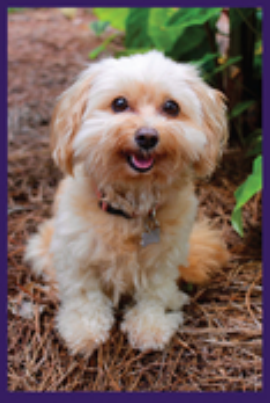
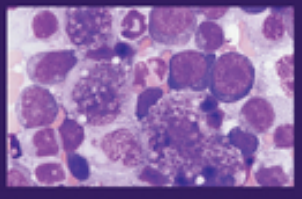
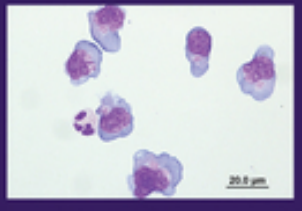
THIRD EDITION

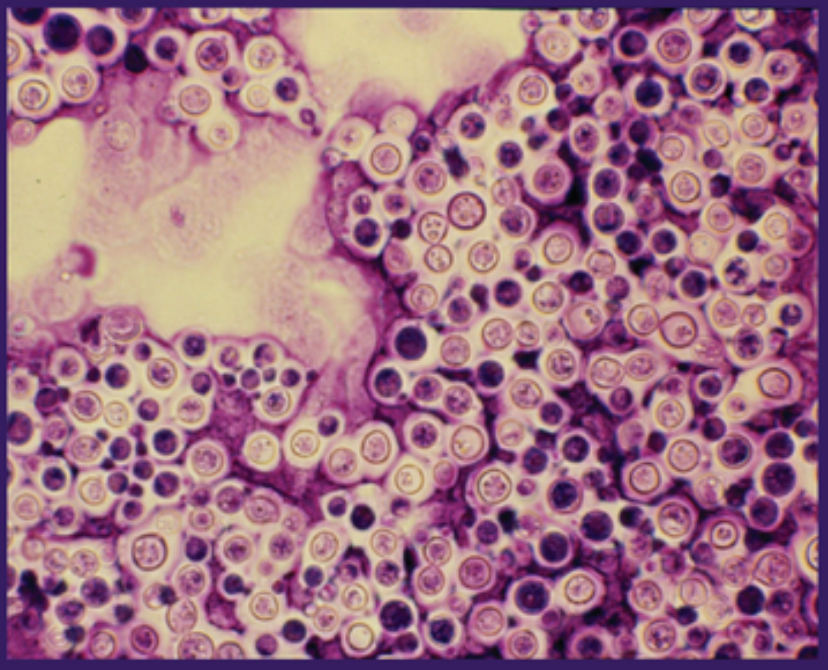
Veterinary Hematology, Clinical Chemistry, and Cytology

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









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Veterinary Hematology, Clinical Chemistry, and Cytology

Third Edition

About the cover

From left: Nala was rescued by Ross University veterinary students in 2016 on the island of St. Kitts, where she was found covered with ticks and ill with ehrlichiosis. Nala adopted Dr. Clarissa Freemyer and now lives in Raleigh, North Carolina. **Middle top:** Bone marrow aspirate from a cat with systemic histoplasmosis. Macrophages have phagocytized numerous *Histoplasma* organisms. **Middle middle:** Cerobrospinal fluid from a dog with Large Granular Lymphocyte lymphoma. Note the azurophilic granules in the large lymphoid cells. **Middle bottom:** A Hyacinth Macaw photographed by Dr. Robin W. Allison in the wilds of the Pantanal, Brazil- the world's largest tropical wetland. **Right:** Imprint of an ulcerative lesion on a cat's paw showing numerous *Cryptococcus* organism. This was Mary Anna Thrall's first attempt at taking a cytologic sample, and the inspiration for her to become a clinical pathologist (circa 1974, new methylene blue stain).

Veterinary Hematology, Clinical Chemistry, and Cytology

THIRD EDITION

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Dr. Mary Anna Thrall dedicates the third edition of this book to her veterinary students, interns, residents, graduate students, and other trainees at Colorado State University and Ross University School of Veterinary Medicine who have made the profession of veterinary medicine and the specialty of clinical pathology extremely rewarding and enjoyable for many years. I also dedicate this edition to my adult children Joseph Bammer, Dr. Anna Freemyer-Brown, Sarah Freemyer, and Dr. Clarissa Freemyer, all of whom are happy, wonderful, and productive people who excelled in spite of my somewhat neglectful parenting.

