SECOND EDITION

VETERINARY HEMATOLOGY AND CLINICAL CHEMISTRY

MARY ANNA THRALL, GLADE WEISER,
ROBIN W. ALLISON, AND TERRY W. CAMPBELL

with website

WILEY-BLACKWELL

Veterinary Hematology and Clinical Chemistry

Second Edition

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SECOND EDITION

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The authors wish to dedicate this book to their mentors, the pioneers in veterinary clinical pathology. In particular, the book is dedicated to Drs. Maxine Benjamin, Oscar Schalm, and J.J. Kaneko for their respective first-generation discovery and textbooks addressing veterinary clinical pathology, hematology, and clinical chemistry and for their inspiration to the many subsequent careers in veterinary clinical pathology.

Dr. Mary Anna Thrall wishes to thank and remember Dr. Maxine Benjamin for her generosity, patience, and friendship. The authors acknowledge and remember Dr. E. Duane Lassen for his important contributions to the first edition of this textbook. He has since lost a hard-fought battle with cancer. He was an outstanding teacher, excellent clinical pathologist, and dear friend across much of the veterinary clinical pathology community.

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Extra PowerPoint presentations available online at www.wiley.com/go/thrall.

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Preface

On behalf of the contributing authors and Wiley-Blackwell we are pleased to introduce the Second Edition of *Veterinary Hematology and Clinical Chemistry*. Our goal is to provide an image-rich, readable resource addressing routine laboratory diagnostics in veterinary practice. The theme of the presentation is applied clinical pathology for veterinary students and veterinary health professional teams in the practice setting. We aimed to maintain our intended target audience and original organizational structure.

Audience

A continuing trend in frontline veterinary medicine is the movement of laboratory diagnostics into the veterinary facility. Evolving technological advancements in point-of-care diagnostic capability drives this trend, which increases the need for education in veterinary clinical pathology. Although this book was written primarily for veterinary students and practitioners, it has applications for a broader audience, serving as a useful adjunct for the educational and reference needs of a variety of other users. The following audiences may benefit from this resource:

- students in professional veterinary medical education programs;
- health professional teams in veterinary care facilities;
- clinical pathologists and clinical pathologists in training;
- product development groups utilizing veterinary clinical pathology.

Organization

Veterinary Hematology and Clinical Chemistry is organized into six sections, arranged as follows:

- I: presents principles of laboratory technology and test procedures used in veterinary laboratories to generate laboratory results. It also presents perspectives on how laboratory data interpretation is used in diagnosis and overall clinical case management.
- II: presents hematology and hemopathology of common domestic species. This includes all aspects of the hemogram or complete blood count, bone marrow, hemostasis, and transfusion medicine.
- III: presents hematology of common nondomestic species encountered in veterinary practice.
- IV: presents clinical chemistry of common domestic species and is organized primarily by organ system.
- V: presents clinical chemistry of common nondomestic species.
- VI: is a compilation of clinical cases. Each case includes a signalment, brief history, and pertinent physical examination findings. Then, relevant laboratory data are presented in tables followed by a narrative interpretation of the data.

Revisions and additions

Some of the more important revisions and additions include the following.

The overview of laboratory technology has been updated to reflect continued advances in and adoption of in-clinic diagnostic instrumentation and capabilities. Some of the historical laboratory procedures that are no longer used have been removed. Next, we comment on data interpretation skills. Our experience indicates that veterinarians are reasonably adept at understanding how laboratory tests relate to pathophysiology, but then don't think probabilistically about the magnitude of data abnormalities and often struggle interpreting complex data sets. Rules for interpreting diagnostic tests assume homogeneity of pathophysiologic responses, or that our animal friends have "read the book"; as imagined in Figure P.1.

However, we know that there are many variables that create considerable biologic variability in expected responses. Chapter 3, Perspectives in Data Interpretation, has been revised to provide introductory guidance to build the skill set required for adroit interpretation of laboratory data. This involves development of flexible, probabilistic thinking skills when solving the complex puzzle formed by the array of clinical findings and laboratory data.

Extensive revision and some additions have been made possible for selected chapters by incorporation of content from new authors. Examples include:

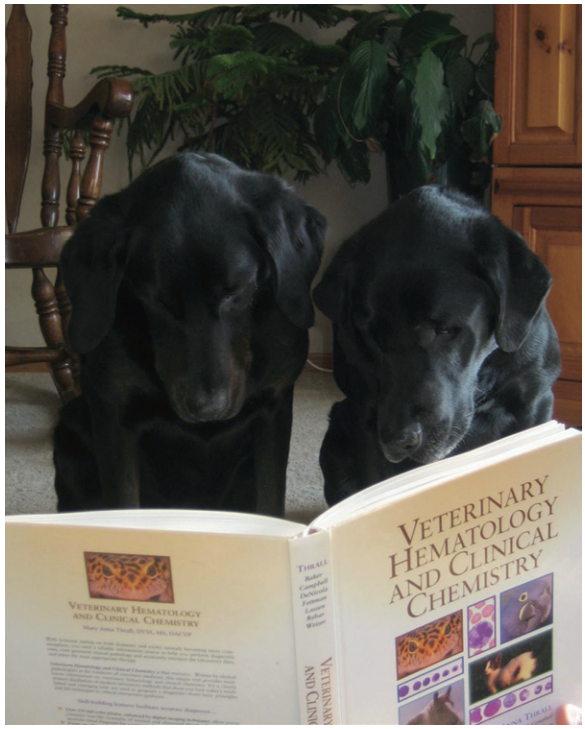

Figure P.1 Dogs caught 'reading the book' in an attempt to make their disease responses predictable. Not all will read the book. (Courtesy of Dr. Sara Hill.)

• Wayne Jensen, Morris Animal Foundation, provides an update of the rapidly evolving area of immunodiagnostics.
• Advances have continued to be made in the diagnosis and classification of hematopoietic cell neoplasia. A new chapter from Anne Avery, Colorado State University, provides an overview of evolving molecular and flow cytometric diagnostics applied to hematopoietic neoplasia. This complements the other chapters detailing leukocyte responses, bone marrow evaluation, and hematopoietic proliferative disorders.
• Robin Allison, Oklahoma State University, has made extensive revision of laboratory diagnostics related to pathology involving liver, pancreas, blood proteins, and muscle.
• Andrea Bohn and Glade Weiser, Colorado State University, have revised the often-dreaded subjects of electrolyte and acid-base pathology, with an attempt to simplify clinical understanding of these laboratory tests.
• Judy Radin, The Ohio State University, has provided a new chapter covering lipid pathology.
• Last, but not least, Don Meuten, North Carolina State University, brings his extensive experience and expertise cultivated from too many years in both anatomical and clinical pathology. He contributes new treatment of renal, endocrine, and calcium metabolic pathologies.

The Clinical Case Presentations were a separately bound supplement to the 1st Edition. In the 2nd Edition, the Clinical Case Presentations are incorporated into this single-bound textbook. These presentations are intended to provide students "practice" to develop interpretive skills by seeing examples of how data are interpreted into pathologic processes and how pathologic processes may culminate in a diagnostic scenario. The original cases are retained because their classical usefulness does not change. In addition, a number of new cases have been added by some of the new contributing authors.

It is our wish that readers not only learn principles and skills from this work, but also enjoy interacting with it. As veterinarians and specialists in bioanalytical pathology, we share our passion for the art and science of laboratory diagnostics applied to animal health.

Respectfully submitted,

Glade Weiser
Mary Anna Thrall
Robin Allison
Terry Campbell

Preface to the First Edition

The publication of Veterinary Hematology and Clinical Chemistry marks a new and unique contribution to veterinary clinical pathology. The product of a collaborative effort by a team of experts in the field, this text combines critical information about performing diagnostic tests, viewing pertinent clinical pathology, and interpreting laboratory data with an innovative approach to incorporating color visual content.

Audience

A current trend in the field is the movement of laboratory diagnostics into the veterinary facility, enabled by technological advancements in point-of-care diagnostic capability. This movement to in-house testing increases the need for education in veterinary clinical pathology. Although this book was written primarily for veterinary students and practitioners, it has applications for a much broader audience, serving as a useful adjunct for the educational and reference needs of a variety of other users. The following audiences will benefit from this resource:

- Students in professional veterinary medical education programs
- Veterinary health professional teams in veterinary care facilities
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Organization

Veterinary Hematology and Clinical Chemistry is organized into six parts, arranged as follows:

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- Part IV presents clinical chemistry of common domestic species and is organized primarily by organ system.
- Part V presents clinical chemistry of common nondomestic species.

Unique art program

Many aspects of veterinary clinical pathology are highly visual. The most unique feature of this book is the quantity and quality of color artwork. This was facilitated by digital image acquisition and processing performed by the authors. Optimization and standardization of images was performed by digital image engineering techniques to achieve an improvement in imagery over what is possible with conventional photomicrography. Our goal was to bring a new level of realism to the visual communication of concepts pertaining to microscopy. In some instances, visual content has been amplified by combining images from multiple microscopic fields into a single figure or showing different levels of magnification within the same figure. Digital image engineering also allows for image manipulation; an example is arrangement of cells that are randomized on a microscope field into a specific order to convey a concept such as cell maturation. We believe that the fidelity of visual imagery, as well as its liberal integration with text content, makes this work the first of its kind.

Author team

Contributing content and expertise to this project are a number of recognized authorities in the field of veterinary clinical pathology. These individuals have helped shape the existing curriculum, train the existing faculty, and create the disciplines of comparative laboratory medicine and diagnostic cytology as we know them today. It is through the combined efforts of so many experts in the field that this book was made possible.

We hope you find this publication to be an excellent resource in the clinical laboratory and for laboratory data interpretation.

M. G. Weiser and M. A. Thrall
Fort Collins, Colorado

I General Principles of Laboratory Testing and Diagnosis

1 Laboratory Technology for Veterinary Medicine

Glade Weiser
Colorado State University

This chapter presents an overview of the laboratory technology used to generate data for hematology and clinical biochemistry. For the procedures and technologies likely to be employed within veterinary hospitals, general instructions and descriptions provide a review of the principles previously learned in laboratory courses. This, in conjunction with the instructions accompanying different devices and consumables, should enable users to reproduce the procedures to a satisfactory performance standard. For technologies more likely to be used only in large commercial or research laboratories, the overview provides familiarity with the basic principles.

