected with reasonable certainty. The detection limit depends on the magnitude of the blank measurements and is related to their imprecision [11].

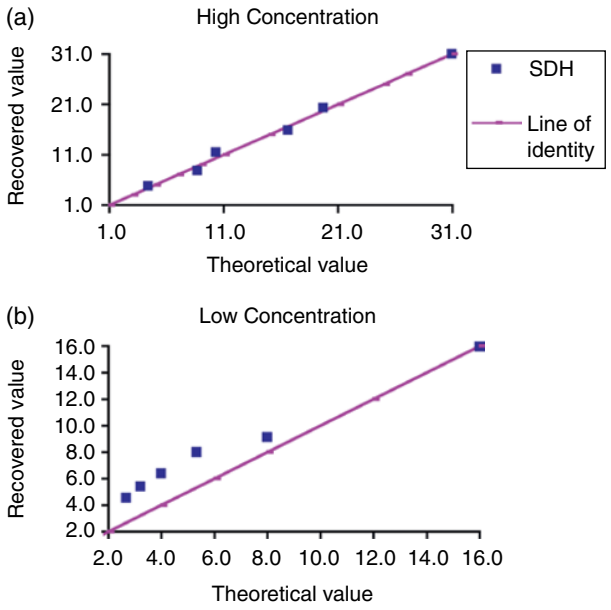

Figure 1.7 Serial dilutions of high and low concentrations of sorbitol dehydrogenase (SDH) to determine assay sensitivity. (a) There is very good correlation between the expected and recovered values in dilutions made from high SDH concentrations. (b) In contrast, at low concentrations of SDH the assay is less sensitive.

Sensitivity measures the change in signal relative to a defined change in the quantity or concentration of an analyte. This is usually accomplished by measuring a series of dilutions of a known amount of analyte (Figure 1.7).

Analytical specificity refers to the ability of a method to detect only the analyte of interest and is related to accuracy. Specificity may be affected by factors such as hemolysis, icterus or lipemia of serum or plasma, or by drugs and other substances that compete for reagents or affect the physical properties of the sample. Interference studies are performed by adding the interfering material directly and measuring its effects or by comparing measurements from hemolyzed, icteric or lipemic samples using the candidate method and one that is not affected by these factors.

Reference values are typically generated at the end of the method validation process and should be included with an instrument after the manufacturer has validated the methodology. When considering a POC instrument for purchase, if the manufacturer has truly validated the instrument for horses, species-specific reference values should be available.

1.5.2 Reference Values

The use of reference values to diagnose or screen for disease implies that health is a relative concept; clinical

examination, evaluation of laboratory data, and diagnostic imaging findings all require comparison to a "normal" standard. "Normality" itself is also relative. What would be considered usual values for a racehorse may vary significantly from values from a cold-blooded working horse. Because health and disease are defined against "normal" or reference standards, the importance of appropriate reference values cannot be overstated. A few general principles regarding the use of reference values should be common knowledge for all veterinary practitioners.

1) When laboratory-specific or instrument-specific reference values are not available, published reference intervals (RI) should be used with caution. Published reference values should provide basic information regarding how health was defined for the population, as well as the general characteristics of the population (including number of animals sampled) and the instrumentation from which the values were derived. The practitioner should attempt to match the population and instruments from which the values were generated as closely as possible to the patient to which they are being applied.
2) Reference values obtained from one type of POC instrument should not be used interchangeably with those for another instrument, especially when different methodologies are involved. Similarly, using RIs generated from diagnostic laboratories analyzers to interpret data from your POC analyzer can be like comparing apples and oranges. If your POC analyzer does not come with RIs provided by the company from which you bought it, look for published RIs which are for similar POC analyzers. If your POC analyzer *does* have RIs provided from the manufacturer, take the time to find information on where the RIs came from. Some POCs may be designed for humans and the RIs provided may not even be from a veterinary species or may pertain only to a given species.
3) If you plan to replace a POC analyzer with a similar but different instrument and want to use the old RIs from your original analyzer, the old RIs should be validated for the new instrument. Validation can be achieved using a small sample (n = 20) of "normal" individuals. The values obtained from these healthy individuals can be tested against the RI to be used with the new instrument; if two or fewer subjects are outside the candidate RI, it is considered transferable. If three or four values fall outside the RI, another 20 patients can be tested and interpreted in the same manner as the original 20 samples. If >4 of the original 20 values fall outside the candidate RI, transference is rejected for that analyte and an alternative RI must be used [10].

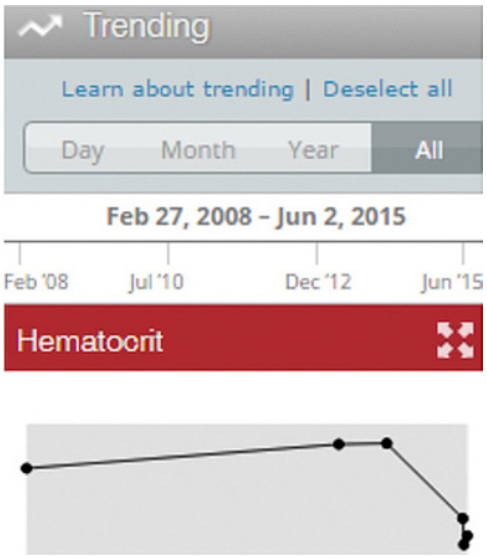

Figure 1.8 A graph from Idexx Laboratories' VetConnect® platform depicting a patient's hematocrits over the course of seven years. The gray zone represents the reference limits for hematocrit. Although the last three values on the graph are within the reference limits for "normal," these values are clearly abnormal for this individual.