Dr. Glade Weiser dedicates this edition to Dr. Gary Kociba for his mentorship and willingness to risk hiring a faculty member as an untrained clinical pathologist coming out of internal medicine. Soon after, Drs. Bob Hall and Don Meuten joined the section as trainees. Truth be known the three of us jointly contributed to each other's training. In addition, I treasure the numerous mentorship and working relationships over the years with faculty, staff, and colleagues at the University of California Davis, the Ohio State University, Colorado State University, Coulter Electronics Inc., Heska Corporation, and the American College of Veterinary Pathologists. Lastly, working with many clinical pathologists in training over the years was a most rewarding renewable source of inspiration.

Dr. Robin W. Allison dedicates this edition to Dr. Mary Anna Thrall, who was my inspiration to become a clinical pathologist somewhat late in life. As a veterinary technician in mixed animal practice taking continuing education classes at Colorado State University, I marveled at her knowledge of cytology and vowed to "become her." I may not have entirely succeeded, but not for lack of trying. I will always treasure the wonderful relationships with so many talented clinical pathologists I've become friends with over the years. Additionally, this edition is dedicated to the trainees and veterinary students that made sure I never stopped learning by asking great questions; you are the future of clinical pathology.

As someone trained in clinical pathology but having a career in clinical exotic animal medicine, Dr. Terry W. Campbell dedicates this edition to his animal patients who have been the fountainhead of his education. It has been a joyfully inspirational experience working with clinical pathologists at Kansas State University and Colorado State University and exploring the world of comparative clinical pathology throughout the many years.

Contents

Editors, ix

Preface, xi

About the Companion Website, xiii

Section I General Principles of Laboratory Testing and Diagnosis, 1

1 Laboratory Technology for Veterinary Medicine, 3
Glade Weiser

2 Sample Collection and Processing, Preparations for Clinical Microscopy, and Analysis of Laboratory Service Options, 30
Glade Weiser

3 Perspectives in Laboratory Data Interpretation and Disease Diagnosis, 46
Glade Weiser and Robin W. Allison

4 Immunodiagnostics: Current Use and Future Trends in Veterinary Medicine, 58
Wayne A. Jensen

5 Laboratory Diagnosis of Infectious Diseases of Animals, 65
Sreekumari Rajeev

Section II Hematology of Common Domestic Species, 85

6 Erythrocyte Production, Function, and Morphology, 87
Mary Anna Thrall

7 Classification of and Diagnostic Approach to Anemia, 100
Mary Anna Thrall

8 Nonregenerative Anemia, 106
Mary Anna Thrall

9 Regenerative Anemia, 110
Mary Anna Thrall

10 Classification of and Diagnostic Approach to Erythrocytosis, 135
Mary Anna Thrall

11 Introduction to Leukocytes and the Leukogram, 139
Glade Weiser

12 Neutrophil Production, Trafficking, and Kinetics, 144
Glade Weiser

13 Interpretation of Leukocyte Responses in Disease, 148
Glade Weiser

14 Molecular Diagnostics of Hematologic Malignancies, 161
Emily D. Rout and Anne C. Avery

15 Laboratory Evaluation of Bone Marrow, 170
Mary Anna Thrall and Glade Weiser

16 Lymphoproliferative Disorders and Myeloid Neoplasms, 183
Mary Anna Thrall

17 Disorders of Hemostasis, 201
James Meinkoth

18 Principles of Blood Transfusion and Crossmatching, 220
Linda M. Vap and Karl E. Jandrey

Section III Hematology of Common Nondomestic Mammals, Birds, Reptiles, Fish, and Amphibians, 235

19 Mammalian Hematology: Laboratory Animals and Miscellaneous Species, 237
Terry W. Campbell

20 Hematology of Birds, 254
Terry W. Campbell

21 Hematology of Reptiles, 292
Terry W. Campbell

22 Hematology of Fish, 314
Terry W. Campbell

23 Hematology of Amphibians, 330
Terry W. Campbell

Section IV Clinical Chemistry of Common Domestic Species, 341

24 Laboratory Evaluation and Interpretation of the Urinary System, 343
Donald Meuten and Saundra Sample

25 Laboratory Evaluation of Electrolytes, 402
Andrea A. Bohn

26 Laboratory Evaluation of Acid-Base Disorders, 417
Glade Weiser

27 Laboratory Evaluation of the Liver, 425
Robin W. Allison

28 Laboratory Evaluation of the Pancreas and Glucose Metabolism, 445
Robin W. Allison

29 Laboratory Evaluation of Gastrointestinal Disease, Digestion, and Intestinal Absorption, 458
Dawn Seddon

30 Laboratory Evaluation of Plasma and Serum Proteins, 484
Robin W. Allison

31 Laboratory Detection of Muscle Injury, 498
Robin W. Allison

32 Laboratory Evaluation of Lipids, 502
M. Judith Radin

33 Laboratory Evaluation of the Thyroid, Adrenal, and Pituitary Glands, 515
Donald Meuten and Saundra Sample

34 Parathyroid Glands and Calcium and Phosphorus Metabolic Pathology, 561
Donald Meuten

Section V Clinical Chemistry of Common Nondomestic Mammals, Birds, Reptiles, Fish, and Amphibians, 587