Hematologic techniques

Basic techniques applicable for any veterinary hospital

The procedures outlined here are most appropriate for the in-house veterinary laboratory in most practice settings. These procedures, with the exception of a cell counting hematology system, require minimal investment in instrumentation and technical training. These basic hematologic procedures include:

- Blood mixing—for all hematologic measurements
- Packed cell volume or hematocrit by centrifugation
- Plasma protein estimation by refractometry
- Cell counting instrumentation
- Preparation of blood films
- Differential leukocyte count and blood film examination

Blood mixing

The blood sample is assumed to have been freshly and properly collected into an ethylenediaminetetraacetic acid (EDTA) tube (as described in Chapter 2). When performing any hematologic procedure, it is important that the blood is thoroughly mixed. Cellular components may settle rapidly while the tube sits on a counter or in a tube rack (Fig. 1.1). As a result, failure to mix the sample before removing an aliquot for hematologic measurement may result in a serious error. Mixing can be performed by manually tipping the tube back and forth a minimum of 10–15 times (Fig. 1.1). Alternatively, the tube may be placed on a rotating wheel or tilting rack designed specifically to mix blood (Fig. 1.2).

Packed cell volume

The packed cell volume value is the percentage of whole blood composed of erythrocytes. It is measured in a column of blood after centrifugation that results in maximal packing of the erythrocytes. Tools for performing the packed cell volume include 75×1.5-mm tubes (i.e., microhematocrit tubes), tube sealant, a microhematocrit centrifuge, and a tube-reading device.

The procedure is performed using the following steps. First, the microhematocrit tube is filled via capillary action by holding it horizontally or slightly downward and then touching the upper end to the blood of the opened EDTA tube (Fig. 1.3).

Next, allow the tube to fill to approximately 70–90% of its length. Hold the tube horizontally to prevent blood from dripping out of the tube, and seal one end by pressing the tube into the tube sealant once or twice (Fig. 1.4). Note that air may be present between the sealant and the blood (Fig. 1.4). This is not a problem, however, because the trapped air is removed during centrifugation.

The tube is then loaded into the microhematocrit centrifuge according to the manufacturer's instructions (Figs. 1.5 and 1.6). The microhematocrit centrifuge is designed to spin the lightweight tube at very high speeds to generate sufficient centrifugal force to completely pack the red cells within 2–3 minutes. With such centrifugal force, most (or all) of the plasma is removed from the layers of packed cells.

Veterinary Hematology and Clinical Chemistry, Second Edition. Edited by Mary Anna Thrall, Glade Weiser, Robin W. Allison, and Terry W. Campbell.

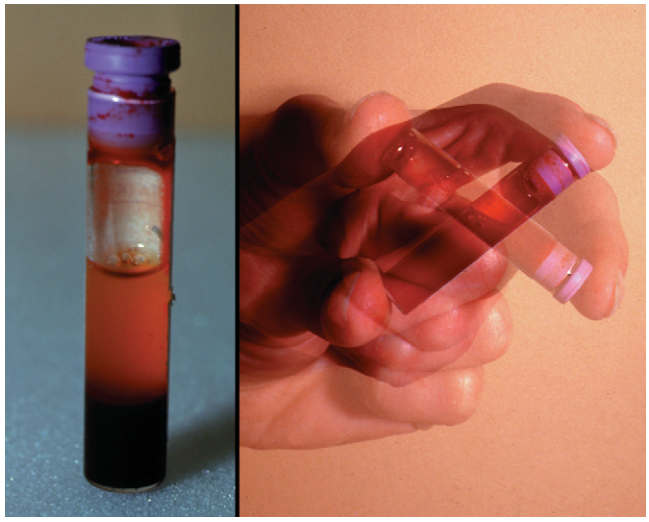

Figure 1.1 Left. Gravity sedimentation of whole blood. Right. A gentle, repetitive, back-and-forth tube inversion technique used to manually mix blood before removing aliquots for hematologic procedures.

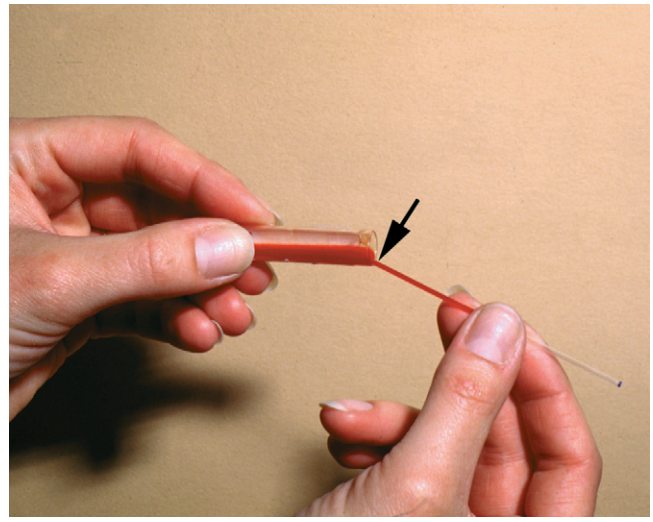

Figure 1.3 Proper technique for filling a microhematocrit tube. The tube should be positioned horizontally or tilted slightly downward to facilitate filling by capillary action. Capillary action is established by touching the upper end of the tube to the blood (arrow).

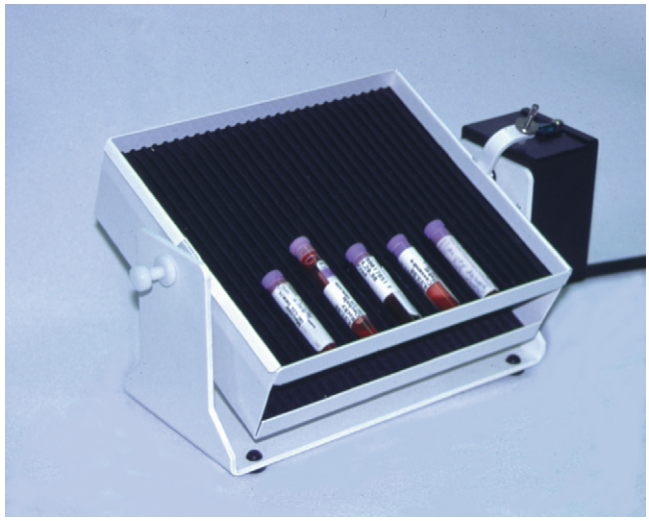

Figure 1.2 Representative mechanical blood-mixing table. The surface holds several tubes on a ribbed rubber surface and tilts back and forth at the rate of 20–30 oscillations per minute.

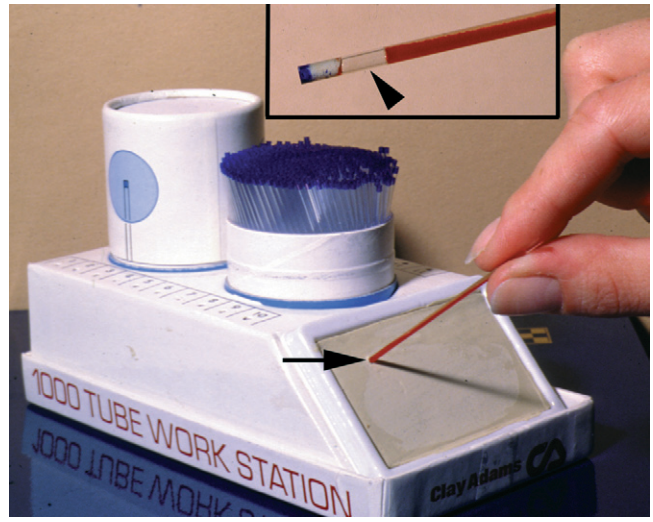

Figure 1.4 A microhematocrit tube is sealed by pressing two to three times into the clay sealant (arrow). Note that a small amount of air trapped between the blood and white clay is not a problem (arrowhead in the inset).

Three distinct layers may be observed in the tube after removal from the centrifuge: the plasma column at the top, the packed erythrocytes at the bottom, and a small, middle white band known as the buffy coat (Fig. 1.7). The buffy coat consists of nucleated cells (predominantly leukocytes) and platelets, and it may be discolored red when the nucleated erythrocyte concentration is prominently increased. Observations of any abnormalities in the plasma column above the red cells should be recorded. Common abnormalities such as icterus, lipemia, and hemolysis are shown in Figure 1.7. Icterus is excessively yellow pigmentation of the plasma column that suggests hyperbilirubinemia; the magnitude of this hyperbilirubinemia should be confirmed by a biochemical determination of serum bilirubin concentration (see Chapter 26). The observation of an icteric coloration to the plasma is diagnostically useful in small animals. It is not reliable in large animal species, however, because their serum usually has a yellow coloration from the normal carotene pigments associated with their herbivorous diet. Lipemia is a white, opaque coloration of the plasma column

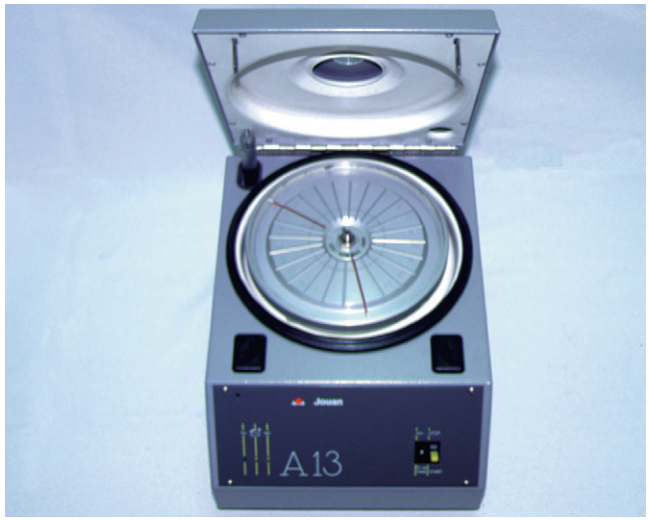

Figure 1.5 Representative microhematocrit centrifuge. The head and motor are designed to spin the tubes at very high speeds to achieve maximal erythrocyte packing.