Reference intervals used for interpretation of laboratory data are population based, using cross-sectional data typically representing 95% of the population chosen. Thus, by definition, any given RI implies that there will always be about 2.5% of the population whose values will normally fall above or below the RI. This fact should be considered when interpreting abnormal data that do not fit the clinical picture.

A population-based RI may not be sensitive enough to detect change in an individual if it is not marked. While this can be true for any analyte, some analytes are much more prone to this effect than others [12]. For these analytes, using the individual as its own normal can be much more effective in identifying abnormalities, especially with particular analytes (Figure 1.8). Patient-based RIs are generated from the individual patient's longitudinal data, if available, and can be assessed by looking at how the data trend from that patient in health. Some diagnostic laboratories and practice information systems provide graphing tools to follow patient data over time. In this manner, significant changes in an analyte can be detected before the values fall outside the RI.

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2

Equine Hematology

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2.1 CBC Interpretation

The complete blood count (CBC) provides information beyond the concentrations of blood cells. Insight into disease processes, their severity, and even diagnoses can be gleaned from a complete evaluation of the CBC, especially in conjunction with a peripheral blood film. A single CBC is merely a "snapshot" in time; therefore, serial CBCs are often beneficial in better understanding a progressing or improving disease process.

Blood submitted for a CBC should be collected and immediately mixed with EDTA, which is the preferred anticoagulant for mammalian blood. Ideally, analysis of the sample should occur promptly to prevent the formation of cellular changes such as swelling and degeneration, which can affect both blood smear analysis and the evaluation of the blood sample with an automated hematology analyzer.

It is not uncommon in equine medicine for delays in sample analysis up to 24 hours to occur as a result of restricted access to diagnostic laboratories. Characteristic changes in blood parameters associated with delayed analysis of equine blood samples using a common hematology analyzer (Advia 120, Bayer Corporation, Tarrytown, NY) include increased numbers of normocytic, hypochromic red blood cells (RBCs), increased numbers of macrocytic, hypochromic RBCs, misclassification of granulocytes as mononuclear cells using the basophil reagent method, and a pseudothrombocytosis due to the categorization of lyzed erythrocytes as platelets. These changes are mitigated by storage at 24 °C rather than at 4 °C [1]. In general, equine blood differential leukocyte counts obtained from the Advia 120 show less precision compared with classic impedance methods and it is recommended that these instrument-derived counts should be verified with manual differentials [2].

It is also suggested that blood samples be warmed to 37 °C prior to analysis. Warming EDTA blood samples reduces pseudothrombocytopenia secondary to platelet clumping, a common preanalytical error for platelet counts [3].

2.1.1 The Erythrogram

In health, erythrocyte lifespan in horses appears to vary between breeds, but is approximately 140–160 days [4]. After a hemorrhagic event, erythrocyte lifespan is shortened to an average of approximately 139 days [5] and 144 days after a hemolytic event [6]. The erythrogram typically comprises the following elements: RBC count ($\times 10^6/\mu L$); hematocrit or packed cell volume (PCV) (%); hemoglobin (Hb) concentration (pg/dL); mean cell volume (MCV) (fL); mean cell Hb (MCH) (pg); mean cell Hb concentration (MCHC) (g/dL).

Calculated indices:

$$\textbf{Hematocrit}(\%) = \frac{\text{MCV} \times \text{RBC}}{10} \tag{2.1}$$

$$\textbf{MCH}(\text{pg}) = \frac{\text{Hb} \times 10}{\text{RBC}} \tag{2.2}$$

$$\textbf{MCHC}(\text{g/dL}) = \frac{\text{MCH}}{\text{MCV}} \text{ or } \frac{\text{Hb}}{\text{PCV}} \tag{2.3}$$

The indices that are measured by the hematology analyzer include RBC count, Hb, MCV, and PCV. Knowledge of which indices are calculated and which are measured will help to determine possible artifacts in the erythrogram. For example, a discrepancy between the hematocrit and PCV (>2% difference) will point to a spurious MCV or RBC measurement. When there is agglutination, the hematocrit may be spuriously low as a result of the measured RBC count being lower than the true RBC count due to the

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presence of RBC aggregates that are not detected by the hematology analyzer. However, agglutination also may spuriously increase the MCV measurement when RBC doublets are measured as individual RBCs. If the spuriously increased MCV is in proportion to the spuriously decreased RBC count, the hematocrit may not be significantly different from the PCV.

Another example of artifact-associated change that may not affect measured values would be erythrocyte swelling associated with lithium heparin anticoagulant. Lithium heparin anticoagulant may cause spuriously high hematocrits as a result of RBC swelling causing spuriously high MCV [7]. However, the increased MCV will similarly affect the centrifuged hematocrit so there may not be a mismatch between the calculated hematocrit and PCV.

As a control for the accuracy of the analyzer hematocrit, a spun hematocrit (PCV) should always be run for comparison with the hematocrit. In the absence of a PCV, the universal relationship between the mammalian Hb concentration and hematocrit can be used to determine the accuracy of the hematocrit; for mammals other than camelids, the hemoglobin should be one-third of the hematocrit. For example, if the Hb concentration is 11 pg/dL, the hematocrit should be approximately 33%.

2.1.1.1 Erythrocytosis

Erythrocytosis is defined as an increased hematocrit and may be relative or absolute. Transient, absolute erythrocytosis in horses may occur as a result of splenic contraction (see Section 2.1.1.6). Hemoconcentration produces a relative erythrocytosis secondary to dehydration. Erythrocytosis due to hemoconcentration will produce concomitant increases in both the PCV and plasma protein concentration, whereas erythrocytosis from splenic contraction is not accompanied by alterations in plasma protein concentration [8].