35 Clinical Chemistry of Mammals: Laboratory Animals and Miscellaneous Species, 589
Terry W. Campbell

36 Clinical Chemistry of Birds, 601
Terry W. Campbell

37 Clinical Chemistry of Reptiles, 617
Terry W. Campbell

38 Clinical Chemistry of Fish and Amphibians, 625
Terry W. Campbell

Section VI Cytopathology of Common Domestic Animals, 633

39 Cytology of Inflammation and Infectious Agents, 635
Robin W. Allison

40 Cytology of Neoplasia, 652
Donald Meuten and Kristina Meichner

41 Cytology of Skin and Subcutaneous Masses, 676
Donald Meuten, Kristina Meichner, and Mary Anna Thrall

42 Cytology of Body Cavity Effusions, 703
Robin W. Allison

43 Cytology of Synovial Fluid, 717
James Meinkoth

44 Cytology of Abdominal Organs, 731
Mary Anna Thrall and Andrea A. Bohn

45 Cytology of Lymph Nodes, 752
Mary Anna Thrall, Donald Meuten, Andrea A. Bohn, and Kristina Meichner

Section VII Clinical Case Presentations, 775

Clinical Case Presentations, 777
Alex Mau
This section includes 117 cases with clinicopathologic data accompanied by an interpretive discussion and diagnostic summary.

Index, 1011

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Preface

On behalf of the contributing authors and Wiley-Blackwell, we are pleased to introduce the Third Edition of *Veterinary Hematology and Clinical Chemistry*, now titled *Veterinary Hematology, Clinical Chemistry, and Cytology*. 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. We believe that the addition of cytology to the textbook makes it a complete and valuable reference for anyone interested in clinical pathology.

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 drive 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 using veterinary clinical pathology.

Organization

Veterinary Hematology, Clinical Chemistry and Cytology is organized into seven 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: Presents cytology of common domestic species and includes cytology of inflammation, neoplasia, skin and subcutaneous tissue, body cavity effusions, joint fluid, internal organs, and lymph nodes.

VII: Provides 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.

Comments, Revisions, and Addition Highlights

Development of data interpretation skills by veterinary students, clinical pathologists in training, and practitioners continues to be the primary focus in this edition. While rules for interpreting diagnostic tests assume homogeneity of pathophysiologic responses, we realize that not all of our animal friends have "read the book," although most do, as seen in Figure P.1.

Revisions of chapters have been made throughout where needed. Chapters that have been extensively revised are the following:

- Glade Weiser updates Chapter 2 to include a section on cytology, with details on fluid and tissue sample collection, preparation, staining, and approach to specimen examination.

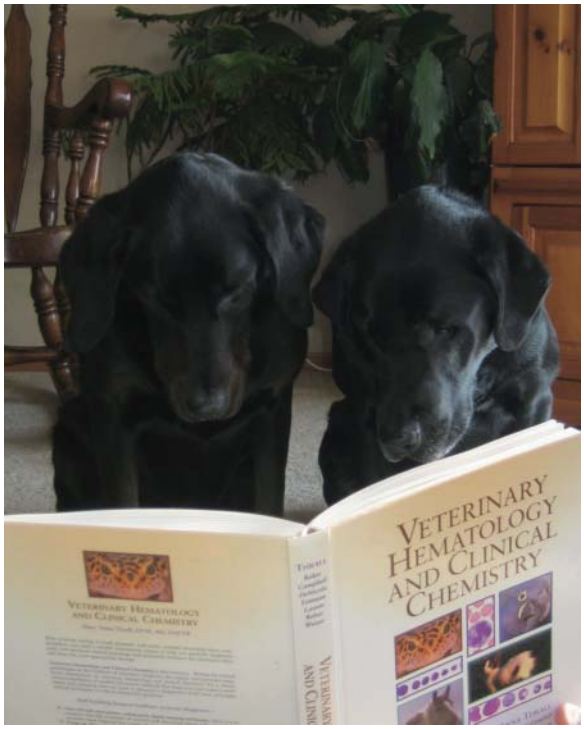

Figure P.1 Dogs caught "reading the book" in an attempt to make their disease responses predictable. Not all will read the book. Source: Courtesy of Dr. Sara Hill.