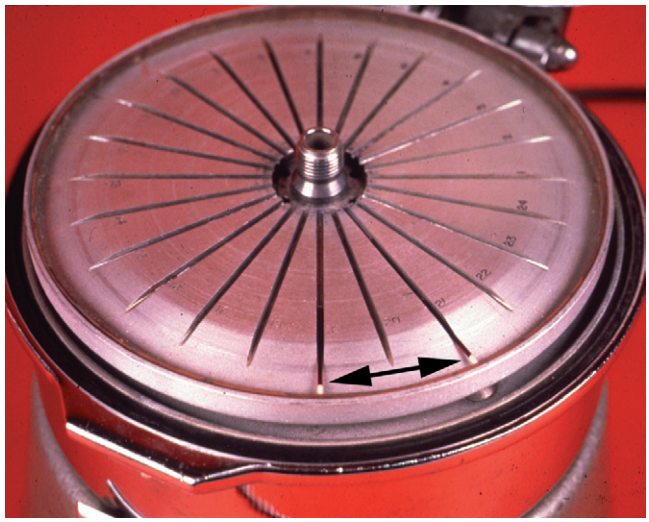

Figure 1.6 Placement of microhematocrit tubes on a microhematocrit centrifuge head. Note the proper orientation of two microhematocrit tubes, with the clay-sealed end positioned at the outer ring of the centrifuge head (double arrow).

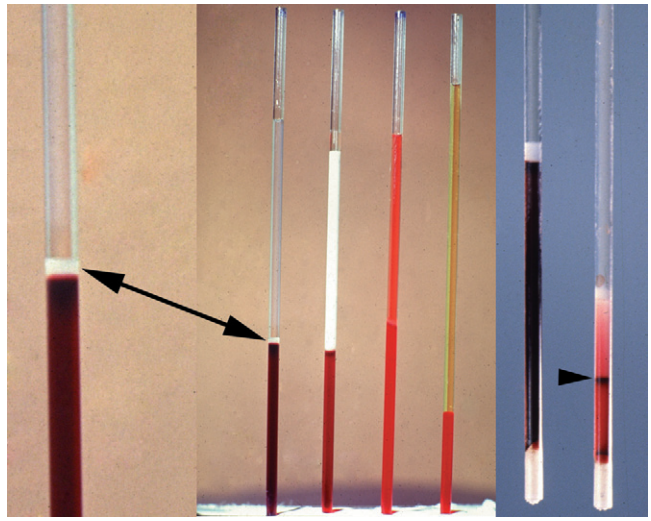

Figure 1.7 Normal and abnormal spun microhematocrit tubes (4 tubes in middle panel). The tube on the left is normal. Note the packed erythrocytes at the bottom, plasma layer at the top, and buffy coat in the middle (arrow; enlarged at left). The second tube illustrates lipemia, the third hemolysis, and the fourth icterus. Note also that the hematocrit is considerably decreased in the fourth tube. Two additional tubes illustrate buffy-coat abnormalities (enlarged at right). The first of these tubes has an increased buffy coat that correlates with an increased leukocyte concentration. The second (right) is from a sheep with leukemia and has a dramatically increased buffy coat. The leukocyte concentration was greater than 400,000 cells/μL. There is also severe anemia. With such major abnormalities in cell concentration, separation of erythrocytes and leukocytes is not complete, and division may be blurred. What is interpreted as being the "top" of the erythrocyte column is indicated by the arrowhead. The red discoloration of the buffy coat may be caused by a prominent increase in nucleated erythrocytes.

because of the presence of chylomicrons. Lipemia most commonly is associated with the postprandial collection of blood, but it also may be associated with disorders involving lipid metabolism (see Chapter 31). Hemolysis is a red discoloration of the plasma column, which usually results from artifactual lysis of red cells induced during the collection of blood. A small quantity of lysed erythrocytes is sufficient to impart visual hemolysis. Therefore, if the hematocrit is normal, one may assume it is an artifact. Less commonly, causes of anemia that result in intravascular hemolysis give rise to observable hemolysis in the plasma fraction, which also is known as hemoglobinemia (see Chapter 8). This will typically also be associated with hemoglobinuria.

The packed cell volume is measured on a reading device, such as a microhematocrit card reader (Fig. 1.8). The procedure is performed by positioning the erythrocyte–clay interface on the 0 line and the top of the plasma column on the 100 line. The position of the top of the erythrocyte column is then read on the scale as the packed cell volume.

Plasma proteins by refractometry

After measurement and observation of the microhematocrit tube, the plasma column may be used to estimate the plasma protein concentration on the refractometer (Fig. 1.9). This instrument may be used to estimate the concentration of any solute in fluid according to the principle that the solute refracts (or bends) light passing through the fluid to a degree that is proportional to the solute concentration. The principle or property being measured is the refractive index relative to distilled water. The scale for a particular solute can

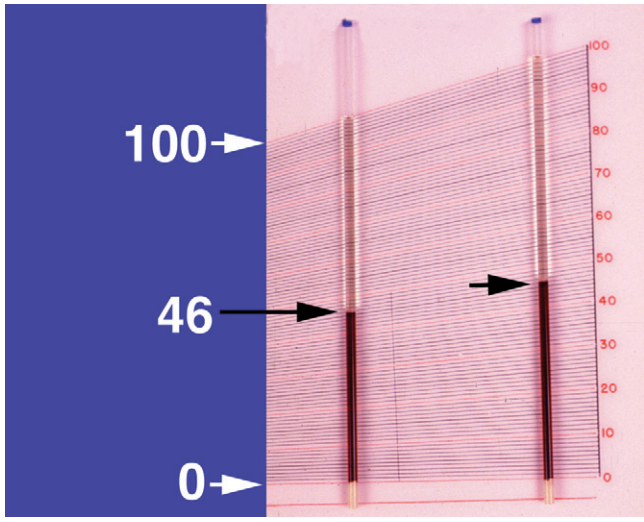

Figure 1.8 Determination of packed cell volume on a microhematocrit tube card reader using two tubes of blood from the same patient sample. Note that the scale allows the tube to be read over a considerable range of filling levels. The steps are to line up the erythrocyte–clay interface with the 0 line, line up the top of the plasma column with the 100 line, and then read the top of the erythrocyte column on the scale. The positions of these steps are indicated by the arrows. Note in this example that the packed cell volume is 46%.

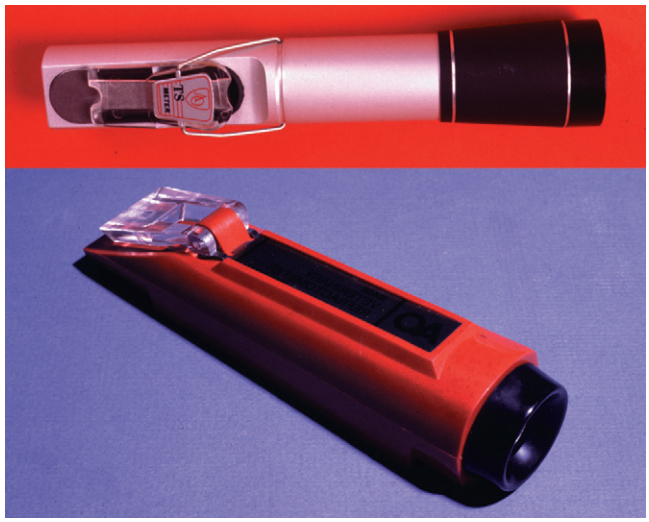

Figure 1.9 Refractometers. The lower refractometer is more rugged, because it is encased in rubber. It is known as a veterinary refractometer, and it has a canine and feline urine specific gravity scale that calibrates for minor differences between species during this determination.

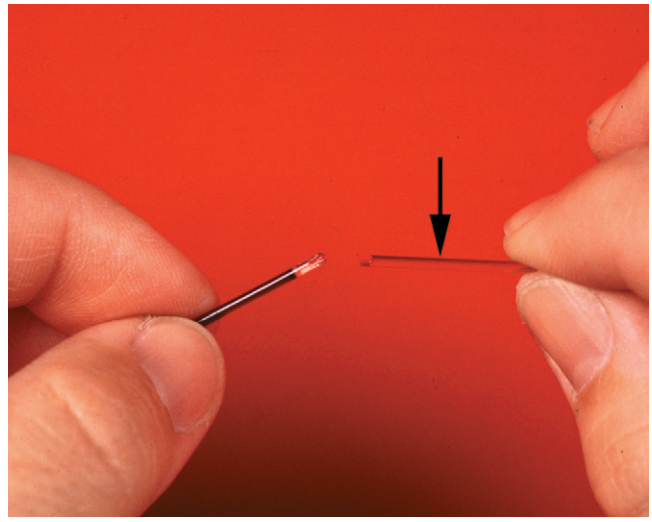

Figure 1.10 Preparation of the microhematocrit tube for measuring plasma protein concentration. The tube is broken just above the buffy coat to yield a column of plasma (arrow).

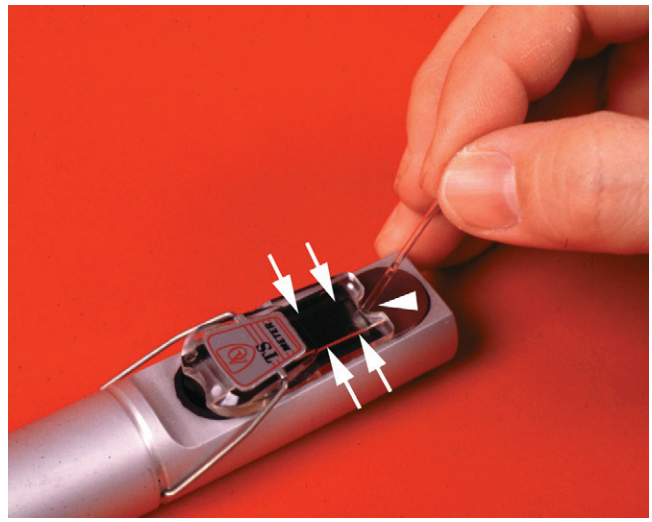

Figure 1.11 Loading plasma from the microhematocrit tube to the refractometer. To wick plasma onto the refractometer, capillary action is established by touching the end of the plasma tube at the notch of the prism cover (arrowhead). Flow should establish a thin layer of plasma under the plastic cover to fill the area delineated by arrows. After reading, the plastic cover is flipped back and wiped clean with a laboratory tissue.

be developed from refractive index measurements calibrated to solutions with known solute concentrations. In clinical diagnostics, refractometry is used to estimate the plasma protein concentration and urine specific gravity.

Plasma protein is measured using the plasma column in the microhematocrit tube. The tube is broken above the buffy coat layer (Fig. 1.10), and the portion of the tube containing the plasma is used to load the refractometer (Fig. 1.11). The instrument then is held so that an ambient light source can pass through the prism wetted with plasma, and the light refraction is read on a scale through an eyepiece (Fig. 1.12).