Paraneoplastic erythrocytosis is rare in horses and has been reported with lymphoma, hepatoblastoma, hepatocellular carcinoma, and a carcinoma of unknown origin as a result of autonomous erythropoietin excretion by the neoplastic cells [9–12]. Neoplastic erythrocytosis (primary erythrocytosis or polycythemia vera) is very rare in equids with only a single confirmed case report in a 2-year old Arabian gelding [34].

2.1.1.2 Anemia

2.1.1.2.1 Regenerative Anemia Similar to other species, equids release erythropoietin in response to hypoxemia caused by decreased erythrocyte circulating mass due to loss or hemolysis. Thus, regenerative anemias in horses occur as a result of hemorrhage or hemolysis from immune-mediated damage, toxins, or oxidative damage. During immune-mediated hemolytic anemia (IMHA), antibodies with or without complement accelerate erythrocyte destruction [13]. Immune-mediated hemolytic anemia may be idiopathic or secondary to medications, neoplasia, or infectious disease. Oxidative damage in horses has been associated with red maple leaf toxicity, glucose-6-phosphate dehydrogenase deficiency, erythrocyte flavin adenine dinucleotide deficiency, onion ingestion, phenothiazine, and equine infectious anemia viral infection (EIAV).

2.1.1.2.2 Nonregenerative Anemia In horses, nonregenerative anemias are most often attributable to decreased erythropoiesis associated with inflammation or disease due to inflammatory cytokine effects, often referred to as "anemia of chronic disease." This type of anemia is now more appropriately termed "anemia of inflammation." The net effects of inflammatory cytokines result in decreased iron availability for erythropoiesis and direct suppression of erythropoiesis (Figure 2.1) [14].

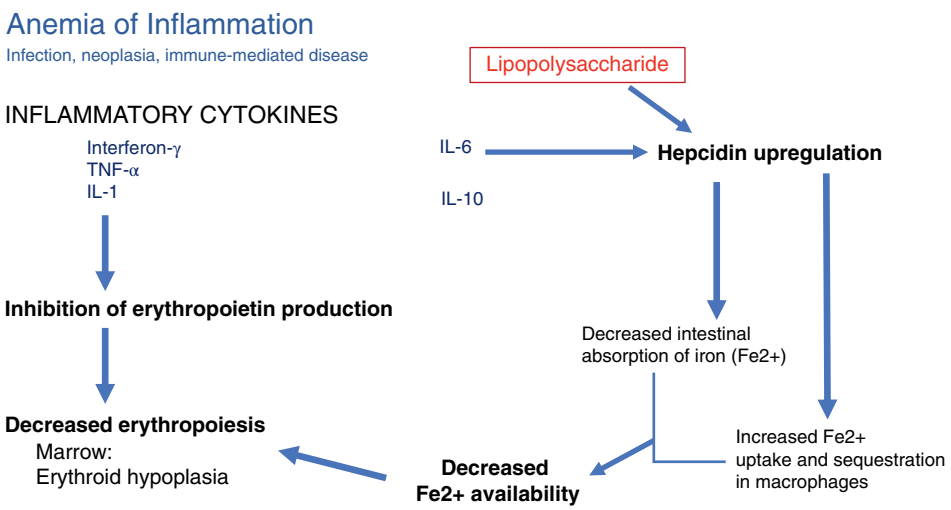

Figure 2.1 Simplified overview of the mechanisms in anemia of inflammation that result in erythroid hypoplasia and nonregenerative anemia.

Decreased plasma iron concentration was shown to have good sensitivity as a marker of systemic inflammation in horses [15]. The decrease in iron is a relative rather than absolute deficiency due to sequestration in macrophages. Absolute iron deficiency in adult horses is very rare.

Inflammation can be associated with neoplasia, infectious disease, and immune-mediated disease, resulting in nonregenerative anemia. However, these diseases can also cause hemolysis or hemorrhage, so some neoplasms, infectious agents, or immune-mediated disease may be associated with regenerative anemia depending upon the net effect of stimulatory and inhibitory forces. Impaired renal function may result in anemia due to decreased production of erythropoietin, but also as a result of inflammation [14, 16].

2.1.1.3 Changes in Erythrocyte Indices in Response to Anemia

The response to increased erythropoietin from most mammalian species is to release marrow reticulocytes into circulation, which can primarily affect MCV, MCH, and MCHC. The classic change in RBC parameters in regenerative anemia is therefore macrocytic and hypochromic in most species. In contrast, the typical regenerative response to anemia in horses is macrocytic and normochromic. Horses are unique amongst domestic mammalian species with respect to the lack of release of reticulocytes following mild to moderate anemia. While reticulocytes are produced within the marrow and increases in marrow reticulocytes are associated with regenerative erythroid responses, too few reticulocytes are released into circulation to be useful as an indicator of regeneration.

Until the advent of automated reticulocyte enumeration methods that evaluate more than 40 times the number of erythrocytes evaluated by manual methods, reticulocytosis was not thought to occur in equine blood. Using laser methodology (Advia 120), small numbers of circulating reticulocytes ($0.5–85 \times 10^3/\mu L$) can be detected in health [2]. Reticulocyte numbers vary slightly depending on breed and age, with cold-blooded horses having approximately 20% fewer reticulocytes compared to other breeds and Thoroughbred foals with approximately 50% more reticulocytes compared to adults. Nonblood diseases such as colic or dysproteinemia resulted in approximately 36% greater numbers of reticulocytes while marked anemia resulted in 120% greater numbers of reticulocytes [17]. However, since there are only low circulating numbers of reticulocytes overall, the clinical use of reticulocyte numbers is limited to severe anemias in select situations such as anemia associated with immune-mediated hemolysis [18] or with high-dose erythropoietin administration [19].