• Jim Meinkoth, Oklahoma State University, provides a complete revision of the chapter on diagnosis of hemostasis disorders.
• Karl E. Jandrey, University of California, who is board certified in veterinary emergency and critical care, provides new information from a criticalist viewpoint as a coauthor of the chapter on blood transfusion and cross-matching.
• Saundra Sample, University of Missouri, is a new coauthor of two chapters on laboratory evaluation of the kidney and laboratory evaluation of the thyroid, adrenal, and pituitary glands.

Additions include the following: Sreekumari Rajeev, who is board certified in both anatomic pathology and microbiology and is a professor of infectious disease at the University of Tennessee, provides a new chapter on laboratory diagnosis of infectious disease, which discusses a logical approach to the use of modern tools that are available to diagnose infectious disease, a topic of increasing importance particularly as it relates to zoonotic diseases.

Another very important addition is a new cytology section, with seven new chapters. Contributors of new chapters include the following:
• Robin W. Allison from Oklahoma State University is the author of two of the new chapters, one on inflammation and infectious agents and one on body cavity effusions.
• Jim Meinkoth, co-author of *Diagnostic Cytology and Hematology of the Dog and Cat,* 3rd edition, is the author of a chapter on cytology of joint fluid.
• Donald Meuten, who is board certified in both clinical and anatomical pathology, and the editor of the 5th edition of *Tumors in Domestic Animals*, brings years of experience and expertise to the chapters discussing cytology of neoplasia, skin masses, and lymph nodes.
• Kristina Meichner, who is board certified in both clinical pathology and internal medicine (oncology), brings her expertise in cytology and oncology to three of the new chapters.
• Mary Anna Thrall is the coauthor of three of the new chapters on cytology, including cytology of abdominal organs, and cytology of lymph nodes.
• Andrea Bohn is the coauthor of two of the new chapters, cytology of abdominal organs and cytology of lymph nodes.

The clinical case presentations in what is now Section VII, edited by Dr. Alex Mau, are intended to provide readers "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, not unlike reading and solving a mystery in a novel. The original cases are retained because their classical usefulness does not change. Forty-three new cases have been added to this edition by several contributors, to whom we are grateful for sharing.

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 W. Allison
Terry W. Campbell

About the Companion Website

This book is accompanied by a website containing:

- Case studies
- Figures
- References and Suggested Reading

www.wiley.com/go/thrall/veterinary

I

General Principles of Laboratory Testing and Diagnosis

1 Laboratory Technology for Veterinary Medicine

Glade Weiser
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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 measuring 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.
- Microscopic differential leukocyte count and assessment of blood film pathology.

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 (Figure 1.1).

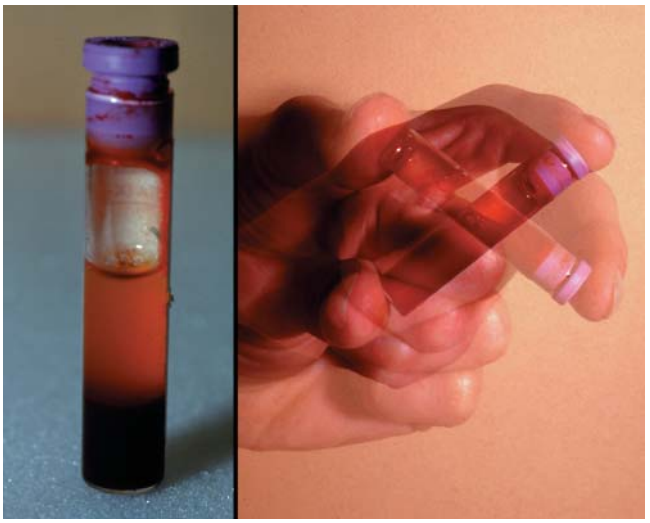

Figure 1.1 Left. Gravity sedimentation of whole blood. Right. A gentle, repetitive, back-and-forth tube inversion technique used to manually mix blood before removing aliquots for hematologic procedures.

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 (Figure 1.1). Alternatively, the tube may be placed on a rotating wheel or tilting rack designed specifically to mix blood (Figure 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

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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.