The protein measurement is regarded as being an estimate based on calibration, assuming that other solutes in the serum are present in normal concentrations. The measure-

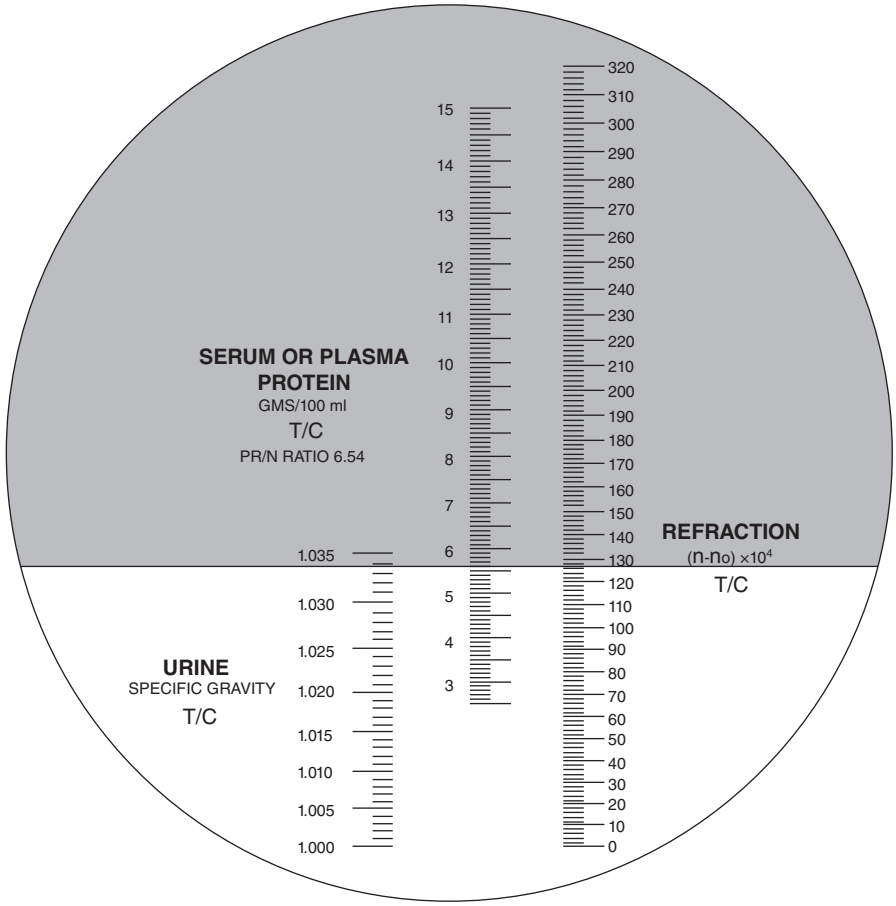

Figure 1.12 Representative refractometer scale as seen through the eyepiece. Light refraction creates a shadow–bright area interface that is read on the appropriate scale.

ment may be influenced by alterations in other solutes. Most notably, lipemia may artificially increase the protein estimate by as much as 2 g/dL. Other alterations of solutes such as urea and glucose influence the protein estimate to a much lesser, and usually negligible, degree.

Determination of total leukocyte concentration

Two general approaches are available to determine the leukocyte concentration. Historically, cell concentrations were measured manually using a blood dilution placed onto a hemocytometer and counted while observing by microscopy. This procedure, and associated consumables, is regarded as obsolete for the veterinary practice setting. Over the past 30 years this procedure has been progressively replaced by automated cell counting hematology systems or alternatively expanded buffy coat analysis technology in which cellular estimates are made from layers in a specialized hematocrit tube. The total leukocyte count is the concentration of nucleated cells, because the techniques detect all the nuclei in solutions from which erythrocytes have been removed by lysis or centrifugation. Therefore, nucleated erythrocytes typically are included in this count. In most cases the concentration of NRBC is negligible, but on rare occasion they may make up an appreciable fraction of the total nucleated cell concentration.

A variety of electronic cell counters operate by enumerating nuclear particles in an isotonic dilution in which a detergent is used to lyse the erythrocytes. These systems must be engineered for animal blood, however, to generate accurate measurements of cell concentrations. There are also continued advances in these hematology systems for performing leukocyte differentiation. Three-, four-, and five-part differential systems exist. The differential capability works best with normal blood, but there are individual exceptions. All systems may produce questionable results when there is leukocyte pathology and none properly detects abnormalities such as left shift, toxic change, and cell types outside the routine five normal cell types (see Chapters 10 and 12). (For principles of hematology system operation, see the discussion of advanced hematologic procedures later in this chapter.) The quantitative buffy coat analysis system (QBC, Becton Dickinson) estimates the leukocyte concentration by

measurement of the buffy coat layer in a specialized microhematocrit tube, in which a float is present to expand the buffy coat region for optical scanning.

In isolation, the total leukocyte count is not particularly useful for interpretive purposes; this measurement is used to determine the concentration of various leukocyte types that make up the differential count. The concentration of individual leukocytes is the most useful value for the interpretation of disease processes. This information is determined by evaluating the stained blood film (discussed below). Because of the limitations in automated leukocyte differentiation described above, it is important to utilize blood film examination in conjunction with automated hematology systems. This is essential not only for leukocyte characterization, but also for evaluation of erythrocytes in cases of anemia and platelets when the instrument produces a decreased platelet concentration value.

Preparation of blood films

The stained blood film is an essential tool for determining the concentrations of individual leukocyte types (i.e., differential count) and for evaluating important pathologic abnormalities involving leukocytes, erythrocytes, and platelets. Successful derivation of information from the blood film requires a proper technique, which both creates a monolayer of individually dispersed cells and a minimal disturbance of relative cell distributions that reflect the cell concentrations in mixed blood. A poorly prepared film presents confusing artifacts and may result in cell distributions on the slide that lead to serious errors in the differential count.

Preparation of a good-quality blood film requires mastery of a specific technique (Figs. 1.13–1.15). The most common procedure is known as the wedge or push technique and uses two glass microscope slides. A drop of blood is placed near one end of the first slide supported on the counter. The second slide is placed on the first in a way that forms a "wedge" consisting of a 30–45° angle in front of the drop of blood. The second slide, which is known as the pusher slide, then is backed into the drop of blood and advanced forward to the end. This should be accomplished in one rapid motion that involves a flip of the wrist holding the pusher slide. Downward pressure on the pusher slide should be minimal.

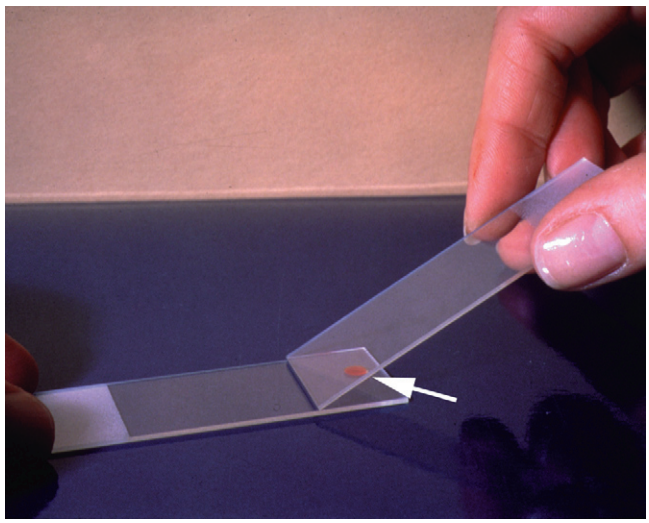

Figure 1.13 Blood film preparation. The blood slide is held on a firm surface, and a drop of blood is placed near the end (arrow). The pusher slide then is placed on the blood slide in front of the drop of blood to form an angle of approximately 30°.

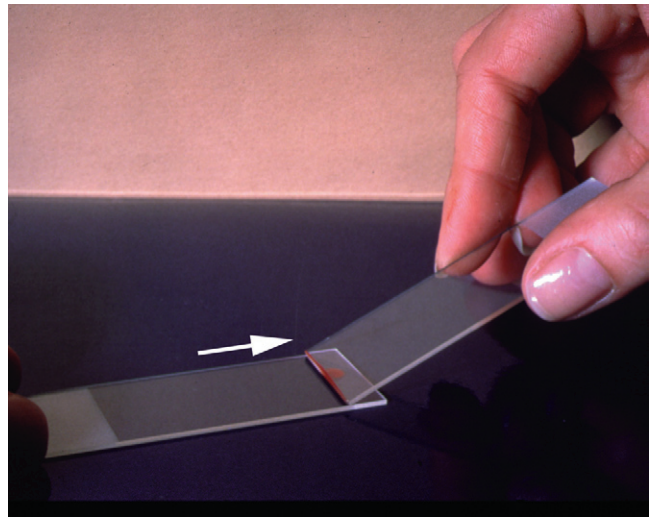

Figure 1.14 Blood film preparation. The pusher slide is backed into the drop of blood with a directional movement (arrow).

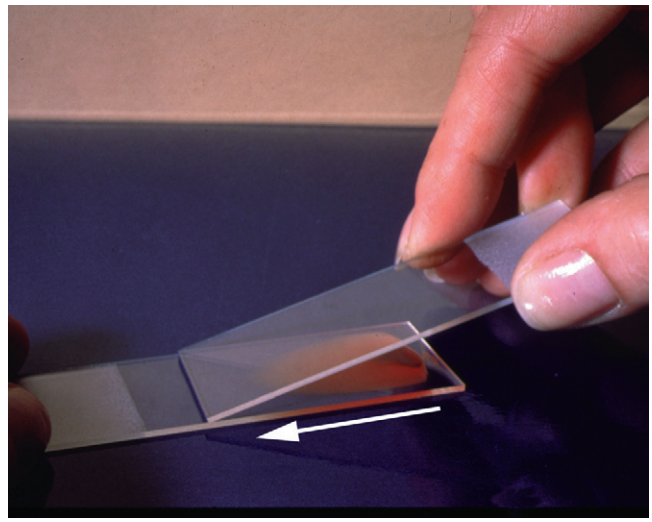

Figure 1.15 Blood film preparation. The pusher slide is pushed forward with a rapid directional movement (arrow). It is important that the movements shown in Figures 1.13 through 1.15 are a single, rapid procedure involving a flip of the wrist. Considerable practice is required to develop this skill. The result should be a uniform film of blood that gets progressively thinner (see Fig. 1.17).