A regenerative response to blood loss anemia in horses is reported to take about four days from the onset of RBC loss, with a maximal response seen at nine days [20]. Recovery to normal values after a hemolytic event takes about 1–2 months [6], whereas recovery from hemorrhagic anemia is of the order of 2–3 months [5]. Historically, the best indicator of a regenerative response in horses prior to increasing hematocrit is evaluation of bone marrow. However, erythrocyte indices can show characteristic changes indicative of a regenerative response, especially in severe hemorrhagic or hemolytic anemias.

2.1.1.3.1 Mean Cell Volume Macrocytosis, characterized by the release of macrocytes that are roughly twice normal size, is part of the maximal erythrocyte regenerative response. This macrocytosis is not strictly related to reticulocytosis as regenerative macrocytosis in horses and other species does not correlate with reticulocytosis [19]. Macrocytosis is one of the first and most consistent parameters to show change following anemia in horses and is a more sensitive indicator of regeneration than hematocrit. However, horses with effective regenerative responses do not always have macrocytosis as defined by increases above reference values, especially with mild blood loss or hemolytic anemias. In these cases, serial evaluation of individual MCVs was more sensitive in detecting macrocytosis than comparison with a population-based reference interval [21]. Widening in the red cell distribution width (RDW) (discussed later) can also identify macrocytic subpopulations before the MCV increases above reference values.

In horses, macrocytosis subsequent to anemia is associated with a decrease in the number of normocytes, which suggests that macrocytes remain large and do not contribute to the normocyte population [22]. Macrocytes persist after hematocrit and RBC counts have returned to preanemia levels, so macrocytosis in the presence of other normal erythrocyte values in horses may be an indicator of a recent regenerative response [21, 22].

Microcytosis is typically associated with absolute or functional iron deficiency or portosystemic shunting in many species. In horses, the most common cause of microcytosis is physiological and age associated, necessitating separate reference values for MCV in horses less than 9 months of age. In horses, microcytosis associated with absolute iron deficiency has not been reported. Documented iron deficiency anemia in a foal was characterized as normocytic and normochromic [23]. Functional iron deficiency attributable to iron sequestration (i.e., anemia of inflammation) may result in microcytosis and does appear to occur in horses. Reported cases of larval cyathostominosis associated with microcytosis attributed the finding to systemic inflammation and/or protein exudation associated with intestinal parasitism [24].

2.1.1.3.2 RDW and the Distribution Histogram Most hematology analyzers will report the RDW with the erythrocyte indices. The RDW is a calculated value assessing the coefficient of variance of the erythrocyte volumes. In other words, it evaluates the amount of variation in the erythrocyte volumes and reflects the degree of anisocytosis.

Increases in RDW are associated with blood loss and hemolytic anemias, as well as with erythropoietin administration [19, 21]. Similar to the MCV, increases in RDW due to macrocytosis are detectable in serial comparisons of individuals, but may not exceed population-based reference intervals. Because the RDW can increase as a result of the emergence of smaller and/or larger erythrocyte populations, the distribution histogram itself can better identify the cause of increases in RDW. The impedance method (see Chapter 1) generates a histogram depicting the distribution of erythrocyte volumes (Figure 2.2). The RBC histogram is valuable in detecting the emergence of macrocytic and microcytic erythrocyte subpopulations. This is best accomplished by comparing serial histograms from a patient at weekly intervals. In horses, the histogram is especially useful because macrocytic subpopulations representing a regenerative response to anemia can be detected before the MCV rises above the reference interval [22]. Moreover, as discussed previously, not all horses with regenerative responses show changes in MCV above the reference interval, but macrocytic subpopulations are detectable on the histogram.

2.1.1.3.3 MCH and MCHC The mean cell hemoglobin (MCH) and mean cell hemoglobin concentration (MCHC) represent the quantity and the concentration of Hb, respectively, per average erythrocyte. Any increase in MCH and/or MCHC indicates artifact since it is not physiologically possible for these indices to increase outside the upper reference limit because Hb synthesis halts when the optimal amount of Hb is present within the erythrocyte cytoplasm.

Mean cell hemoglobin and MCHC are indices calculated from RBC and Hb concentrations, thus increases are associated with spurious RBC or Hb measurements. RBC