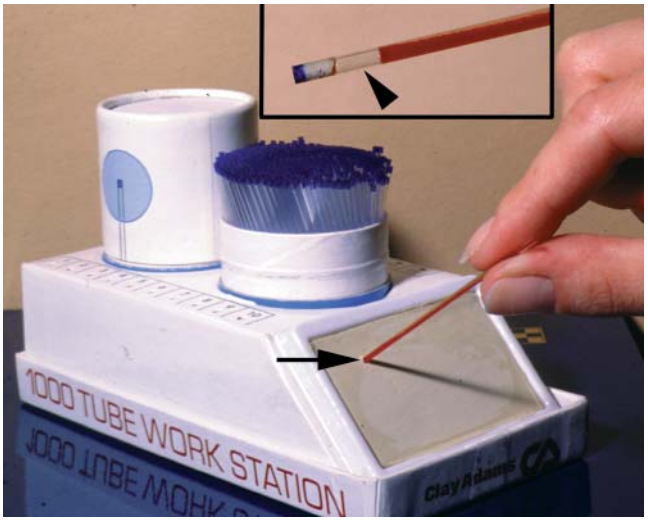

Figure 1.4 A microhematocrit tube is sealed by pressing two to three times into the clay sealant (arrow). Note that a small amount of air trapped between the blood and white clay is not a problem (arrowhead in the inset).

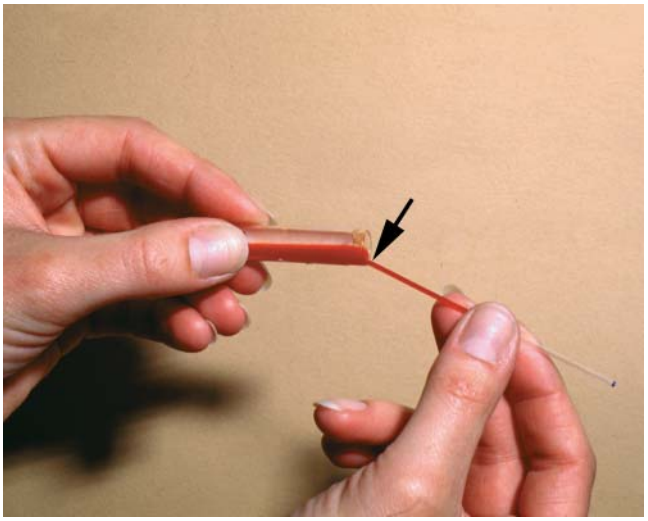

Figure 1.3 Proper technique for filling a microhematocrit tube. The tube should be positioned horizontally or tilted slightly downward to facilitate filling by capillary action. Capillary action is established by touching the upper end of the tube to the blood (arrow).

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

by holding it horizontally or slightly downward and then touching the upper end to the blood of the opened EDTA tube (Figure 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 (Figure 1.4). Note that air may be present between the sealant and the blood (Figure 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 (Figures 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.

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 (Figure 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

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).

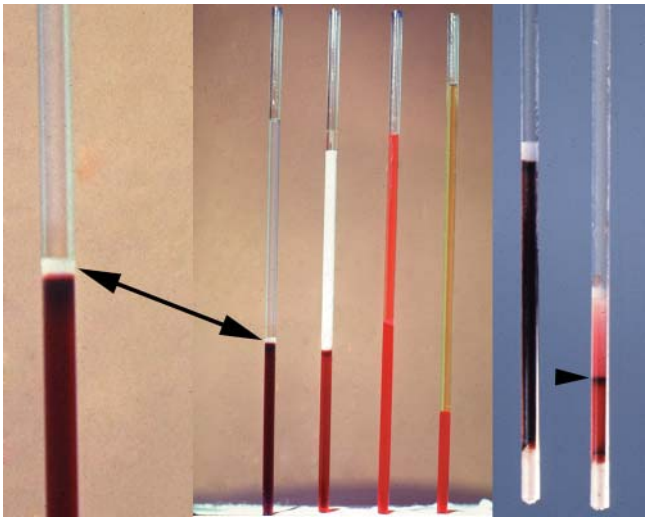

Figure 1.7 Normal and abnormal spun microhematocrit tubes (four tubes in middle panel). The tube on the left is normal. Note the packed erythrocytes at the bottom, plasma layer at the top, and buffy coat in the middle (arrow; enlarged at left). The second tube illustrates lipemia, the third hemolysis, 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.

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 27). 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 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 32). 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 9). This will typically also be associated with hemoglobinuria.

The packed cell volume is measured on a reading device, such as a microhematocrit card reader (Figure 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 (Figure 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 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 (Figure 1.10), and the portion of the tube containing the plasma is used to load the refractometer (Figure 1.11). The instrument then is held so that an ambient light source can pass through the prism wetted with