Learning this technique in the presence of someone experienced with making good films is helpful, and considerable practice is advised. A common poor technique is to push the pusher slide too slowly, thereby creating a film that is too thin. This results in very poor distribution of leukocytes at the end of the film and artifacts in the evaluation of erythrocytes. In blood with reduced viscosity, such as that from patients with severe anemia, increasing the angle to avoid a slide that is too thin is useful.

Staining

After preparation, the blood film is usually stained within minutes. However, it may be stained within hours to days if it is being sent to a diagnostic laboratory. The staining system used for microscopic evaluation of cellular elements is the Wright stain, or a Wright stain modified by the addition of Giemsa. This is a relatively complex procedure that requires care and maintenance, thus often being limited to larger laboratory facilities. Quick-stain procedures that mimic the classical Wright stain are available, however, and for convenience, these are the most commonly used stains in the veterinary practice setting. The best-known stain kit is Diff-Quick (Dade Behring Inc., Newark, DE). Quick stains may result in nuclear overstaining and blurring of chromatin detail, but they provide sufficient quality for differential leukocyte counting and screening for morphologic abnormalities. Examples of manual to automated staining systems are shown in Figure 1.16.

Figure 1.16 Blood film and cytology staining apparatus. Top. Manual staining jars containing Diff-Quick stain. Slides are manually moved from one jar to the next according to the manufacturer's instructions. Bottom. An automated stainer used for higher-throughput situations. Note the mechanical arm that moves a rack of slides (not shown) through the sequence of staining procedure baths (arrow). The stainer may be programmed to control the timing in each bath. Most such machines provide the ability to stain as many as 20–25 slides per cycle.

Expertise for examination of blood films

Once stained, the anatomy of a blood film must be known to properly orient the slide for microscopic viewing (Fig. 1.17). The largest part of the film is the thick area or body, in which cells are superimposed and leukocytes are rounded up, thereby making microscopic evaluation of all components difficult. The feathered edge occurs at the end of the film. Artifacts in this area include broken leukocytes and the inability to evaluate the erythrocyte central pallor. The counting area is a small area between the thick portion and the feathered edge, and it consists of a monolayer of cells in which microscopy is optimal. Leukocytes are flattened out so that the internal detail is most evident.

The amount of interpretive disease relevance that can be gained from examination of the blood film is proportional to the expertise available for the examination. Success in dealing with all components of such examination depends on the quality of film making, stain maintenance, ability to look in the correct place, ability to differentiate preparation artifacts from morphologic abnormalities, and experience with interpretive blood film pathology. To the extent that the user cannot make these distinctions, abnormal blood films should be referred to a specialist for examination and/or second opinion.

It is important to examine the gross appearance of blood films as a correlate to artifact recognition. Improper preparation can be recognized, thereby alerting the observer to

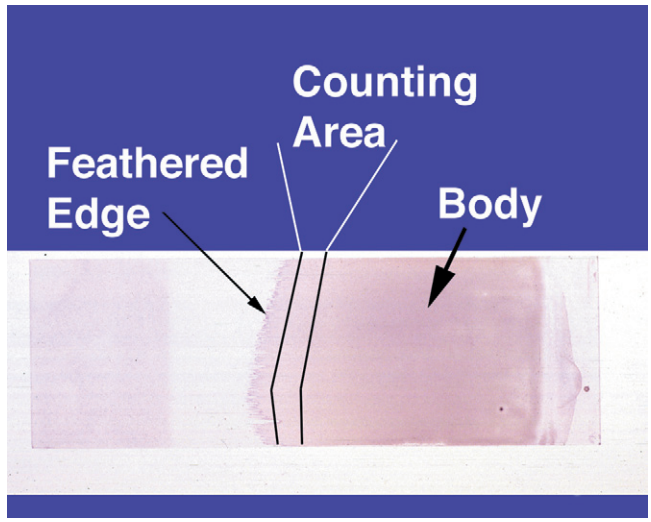

Figure 1.17 Anatomy of a stained blood film. Note the feathered edge (thin arrow) and the thick area or body of the slide (thick arrow). The counting area containing a monolayer of cells is present in a relatively small area, which is delineated approximately by the lines across the slide. This gross examination of the slide is very helpful in orienting the observer before placing the slide on the microscope stage. This facilitates alignment of the optics over the proper area of the slide, making it easier and faster to perform low-magnification observations and to find the counting area.

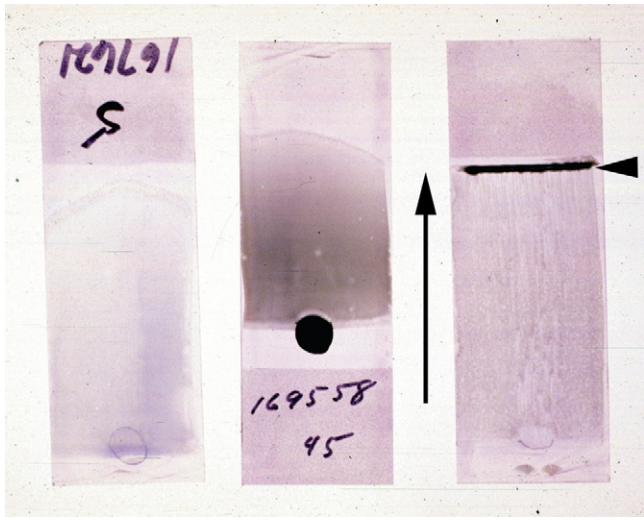

Figure 1.18 Gross appearance of blood films. All three of these films are oriented the same way. The drop of blood was placed near the bottom of the picture, and the film was made by pushing in the direction of the arrow. The middle film has a normal appearance and intensity of color. The appearance is homogeneous but gets progressively thinner as one approaches the feathered edge. The film on the left is very pale; this is the appearance when severe anemia is present. With severe anemia, blood viscosity is reduced, resulting in a much thinner film. The film on the right is made improperly and does not yield accurate information. The pusher slide was pushed too slowly, making a thin film with streaks. Note the streaking and irregularity over most of the slide. Blood was still present at the end of the slide as well, resulting in a line of densely concentrated cells (arrowhead). It is not possible to find a good monolayer for evaluation of erythrocyte morphology on this slide. In addition, the leukocytes are disproportionately concentrated at the end of the slide, which ordinarily has a feathered edge. Performing a differential count will be difficult in this case—and likely not accurate. A thin slide as a result of pushing too slowly is the most common problem in technique found at veterinary facilities.

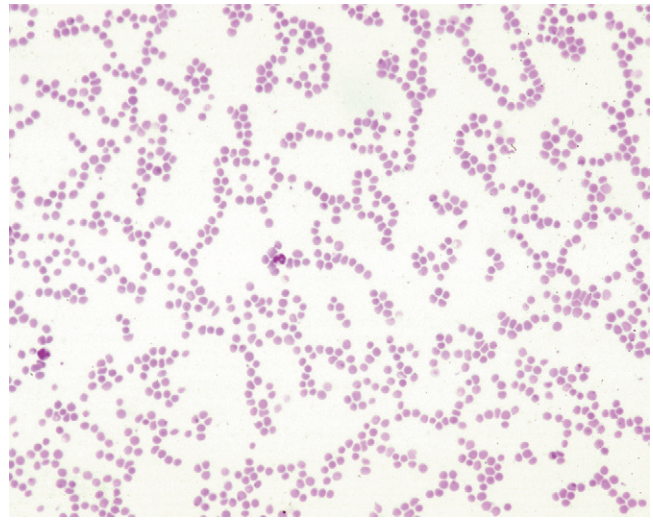

Figure 1.19 Low-magnification appearance of the feathered edge. Note the reticulated pattern of erythrocyte distribution. Artifactual loss of central pallor makes evaluation of erythrocyte morphology difficult, and false interpretation of pathologic abnormalities is likely to occur in this area.

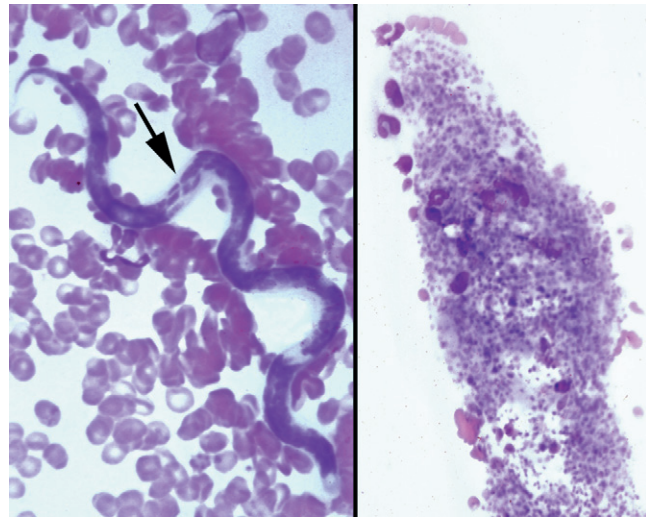

Figure 1.20 Large items pushed to the feathered edge. Left. Microfilaria (arrow) in an animal with heartworm disease. Right. A large clump of platelets with trapped leukocytes. Several hundred platelets are contained in this microclot.

artifacts that can be avoided and preventing any associated, errant interpretations. Common abnormalities that may be recognized grossly are presented in Figure 1.18. The most common and important abnormality is a slide that is too thin, which can be recognized by streaks progressing toward the feathered edge. This results in a leukocyte distribution that presents major errors in the differential count. In addition, there is not an area adequate for the evaluation of erythrocyte abnormalities.

The observer should locate the counting area using the 10× objective. The feathered edge is recognized by a loss of erythrocyte central pallor and a reticulated pattern of erythrocyte distribution on the film (Fig. 1.19). Quick, low-power examination of the feathered edge is useful for the detection and identification of abnormalities such as microfilaria, platelet clumps, and unusual, large cells that are preferentially deposited here (Fig. 1.20). The thick area is recognized by a progressive superimposition of erythrocytes as the observer moves further into the thick area of the slide. In very thick areas, the evaluation of cells is severely compromised (Fig. 1.21). The counting area is recognized by a monolayer of evenly dispersed cells (Figs 1.22 and 1.23).