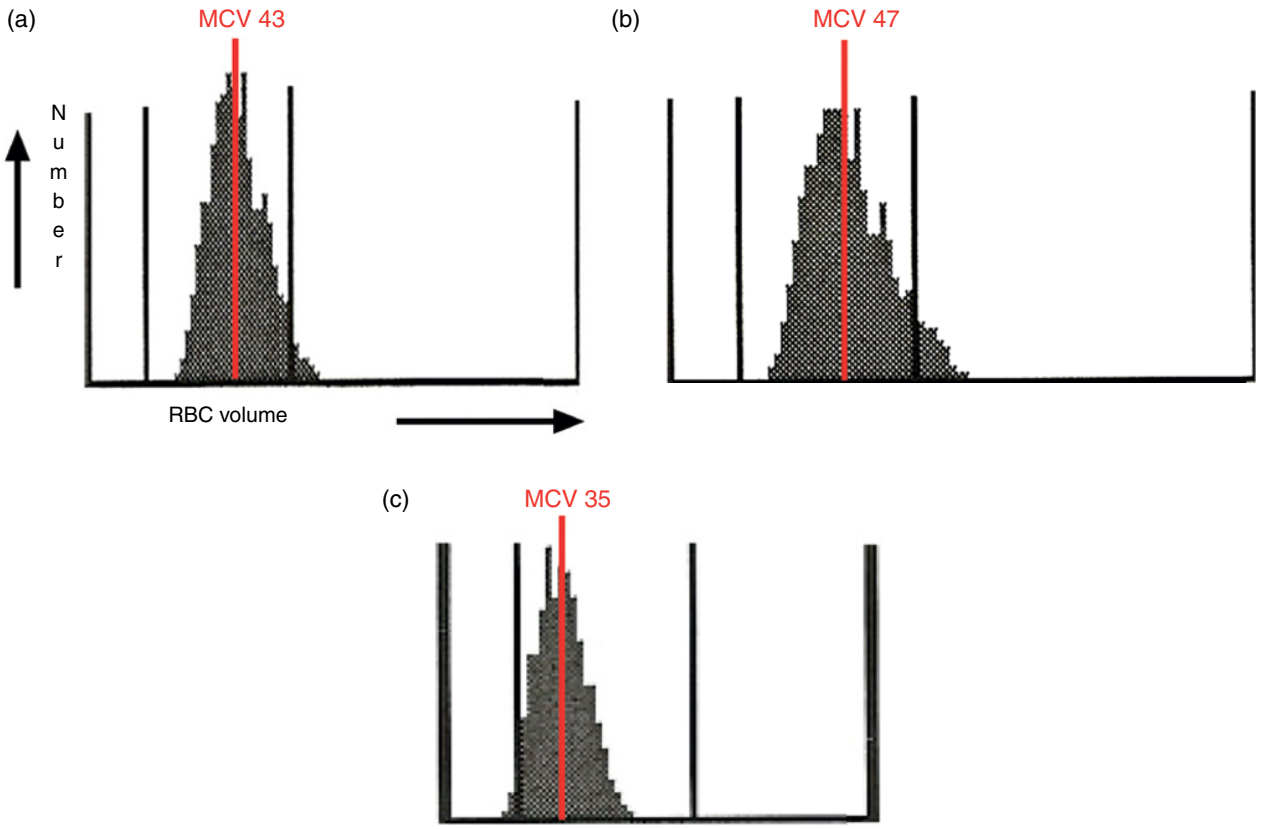

Figure 2.2 Red blood cell (RBC) histograms from the Advia 120 (Bayer Corporation) hematology analyzer. (a) Histogram from a hematologically normal horse. The red line shows the mean cell volume (MCV) in femtoliters; the black lines represent the instrument's preset range of equine RBC volume. (b) Histogram from a horse with a macrocytic anemia. Note the widening of the histogram to include a right shoulder. The MCV is still within the reference limits established for this instrument (38–55 fL), but there is an emerging population of macrocytes suggesting a regenerative response. (c) Histogram from a horse with a microcytic anemia. The whole population of RBCs is microcytic, resulting in a shift of the entire histogram to the left. This horse had an anemia of chronic disease.

agglutination may cause increases in MCH or MCHC due to a spuriously low RBC count. However, as discussed previously, decreases in RBC count due to agglutination may be countered by spuriously increased MCV measurements, resulting in minimal impact on the MCH and/or MCHC. Another common cause of increased MCH and/or MCHC is the presence of lipemia, which results in spurious increases in the Hb measurement. Heinz bodies will also falsely increase MCH and MCHC when determined by laser hematology analyzers and will spuriously increase the Hb measurement with spectrophotometric methods. *In vitro* hemolysis will also increase the MCH and MCHC because the number of intact RBCs is disproportionately low for the amount of Hb measured.

Decreases in MCH and/or MCHC are typically associated with regenerative responses to anemia in species that release reticulocytes in large numbers. Since horses do not usually release substantial numbers of reticulocytes into circulation in a regenerative response, the regenerative response is often normochromic. In other species, decreased hemoglobin concentration as a result of iron deficiency causes hypochromic (and microcytic) anemia, but in horses iron deficiency anemia is reported to be normochromic [23].

2.1.1.4 Age and Breed Effects on RBC Parameters

Relative to adults, erythrocyte number, Hb, and hematocrit (Hct) are increased at birth, decline sharply within 12–24 hours, and then show a gradual decline over the subsequent two weeks to levels at the lower end of adult reference intervals. This change is suspected to be due to the transfusion of placental blood to the foal with subsequent catecholamine release and fluid balance adjustment due to osmotic effects from absorption of immunoglobulins in colostrum [25]. Continued decline is thought to be due to factors such as decreased erythrocyte circulating lifespan, decreased iron delivery to the bone marrow, and reduced stimulation for erythropoietin production from higher hemoglobin saturation.

The MCV is high at birth and decreases to reach a nadir at 3–5 months of age; values are microcytic relative to adult reference intervals until 9 months to 1 year of age [26]. The microcytosis is thought to be due to a relative iron deficiency from limited storage of body iron or low concentration of iron in the dam's milk [23].

Breed effects on erythrocyte indices are reflected in higher Hct, Hb, and RBC counts in "hot-blooded" breeds (Arabians and Thoroughbreds) compared with the "cold-blooded" draught horse and pony breeds. In addition, Thoroughbreds have a smaller reported MCV compared to draught horses [26]. The use of breed-appropriate and age-specific reference values is therefore very important.

2.1.1.5 Splenic Effects

The equine spleen can store up to a third of the RBC mass and rapidly transfer large numbers of erythrocytes into the systemic circulation following epinephrine-induced splenic contraction [25, 27]. Epinephrine-induced splenic contraction is associated with excitement or strenuous exercise. Depending upon the baseline PCV, splenic contraction may result in erythrocytosis or a normal PCV. The time taken for the PCV to return to baseline following contraction may be 40–60 minutes to up to several hours, depending upon the magnitude of the stimulus.