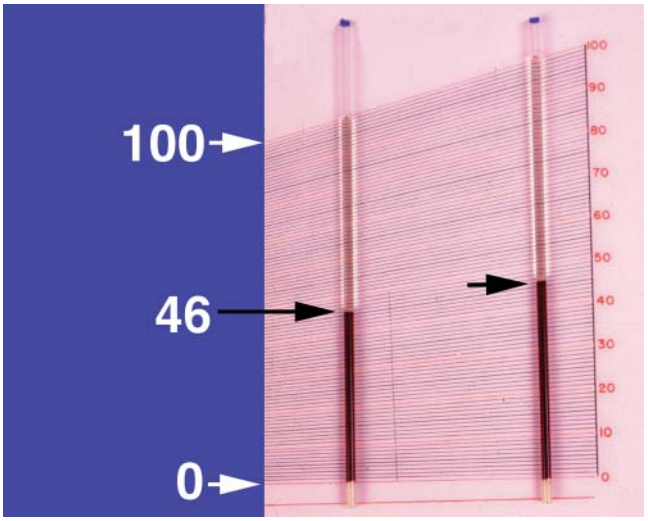

Figure 1.8 Determination of packed cell volume on a microhematocrit tube card reader using two tubes of blood from the same patient sample. Note that the scale allows the tube to be read over a considerable range of filling levels. The steps are to line up the erythrocyte–clay interface with the 0 line, 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.

plasma, and the light refraction is read on a scale through an eyepiece (Figure 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 measurement may be influenced by alterations in other solutes. Most notably, lipemia may artificially increase the protein

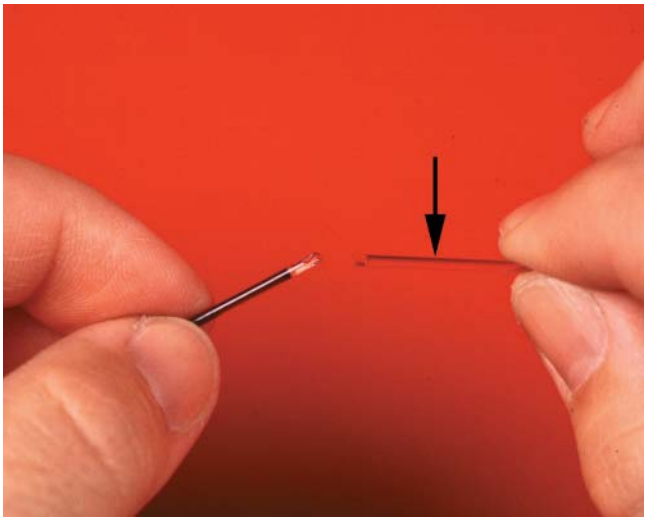

Figure 1.10 Preparation of the microhematocrit tube for measuring plasma protein concentration. The tube is broken just above the buffy coat to yield a column of plasma (arrow).

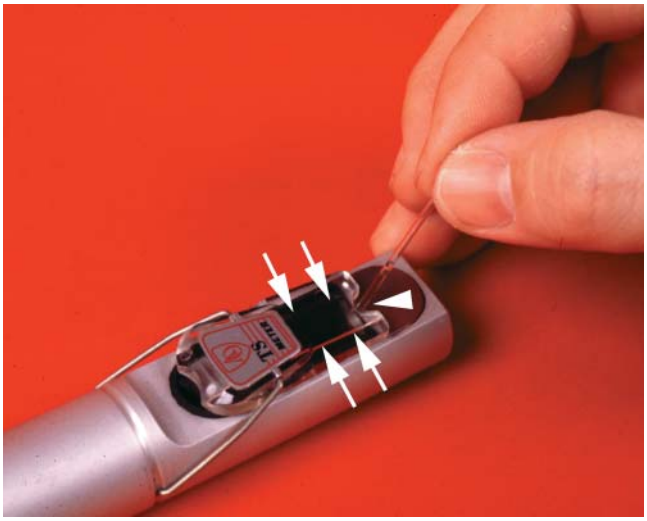

Figure 1.11 Loading plasma from the microhematocrit tube to the refractometer. To wick plasma onto the refractometer, capillary action is established by touching the end of the plasma tube at the notch of the prism cover (arrowhead). Flow should establish a thin layer of plasma under the plastic cover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