Once the counting area is located, the experienced observer can estimate the leukocyte concentration on a well-prepared blood film. This is useful as a gross quality-control measure, and it is recommended that the observer

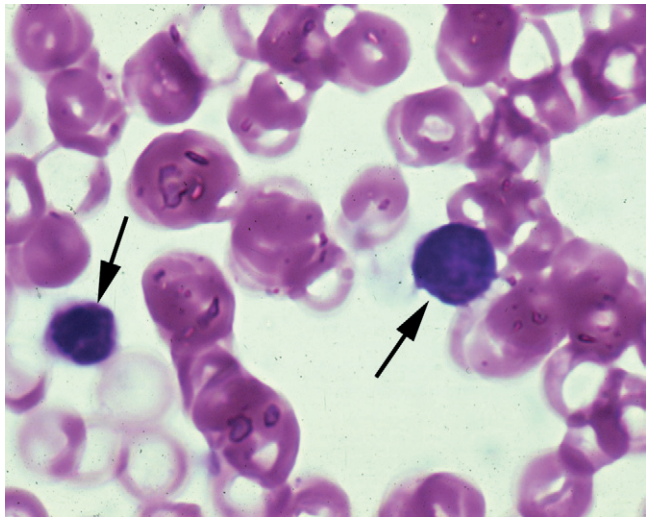

Figure 1.21 High-magnification appearance of cells in the thick area or body of slide. Note the superimposition of erythrocytes, thus making evaluation of erythrocyte morphology difficult. In addition, specifically identifying leukocytes (arrows) is difficult to impossible. In this area, leukocytes are spherical or rounded-up rather than flattened. It is not possible to see intracellular detail or even the delineation between the cytoplasm and the nucleus.

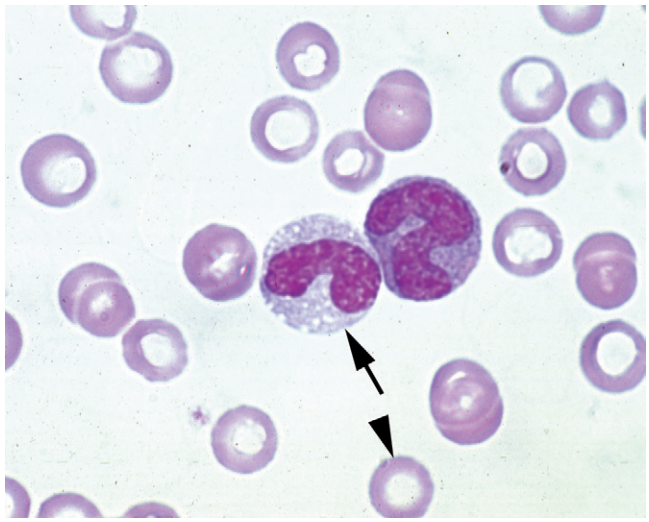

Figure 1.22 High-magnification appearance of cells in the counting area or monolayer. Note the minimal superimposition of erythrocytes, which facilitates evaluation of erythrocyte morphology (arrowhead). Leukocytes (arrow) are flattened on the slide, which makes it possible to see details of the cytoplasm and nucleus. Note that the nuclear borders are sharply delineated from the surrounding cytoplasm.

gain experience at this by repetitive comparison of leukocyte density on well-prepared blood films with total leukocyte counts from a cell counter. The low-power appearances of a leukocyte count in the normal range, marked leukopenia, and marked leukocytosis are shown in Figures 1.23, 1.24, and 1.25, respectively.

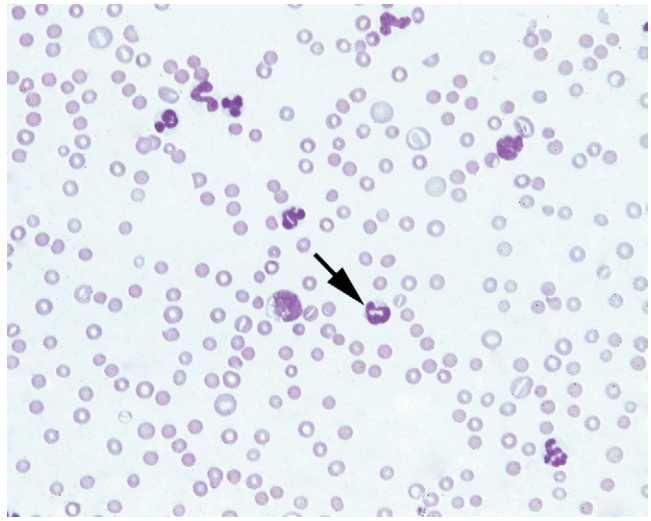

Figure 1.23 Low-magnification appearance of the counting area. Note the evenly dispersed cells and the ability to visualize the erythrocyte central pallor. The density of leukocytes (arrow) is that expected with a leukocyte concentration in the normal range.

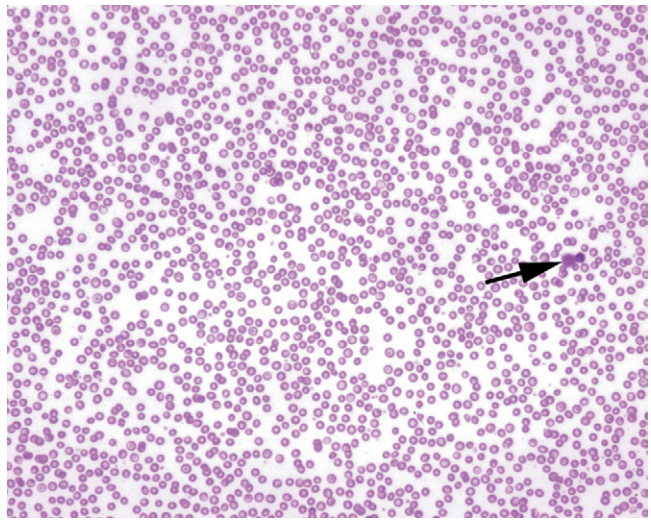

Figure 1.24 Low-magnification appearance of the counting area with a marked decrease in the leukocyte concentration. A rare leukocyte per field is present (arrow).

Procedures using the 100×, oil-immersion objective

Once the counting area is located and these assessments are completed the microscope should be adjusted for oil immersion, high magnification observation. The observer will then perform a systematic evaluation of the three major cell lines. This includes a differential count for leukocytes with notation about any abnormal cells, evaluation of erythrocyte morphology, and evaluation of platelets.

Within the counting area, the observer will move across fields and obtain the differential leukocyte count by classifying a

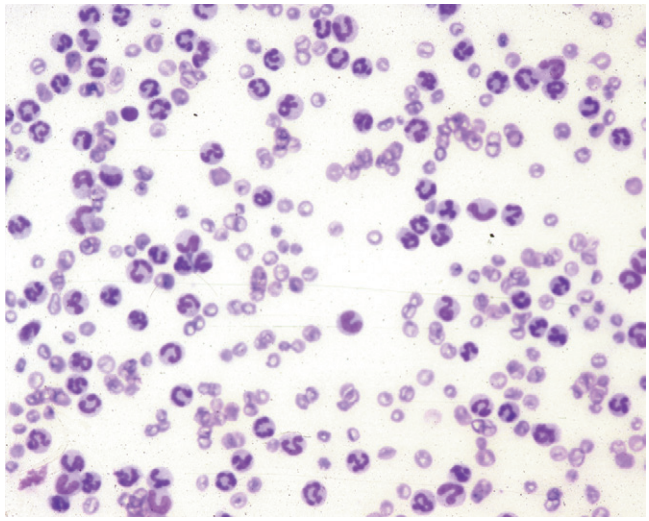

Figure 1.25 Low-magnification appearance of the counting area with a marked increase in leukocyte concentration. The density of leukocytes is considerably greater than that seen in Figure 1.23.

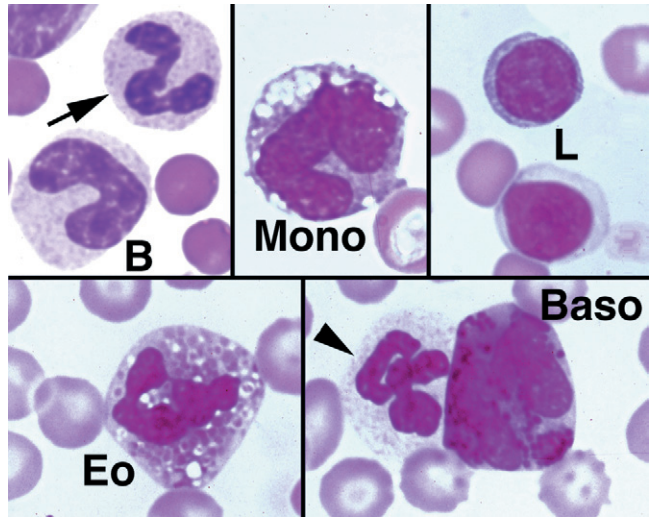

Figure 1.26 Basic leukocytes encountered in the differential count. Upper left. Neutrophils. Note the segmented neutrophil (arrow) and the constrictions in the nuclear contour. The band neutrophil (B) has smooth, parallel nuclear contours. Upper middle. Monocyte (Mono). The nucleus may have any shape, from round to bean-shaped to ameboid and band-shaped, as in this example. The cytoplasm is blue-gray and may variably contain vacuoles. Upper right. Two lymphocytes (L). Lower left. An eosinophil (Eo). Note that granules stain similar to the surrounding erythrocytes. Occasionally, granules may wash out in the staining procedure, leaving vacuoles. Lower right. Basophil (B) with dark granules that stain similar to nuclear chromatin. Note the adjacent neutrophil (arrowhead) and that neutrophils may have small, poorly staining granules that are much smaller than those of eosinophils or basophils.

minimum of 100 consecutively encountered cells. Cells are classified into a minimum of five to six categories, with the presence of abnormal cells being recorded into a category of "other," in which a specification is made for the individual sample. The common six categories of normal cells—neutrophil, band neutrophil, lymphocyte, monocyte, eosinophil, and basophil—are shown in Figure 1.26. (See Chapter 10 for additional visual details regarding leukocyte identification that may be helpful in differential counts.)