In contrast, splenic RBC sequestration and congestion following barbiturate, alpha-2 agonist, or halothane anesthesia may drop the PCV below baseline values [28]. Thus, the spleen's large storage capacity may impact significantly on the circulating RBC mass. Anemia could potentially be masked following splenic contraction or simulated secondary to anesthetic-induced splenic congestion and RBC sequestration.

2.1.2 The Leukogram

The leukogram includes the numeric and morphological data pertaining to white blood cells. The leukogram, like erythrocyte indices, can provide information regarding the presence of a pathological or pathophysiological process, but rarely leads to a specific diagnosis. There are distinct leukogram profiles associated with inflammation, corticosteroids, and epinephrine.

2.1.2.1 Leukogram Patterns

2.1.2.1.1 Inflammation Acute inflammation results in the release of mature neutrophils and bands from the marrow storage and maturation pools, so neutrophilia with a left shift is characteristic of an active need for neutrophils. The marrow responds to inflammatory cytokines released into the blood by replenishing the storage and maturation pools from the stem cell and proliferation pools, resulting in a chronic or compensated inflammatory leukogram characterized by a mature neutrophilia. Inflammatory mediators stimulate neutropoiesis and subsequent granulocytic hyperplasia in the bone marrow. The total 7–9 days neutrophil transit time in health decreases to 3–4 days with inflammatory cytokine stimulation. When there is peracute, severe inflammation, neutropenia may occur as marked tissue demand depletes the storage pool before enhanced neutropoiesis can replenish the storage and maturation pools. A simplified depiction of the growth factors responsible for stimulation of neutrophil production and release is presented in Figure 2.3.

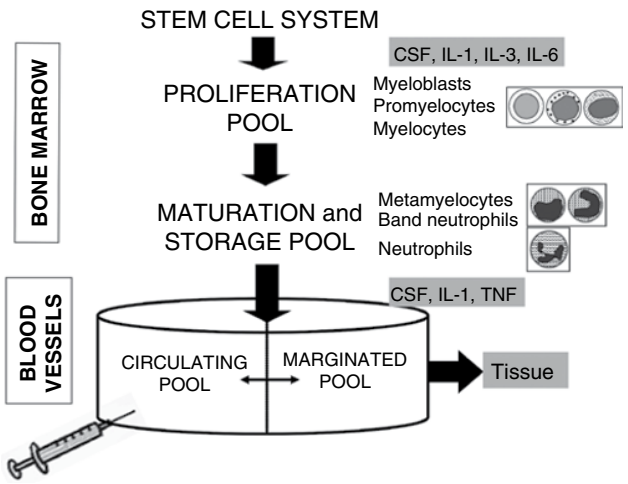

Figure 2.3 Schematic diagram of bone marrow and blood neutrophil pools. Inflammatory mediators released into the blood stimulate the marrow to produce neutrophils via an increase in growth factors and cytokines, mainly colony-stimulating factors (CSF) and interleukins (IL). G-CSF, GM-CSF, and IL-1, IL-3, and IL-6 are the most prominent in neutropoiesis. Inflammatory mediators and cytokines such as tumor necrosis factors (TNF), IL-1, and CSF increase neutrophil release from marrow sinuses and migration from blood into tissue. The most mature neutrophil forms preferentially leave the marrow; these forms also preferentially migrate into tissue.

The equine neutrophil storage pool is intermediate in size compared to the canine and bovine pools, which have the largest and smallest pools, respectively. Horses may have little to no neutrophilia or left shift during inflammation. Inflammatory neutrophilias in horses only occasionally exceed 20,000/μL and it is uncommon to see neutrophilias greater than 30,000/μL.

Monocytosis may be a feature of both acute and compensated (chronic) inflammation and generally reflects a need for macrophages. Thus, inflammatory processes that elicit histiocytic responses are associated with monocytosis.

2.1.2.1.2 Corticosteroid Response Endogenous and exogenous glucocorticoids produce a characteristic leukogram pattern consisting of a mature neutrophilia and lymphopenia. Unlike dogs and cats, horses do not have monocytosis as part of the glucocorticoid response. The neutrophilia is caused by release of marginated neutrophils into circulation. The ratio of marginated to circulating neutrophils in horses is 1:1, thus the maximum increase in neutrophil concentration due to demargination does not exceed twofold. Lymphopenia is considered the hallmark of the glucocorticoid response and is attributed to margination and emigration of lymphocytes to tissues and lymph nodes; chronic glucocorticoid effects include lymphoid hypoplasia, which contributes to the lymphopenia.

2.1.2.1.3 Physiological (Catecholamine) Response A physiological leukogram results from catecholamine release due to excitement, fear, or vigorous exercise. Catecholamine-associated leukocytosis occurs more often in young horses and stallions. A physiological leukogram, promoted by the effects of catecholamines, is characterized by lymphocytosis and a mature neutrophilia. Catecholamines promote an increase in circulating lymphocytes via demargination, especially from the spleen. The concomitant mature neutrophilia is also due to demargination. A modest mature neutrophilia and a lymphocytosis are characteristic of this leukogram. A lymphocytosis of 6000–14,000/μL is not uncommon. Physiological leukogram responses are transient (20–30 minutes) [29].

2.1.2.2 Changes in Individual Leukocyte Parameters

Sometimes, the changes to the leukogram do not correspond with a particular pattern and an abnormality must be assessed individually.