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

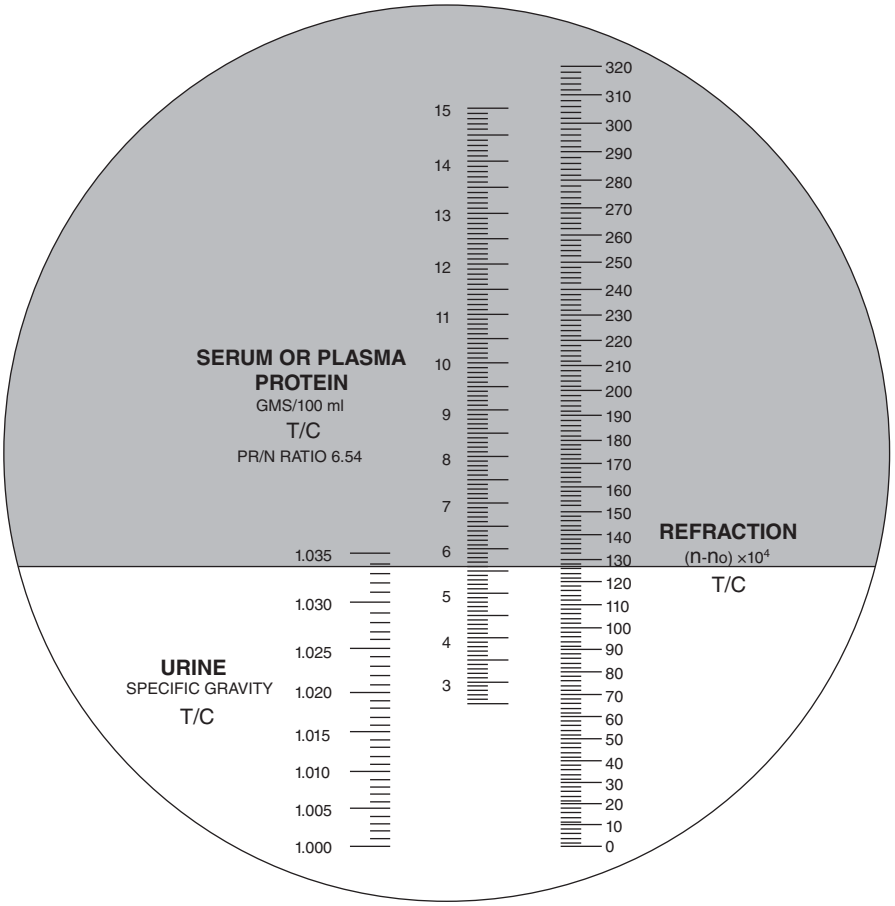

Figure 1.12 Representative refractometer scale as seen through the eyepiece. Light refraction creates a shadow–bright area interface that is read on the appropriate scale.

microscopy. This procedure, and associated consumables, is regarded as obsolete for the veterinary practice setting. This procedure has been replaced by automated hematology cell-counting 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 detect abnormalities such as left shift, toxic change, and cell types outside the routine five normal cell types (see below). (For principles of hematology system operation, see the discussion of advanced hematologic procedures later in this chapter.) The quantitative buffy-coat analysis system (Idexx Autoread™, Idexx Laboratories) 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 leukocyte types is the most useful value for the interpretation of disease processes. This information is determined by evaluating the stained blood film. Because of the limitations in automated leukocyte differentiation described above, it is important to utilize blood film examination in conjunction with automated hematology systems when blood is abnormal. 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. See further detail in the next section.

Microscopic differential count and assessment of morphology

The microscopic differential count and blood film examination is not necessarily required for all complete blood count (CBC) samples. Many samples can be classified as normal when analyzed by modern hematology instruments, especially when the CBC is part of a wellness examination. When all the data are normal, it is very unlikely to find additional useful information from the blood film examination. The need for blood film examination may be determined by examination of the instrumentation data output. The examples of data abnormalities that should prompt blood film evaluation include anemia, any data abnormality in the automated total leukocyte count or automated differential, and any concern about platelet concentration. Almost all automated instrument measurements are highly reliable on well-maintained systems. The measurement most likely to have inaccuracies is the automated differential count. This is because these systems are less likely to recognize and/or properly classify all of the nucleated cell types that do not belong in normal blood. Examples include nucleated erythrocytes, immature and blast forms of any cell lineage, and left shifted neutrophils. Therefore, it is important to perform a microscopic differential whenever the total leukocyte concentration is abnormal and/or when the distribution of cells determined by the instrument differential is abnormal. The next most likely problem is instrument counting of platelets. This is because platelet microclots are common in animal blood samples, especially from cats. The hematology instrument system will not count platelets in microclots. The blood film is helpful for identifying platelet microclots, qualitatively assessing their impact on an instrument platelet measurement value that is abnormally decreased. The examination procedures are described below.

Preparation of blood films and initial approach to blood film examination is detailed in Chapter 2, under "Collection, Preparation, and Examination Techniques for Clinical Microscopy Samples." Using the blood film monolayer area (aka counting area), the microscope should be adjusted to 100× objective oil immersion or high magnification observation for these procedures. 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 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." The specification of "other" is described or defined for the sample being examined. The common six categories of normal cells – neutrophil, band neutrophil, lymphocyte, monocyte, eosinophil, and basophil – are shown in Figure 1.13. (See Chapter 11 for additional visual details regarding leukocyte identification that may be helpful in differential counts.)