The result of counting 100 cells is that the number of each leukocyte type is a fraction of 100, or a percentage of the leukocyte population. Once cells are categorized into percentages, they must be converted to absolute numbers for interpretation purposes. This is done by multiplying the total leukocyte concentration by the percentage of each leukocyte type, which yields the absolute number or concentration of each leukocyte in the blood sample. The following example illustrates the conversion of percentages to absolute numbers:

Example 1.1. Conversion of Percentage Counts to Absolute Concentrations

Total white-blood-cell count = 10,000/μL
Differential white-blood-cell count:

	Percentages	Absolute Numbers/μL
Neutrophils	60%	(6000)
Lymphocytes	30%	(3000)
Monocytes	5%	(500)
Eosinophils	5%	(500)

Any abnormalities in leukocyte morphology also should be noted. Important morphologic abnormalities are detailed in Chapter 12.

Erythrocyte morphology then is systematically evaluated. The observer should note any important erythrocyte shape or color abnormalities; this is particularly important for evaluating anemias. (See Chapter 5 for a review of morphologic erythrocyte abnormalities.)

The presence of platelet adequacy may be interpreted from a properly prepared blood film. A minimum of 8–12 platelets per oil immersion high-power (1000×) field may be interpreted as adequate. The number seen may be considerably greater than described, however, because of the wide range of normal platelet concentrations. This number is only a guideline for most microscopes with a wide field of view. It should be adjusted downward when using a microscope with a narrow field of view and upward if using one with a superwide field of view. If the platelets appear to be decreased, a search for platelet clumps on a low-power setting at the feathered edge should be performed. The ability to look for platelet clumps is also important when a cell counter produces a decreased platelet concentration value; this is a frequent problem in cats. Morphology of platelets also may be noted. Platelets that approach the

diameter of erythrocytes or larger are referred to as macroplatelets or giant platelets. In dogs, these suggest accelerated platelet regeneration, but this interpretation usually is not applied to macroplatelets in cats.

Advanced hematologic techniques

Historically, these capabilities were limited to central laboratories. Over the past 20 years there has been rapid technological evolution resulting in reduced cost and complexity such that these capabilities are now available to the common veterinary facility. Currently, the predominant differences of the larger, more expensive systems used by commercial laboratories are higher throughput rate, automated tube handling, and more sophisticated differential counting technology. (See Chapter 2 for additional discussion of equipment and laboratories.) Hemograms performed on modern hematologic instrumentation provide the following additional measurements.

Items determined by spectrophotometry or calculation:

- Hemoglobin concentration of blood, g/dL
- Mean cell hemoglobin content, pg
- Mean cell hemoglobin concentration (MCHC), g/dL

Items determined by cell (particle) counting and sizing:

- Erythrocyte concentration of blood, $\times 10^6$ cells/μL
- Mean cell volume (the average size of erythrocytes; MCV), fL
- Hematocrit (equivalent to the packed cell volume), %
- Platelet concentration of blood, $\times 10^3$ cells/μL
- Mean platelet volume (MPV), fL
- Total and differential leukocyte concentrations, $\times 10^3$ cells/μL
- Reticulocyte concentration, $\times 10^3$ cells/μL

The method and applicability for each of these measurements are now described.

Items determined by spectrophotometry or calculation

Hemoglobin concentration

This measurement of the quantity of hemoglobin per unit volume, expressed as g/dL, is performed in conjunction with the total leukocyte count. Briefly, a blood sample is diluted, and a chemical agent is added to rapidly lyse cells, thereby liberating hemoglobin into the fluid phase. Nucleated cells remain present in the form of a nucleus with organelles collapsed around it. The absorbance of light at a specific wavelength then may be measured by spectrophotometry in a small flow cell known as a hemoglobinometer. The absorbance of light is proportional to the concentration of hemoglobin. The system is calibrated with material of known hemoglobin concentration using reference techniques.

Interpretation of the hemoglobin concentration is the same as that of the packed cell volume, or hematocrit. It is an index of the red cell mass per unit volume of blood in the patient. Because it is roughly equivalent to the packed cell volume, however, it is not particularly useful for clinical interpretations. Most clinicians are more familiar or experienced with interpreting packed cell volumes. The hemoglobin value is always proportional to hematocrit and is a separate, independent measurement. Therefore, the hemoglobin value may serve as a quality-control adjunct for laboratory personnel when used to calculate the MCHC.

Mean cell hemoglobin

The mean cell hemoglobin is calculated from the hemoglobin concentration and erythrocyte concentration. It is regarded as being redundant to other measurements and, therefore, is not useful.

Mean cell hemoglobin concentration

The MCHC is calculated from the hemoglobin concentration and the hematocrit. It provides an index for the quantity of hemoglobin (HGB) relative to the volume of packed erythrocytes (expressed as g/dL):

$$\frac{\text{HGB (g/dL)}}{\text{PCV (\%)}} \times 100 = \text{MCHC (g/dL)}$$

where PCV is the packed cell volume. An example calculation is

$$\frac{10\ \text{g/dL}}{30\%} \times 100 = 33.3\ \text{g/dL}$$

A universal relationship among mammalian species, other than the camel family, is that the hemoglobin value normally is approximately one-third of the hematocrit value. Thus, from the relationship described, the MCHC for all mammalian species ranges from approximately 33 to 38 g/dL. Because members of the camel family (camel, llama, alpaca, vicuna) have relatively more hemoglobin within their cells, their MCHCs are expected to range from 41 to 45 g/dL.

The MCHC is not particularly useful for clinical interpretations; however, it is useful to laboratorians for monitoring instrument performance. The rationale is that the hematocrit and hemoglobin are determined on different blood aliquots, which are diluted in two different subsystems of the instrument. A malfunction in either of these subsystems may result in a mismatch between the hemoglobin and the packed cell volume, which is reflected by a deviation from the reference interval. In addition, some abnormalities of blood can result in an artifactually increased MCHC, and these can include any factor that causes a false increase in the spectrophotometric determination of hemoglobin relative to the hematocrit. Severe hemolysis in the sample is a common cause of an increased MCHC. Alternatively, common examples of increased turbidity that interfere with

light transmittance are lipemia and a very large number of Heinz bodies (see Chapter 8) in cats. Erythrocyte agglutination, as may occur in immune-mediated hemolytic anemia, may result in a false high MCHC. In this situation, the hemoglobin measurement is accurate, but the hematocrit is falsely low because the agglutinated erythrocytes are out of the system's measuring range and are therefore not counted or sized in derivation of hematocrit.

Two erythrocyte responses related to anemia may be associated with a slightly decreased MCHC. The first is marked regenerative anemia. Reticulocytes or polychromatophilic cells are still synthesizing hemoglobin and, therefore, have not yet attained the cellular hemoglobin concentration of a mature erythrocyte. A very high fraction of reticulocytes is required, however, such as greater than 20%, to develop a detectable decrease in MCHC. The second is severe iron deficiency, in which cells have a reduction in hemoglobin content because they are smaller (i.e., microcytic) but also may have a minor reduction in cellular hemoglobin concentration. There are no causes of a dramatically decreased MCHC (<28 g/dL) other than an analytic instrument error.

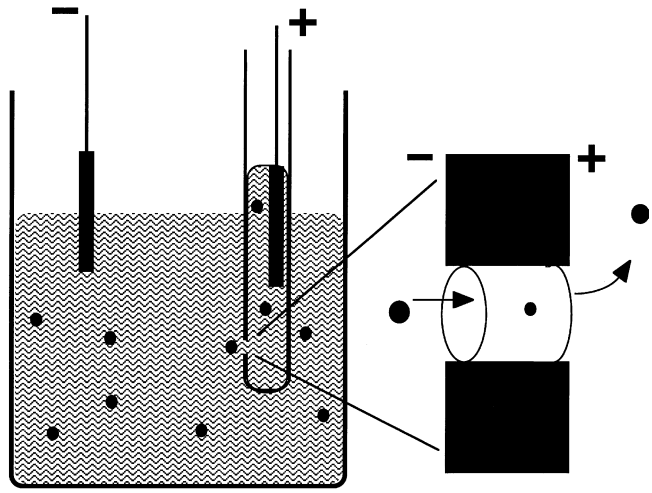

Figure 1.27 Principle of electronic impedance cell counting. Left. Overview of the fluidic chamber. Cells (dots) are diluted in an isotonic fluid (wavy lines). Two electrodes (+ and –) are separated by a glass tube containing a small opening or aperture. Electric current is conducted by the isotonic fluid across the electrodes via the aperture. Vacuum is applied to move the fluid and cells through the aperture. Right. Magnified, diagrammatic view of the aperture. Cells flow through the aperture (arrows). The aperture is a cylindric shape with a volume called the sensing zone. While occupying space within the aperture, cells transiently impede the flow of current. Cell passages are counted as deflections in the current voltage. In addition, the magnitude of voltage deflection is proportional to the volume of the cell.

Items determined by cell (particle) counting and sizing

Cell counting and sizing technologies

A brief overview of cell counting and sizing technology common to all of these measurements is appropriate. One of two technologies is used by most hematology instrument systems.

The first is light-scatter measurement of cells passing through a light source. Cells are passed through a flow cell that is intersected by a focused laser beam. The physical properties of the cell scatter light to different degrees and at different angles relative to the light source. Cell passages eliciting scatter events may be counted to derive the cell concentration. The degree of scatter in the direction of the light beam, which is known as forward-angle scatter, is proportional to the size of the cell. In addition, measurement of light scattered to different angles may be correlated with cellular properties, which leads to the ability to differentiate nucleated cell types.

The second is more common and incorporated into a wider range of instrument designs and may also be used as a second measuring principle in light-scatter systems. This is electronic cell counting, which is also known as impedance technology or Coulter technology (after the original inventor). It is based on the principle that cells are suspended in an electrolyte medium, such as saline, that is a good conductor of electricity. The suspended cells, however, are relatively poor conductors of electricity. Thus, these cells impede the ability of the medium to conduct current in a sensing zone known as an aperture. By simultaneously passing current and cells through a small space or aperture, deflections in current can be measured (Fig. 1.27). In addition, the size of the cell is proportional to the resultant deflection in current. This volumetric size discrimination may be used to measure the size distribution of erythrocytes, to discriminate platelets from erythrocytes, and to partially differentiate leukocytes. Cells within a given population are counted and assigned to a size distribution by particle-size analyzer circuitry (Fig. 1.28). The particle-size analyzer assigns each cell to a size scale that is divided into a large number of discrete size "bins" of equal size. The size scale is calibrated with particles of known size. By rapidly accumulating several thousand cells, a frequency distribution of the sizes of the cell population may be constructed (Fig. 1.29).

The size distribution curve is most useful for the evaluation of erythrocytes in the laboratory. It also may be used to derive leukocyte differential and platelet information.