2.1.2.2.1 Neutropenia Neutropenia is clinically significant since it predisposes the patient to infection. In horses, neutropenia is most often due to increased distribution into tissues and/or rapid margination of circulating neutrophils secondary to overwhelming inflammation and endotoxemia, respectively. Less commonly, neutropenia is due to decreased neutrophil production in the bone marrow, which has been reported due to displacement of marrow by neoplastic cells (myelophthisis), myelonecrosis, and possible immune-mediated disease [29–32]. A rare cause of cyclic neutropenia was reported in related Standardbred horses believed to be caused by bone marrow microenvironment or growth factor defects [33].

2.1.2.2.2 Lymphocytosis Antigenic stimulation may produce lymphocytosis, but lymphocytosis in horses is more commonly attributable to epinephrine-associated responses than to antigenic stimulation. The presence of reactive lymphocytes supports an interpretation of antigenic stimulation even in the absence of absolute lymphocytosis.

Leukemias are rare in horses, though lymphocytic leukemia is the most common leukemia reported [34]. Leukemias are characterized as acute or chronic and by the cell line affected. Acute leukemias are represented by blast-like cells, whereas neoplastic cells in chronic leukemias appear fully differentiated. Chronic lymphocytic leukemia (CLL) manifests as peripheral mature lymphocytosis. Both B- and T-cell chronic lymphoid leukemias have been described [35–37]. Reported lymphocyte counts in blood range from 30,000 to 492,300/μL.

Immunophenotyping of CLL can be accomplished via flow cytometric analysis of the blood [35, 37, 38].

Small cell lymphoma (SCL) typically arises within tissue (lymph nodes and viscera), and peripheral blood involvement may occur in advanced stages when the marrow is infiltrated (secondary leukemia). In primary leukemia, neoplastic lymphocytes originate in the bone marrow and secondarily spread into the peripheral blood. In advanced cases of CLL, neoplastic lymphocytes may infiltrate lymph nodes and other tissues, making differentiation between CLL and SCL difficult or impossible. However, in most species, the distinction between SCL and CLL is not important. In the WHO classification of hematopoietic tumors of domestic animals, small cell lymphocytic leukemias and lymphomas derived from the same neoplastic clone are classified in the same category (i.e., B/T-cell SCL/CLL). By convention, if the neoplastic cells are predominantly in blood and bone marrow, the disease is referred to as leukemia, whereas if the neoplastic proliferation manifests primarily in tissue, the disease is referred to as lymphoma. CLL and SCL are indolent diseases with prolonged survival.

2.1.2.2.3 Lymphopenia In addition to corticosteroid affects from stress or administration of steroids or acute inflammation, lymphopenia (including both B- and T-cells) may also be seen with combined immunodeficiency. Combined immunodeficiency has been described in Arabian and Arabian-cross foals and results in a severe lymphopenia (<1000/μL) and the lack of IgM production [39]. Severe combined immunodeficiency has also been described in a Caspian filly [40]. Lymphopenia has also been noted with infections with *Anaplasma* spp. and with early viral infections such as herpesvirus type I (HV-1).

2.1.2.2.4 Eosinophilia Production of eosinophils from the bone marrow takes 2–6 days. Eosinophilia is uncommon in horses but is most often inflammatory, associated with parasitic, fungal, and allergic (hypersensitivity) conditions, and the degree can be variable depending on the organ(s) affected. Eosinophilic myeloproliferative disease is rare but has been reported [41]. Paraneoplastic eosinophilia has been reported with intestinal lymphoma in a horse [42]. Multisystemic eosinophilic epitheliotropic disease, while associated with eosinophilic abdominal effusions and eosinophilic marrow hyperplasia, is not usually associated with peripheral eosinophilias [43, 44].

2.1.2.2.5 Monocytopenia, Eosinopenia, and Basopenia Monocytopenia, eosinopenia, and basopenia are difficult to document due to relatively low numbers being present in healthy animals. These conditions are not considered to be clinically significant, though eosinopenia may be appreciated with exposure to corticosteroids.

2.1.2.2.6 Pancytopenia Pancytopenia refers to reduced numbers of all three cell lines (anemia, neutropenia, and thrombocytopenia). Pancytopenia may occur secondary to myelophthisis, defined as crowding of the bone marrow, usually by neoplastic cells. Pancytopenia may also occur due to destruction or suppression of early multipotential hematopoietic stem cells from viral infections, necrosis, medications (e.g., trimethoprim; sulfa drugs; pyrimethamine) [45], or toxins.

2.1.3 The Thrombogram

In horses, platelet number and size are shown to be directly proportional rather than inversely proportional as in some species. Normal platelet counts in horses are the lowest of the common domestic species. Equine platelets are also smaller compared to dog and cat platelets with a mean platelet volume of approximately 5.0 fL [46].

2.1.3.1 Thrombocytopenia

The causes of thrombocytopenia are increased platelet utilization, decreased production, and increased destruction and/or sequestration. Of these etiologies, equine thrombocytopenia is most commonly attributable to consumptive processes related to inflammation and endotoxemia [47]. Prothrombotic stimuli, especially potent platelet activators such as thrombin and platelet activating factor (PAF), are produced subsequent to endotoxemia and with severe inflammation. Systemic activation of the coagulation system associated with severe inflammation and/or endotoxemia may result in thrombocytopenia from platelet activation and consumption [48]. Thrombocytopenia may be present in colic horses with or without disseminated intravascular coagulation (DIC) [49]. Thrombocytopenia is a common sequela of snake envenomation, likely through consumption and sequestration due to inflammation caused by venom components [50, 51].