The following measurements derive from the described cell counting and sizing technology. Because of the considerable differences in erythrocyte and platelet sizes between species, instrument systems require careful design and/or adjustment to accurately obtain the various measurements. For example, instruments manufactured for the analysis of human blood do not perform accurately for most animal species without modification.

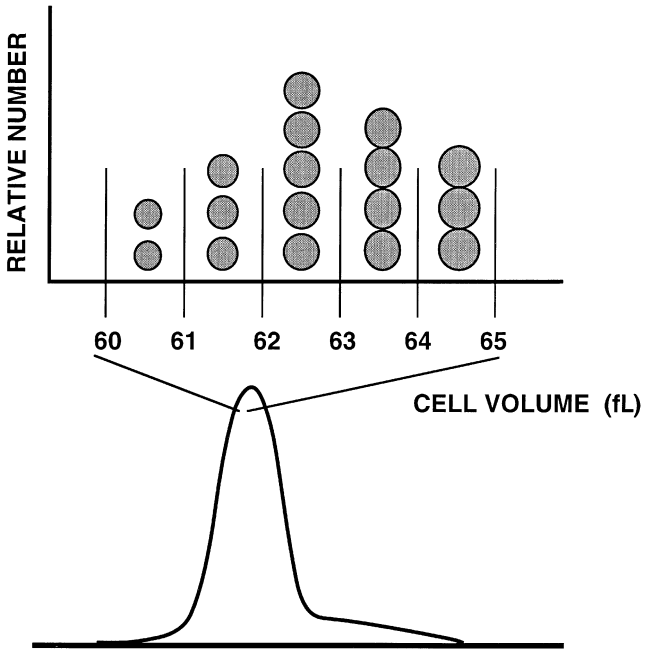

Figure 1.28 Cell volumes assigned to size bins. In the case of erythrocytes, a cell volume scale of approximately 30 to 250 fL is divided into a large number of discrete size bins (e.g., 60–61 fL, 61–62 fL). As the cells are counted, they are assigned to size bins (circles). Accumulation of many cells allows the construction of a size distribution histogram on the cell-volume scale (curve tracing at bottom). The drawing of bins at the top would represent a small area of the total curve.

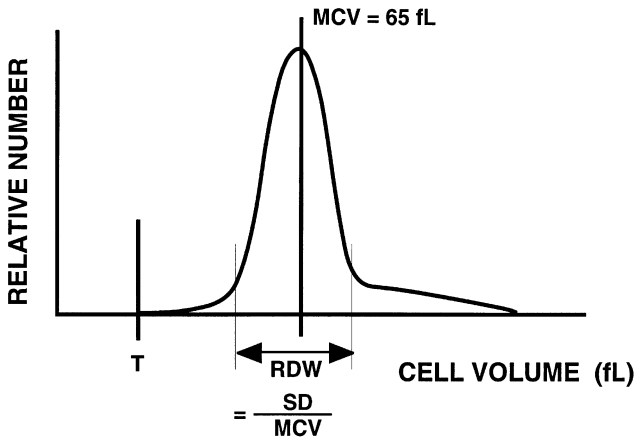

Figure 1.29 Histogram of erythrocyte size distribution. The x-axis is the cell volume, and the y-axis is the relative number of cells at each volume. Only cells above a specified volume or threshold are included in the analysis; this is indicated by the vertical bar (T). The mean cell volume (MCV) is indicated by the large vertical bar. The RDW (red-cell distribution width) value, an index of volume heterogeneity, is the standard deviation (SD) divided by the MCV, with the SD being that of the volumes of erythrocytes within the region indicated by the fine lines marked by the double arrow.

Erythrocyte concentration

The erythrocyte concentration is measured directly by counting the erythrocyte particles in an isotonic dilution of blood. This value is not useful for purposes of clinical interpretation. It generally parallels the packed cell volume and hemoglobin concentration, but the packed cell volume is the preferred value for the interpretation of erythrocyte mass. The erythrocyte concentration is used by the instrument to calculate the packed cell volume (described later).

Mean cell volume, erythrocyte histogram, and red cell distribution width

As the erythrocytes are counted, their size distribution is simultaneously constructed (Fig. 1.29), and from this size distribution, the MCV is easily calculated. The red cell distribution width (RDW) is a mathematic index describing the relative width of the size distribution curve. It is the standard deviation of most the erythrocytes divided by the MCV. The tails of the erythrocyte distribution usually are excluded from this mathematic treatment.

These values are useful for the evaluation of anemia. Iron deficiency results in the production of microcytic erythrocytes, and accelerated erythrocyte regeneration results in the production of macrocytic erythrocytes. Early in these responses, a widening of the erythrocyte size distribution and RDW value may be observed (Fig. 1.29). As a larger proportion of these cells accumulate during the response, the curve shifts in the respective direction, and eventually, the MCV may fall out of the reference interval. The RDW is more useful in the laboratory, in conjunction with the examination of blood films, whereas the laboratorian and the clinician both may interpret the MCV. Examples of interspecies variation and representative reference intervals for MCV are

Humans	80–100 fL
Dogs	60–72 fL
Cats, horses, and cows	39–50 fL
Sheep	25–35 fL
Llama	21–29 fL
Goat	15–25 fL

For additional detail on microcytic and macrocytic anemias and other breed-specific information regarding erythrocyte size, see Chapter 6.

Hematocrit

One of the advantages of hematology instrumentation is that the hematocrit may be determined by calculation, thereby avoiding the need for microhematocrit centrifugation. The instrument calculates hematocrit (HCT) using the erythrocyte concentration (RBC) and the MCV:

$$(\text{MCV} \times 10^{-15}\ \text{L}) \times (\text{RBC} \times 10^{12}\ \text{L}) = \text{HCT}$$

Or, simplified:

$$\frac{\text{MCV} \times \text{RBC}}{10} = \text{HCT}$$

Thus, for example:

$$\frac{\text{MCV 70 fL} \times 7.00\ \text{RBC}}{10} = \text{HCT 49\%}$$

Platelet concentration

Platelets may be counted simultaneously with erythrocytes. Because platelets are considerably smaller than erythrocytes, however, they are analyzed in a separate area of the particle-size analyzer scale. Most species have little or no overlap between platelet and erythrocyte volume, thereby making such analysis both simple and accurate. Cats are an exception, in that their platelets are approximately twice the volume of those in other domestic species. In addition, macroplatelet production is a frequent response during most hematologic disturbances in cats. This response is not specific for any specific disease pattern, but it results in considerable overlap between erythrocyte and platelet size distributions, thus making determination of accurate counts difficult. Therefore, feline platelet counts should be regarded as being estimates only. Because large platelets tend to get counted as erythrocytes, the platelet concentration frequently may be artifactually low. In general, if the platelet concentration falls in the reference interval, it may be regarded as being adequate. If the platelet concentration is decreased, however, the blood film should be examined by a laboratorian to confirm this finding.

White blood cell and differential leukocyte concentrations

To analyze leukocytes, a lytic agent is first added to a dilution of blood. This agent rapidly lyses or dissolves cytoplasmic membranes, thereby making the erythrocytes and platelets "invisible" to the detection technologies. Only nuclear particles of nucleated cells remain, around which is found a "collapse" or condensation of cytoskeletal elements and any attached organelles. These particles are measured by one of the detection technologies previously described to obtain the total leukocyte concentration. Using specially formulated lytic reagents, the degree of collapse may be controlled to different degrees in different leukocyte types. The result is a differential size that can be measured by a particle-size analyzer or light-scatter technology. Automated differential leukocyte counting is not as perfected in domestic animals as in humans; however, the procedure is reasonably accurate for normal blood and, therefore, is very useful in situations such as safety assessment trials, in which most (or all) of the blood samples to be analyzed are normal. When blood is abnormal, however, the frequency of analytic error in the differential count increases considerably. Analytic errors are handled by using the blood film for comparison and the visual differential count whenever an instrument analytic error is either present or suspected. It is essential to monitor instrument performance by visual inspection of the histogram or cytogram display for each sample to know when analytic failure occurs. It is very difficult, if not impossible, to determine this simply by monitoring numeric data from the instrument. Therefore, use of this technology requires considerable training and expertise by the operator to monitor the instrument performance and appropriately intervene with visual inspection of the blood film.

Summary of blood analysis by automated or semiautomated instrumentation

The flow of dilutions, analysis, and calculations within an automated hematology instrument is summarized in Figure 1.30. This flow has two main pathways. In one, an isotonic dilution of blood is made for erythrocyte and platelet analysis. In the other, a dilution is made, into which a lytic agent is added; in this pathway, leukocytes and hemoglobin are measured.

Reticulocyte concentration

Reticulocyte enumeration

The reticulocyte concentration is very useful in the evaluation of anemias. The rate of release of reticulocytes from the bone marrow is the best assessment regarding the function of the erythroid component of bone marrow. (See Chapters 6–8 for a more detailed discussion of the anemias.)

The basis for the reticulocyte count involves the events in the maturation of erythroid cells. The developing erythroid cell is heavily involved in aerobic metabolism and protein (i.e., hemoglobin) synthesis. As it nears the final stages of

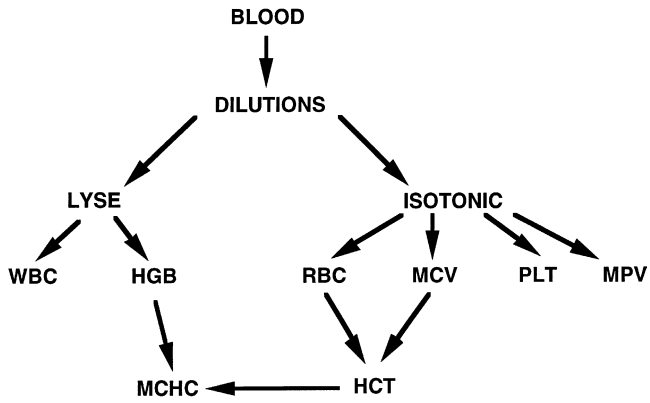

Figure 1.30 Summary of blood analysis pathways in an automated instrument. Two major dilutions are made (see text). In the left pathway, a lytic agent is added, and leukocytes are counted and the hemoglobin concentration measured. In the right pathway, erythrocytes and platelets are counted and sized. From the direct measurements, the hematocrit is calculated. A cross-check between the two pathways is provided by calculation of the mean cell hemoglobin concentration (MCHC).