A less frequent cause of equine thrombocytopenia is immune-mediated destruction (IMT). Etiologies associated with IMT include infectious, neoplastic and idiopathic. In equine infectious anemia (EIA), infection immune complexes consisting of EIA virus particles and antibodies deposit on platelets, targeting them for destruction. In addition, EIA-induced IMT shows a lack of compensatory megakaryocytopoiesis, which contributes to the development of thrombocytopenia [52, 53]. The mechanism of thrombocytopenia in *A. phagocytophilum* infection may also be immune mediated [54]. IMT has also been reported in horses with lymphoma, secondary to drugs

(e.g., trimethoprim, penicillin) and as an idiopathic disorder [47, 53, 55]. Intermittent thrombocytopenia attributable to decreased production is unusual, but has been reported in conjunction with myeloid and megakaryocytic hypoplasia in related Standardbreds [33].

Pseudothrombocytopenia occurs when platelets are not counted in a blood sample. This may be the result of traumatic venipuncture resulting in platelet aggregation and clumping or EDTA-associated platelet clumping [56]. If the latter is suspected, reevaluation of the blood using lithium heparin anticoagulant is recommended.

2.1.3.2 Thrombocytosis

Thrombocytosis in most species is most commonly attributable to physiological or reactive processes. Physiological thrombocytosis occurs secondary to epinephrine-induced splenic contraction. Reactive thrombocytosis is reported secondary to inflammation, infection, or neoplasia. In one study population, thrombocytosis was reported in 1% of horses over a five-year period and was highly associated with inflammatory/infectious disease [57]. Thrombocytosis was also more likely to occur in younger horses and stallions.

2.2 Blood Film Evaluation

A blood film can be dissected into three parts: the body, the monolayer, and the feathered edge (Figure 2.4). The general approach to a blood film can be summed up as follows.

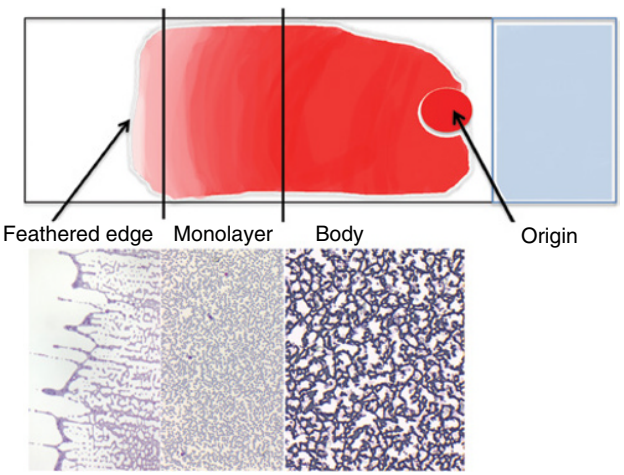

Figure 2.4 Anatomy of a blood smear. The feathered edge and monolayer should be scanned at first low power (4–10×) and the monolayer then evaluated at high magnification (50–100×). Note that leukocytes and erythrocytes cannot be evaluated adequately in the smear body due to the thickness of the preparation in this area.

1) Low-power scan (4× or 10×) of the feathered edge for platelet clumps, large cells, or microorganisms (Figure 2.5).
2) Low-power scan (10×) of the monolayer to estimate RBC and WBC density (Figure 2.6).
3) High-power (40× or 100×) scan of the monolayer to evaluate cellular morphology and perform leukocyte differential and platelet estimation. Note that the 40× objective was designed for use with coverslipped samples and will not focus properly unless the slide has a coverslip. A coverslip may be temporarily placed on the slide for viewing purposes or permanently fixed to the slide with mounting medium.

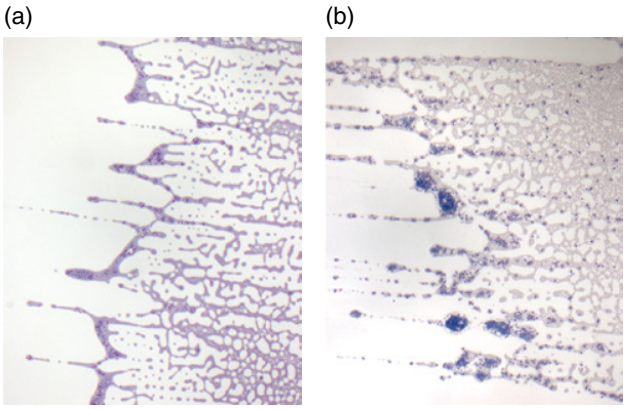

Figure 2.5 Comparison of the feathered edge from two different blood smears. (a) Normal blood smear feathered edge. (b) Note the presence of large platelet clumps that can interfere with both the automated platelet count and manual platelet estimation.

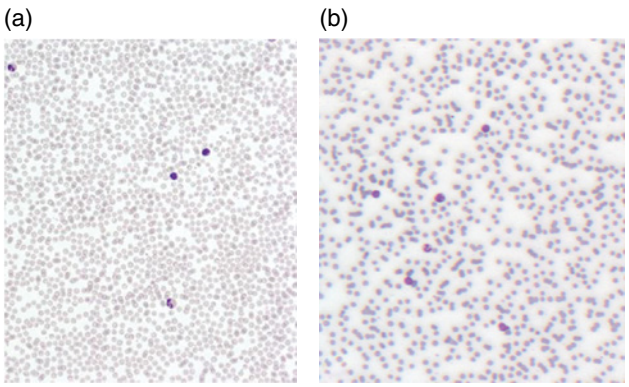

Figure 2.6 Evaluation of red blood cell density at low magnification (10×). (a) Monolayer of a normal blood smear. (b) Monolayer from an anemic blood smear. Note the difference in the concentration of erythrocytes in the monolayers.