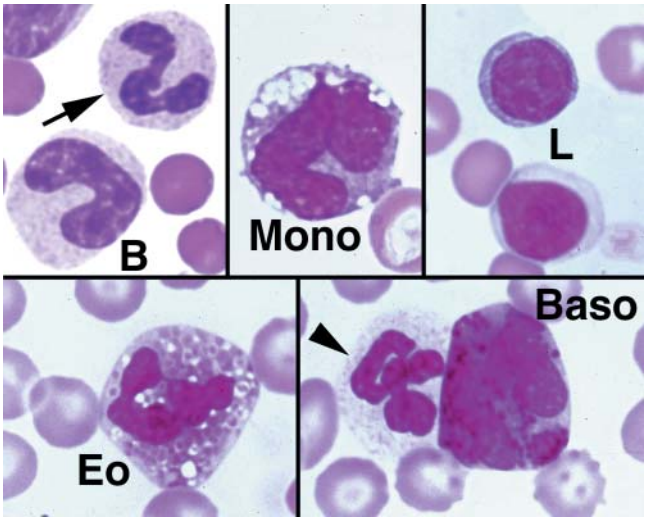

Figure 1.13 Basic leukocytes encountered in the differential count. Upper left. Neutrophils. Note the segmented neutrophil (arrow) and the constrictions in the nuclear contour. The band neutrophil (B) has smooth, parallel nuclear contours. Upper middle. Monocyte (Mono). The nucleus may have any shape, 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 (Baso) 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.

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: (See Example 1.1, next page)

Any abnormalities in leukocyte morphology also should be noted. Important morphologic abnormalities are detailed in Chapter 13.

Erythrocyte morphology is then systematically evaluated. The observer should note any important erythrocyte shape or color abnormalities; this is particularly important for evaluating anemias. (See Chapter 6 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

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)

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 (MCV; the average size of erythrocytes), 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 33–38 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 9) 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.

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 (Figure 1.14). 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 (Figure 1.15). 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 (Figure 1.16).

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.

Erythrocyte concentration

The erythrocyte concentration is measured directly by counting the erythrocyte particles in an isotonic dilution of blood.

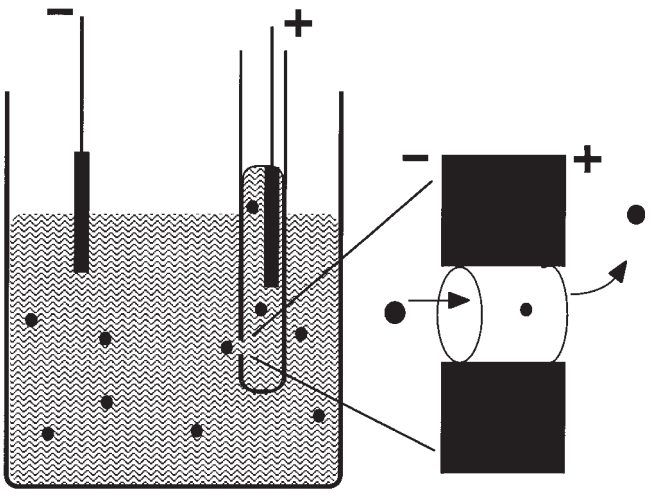

Figure 1.14 Principle of electronic impedance cell counting. Left. Overview of the fluidic chamber. Cells (dots) are diluted in an isotonic fluid (wavy lines). Two electrodes (+ and –) are separated by a glass tube containing a small opening or aperture. Electric current is conducted by the isotonic 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.

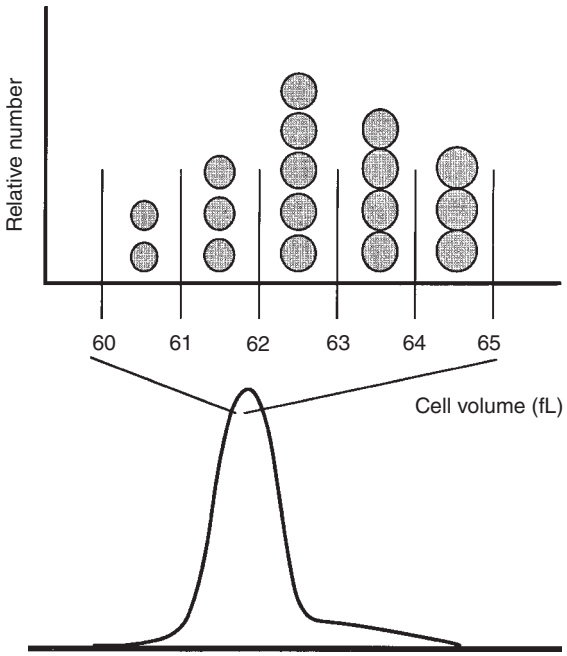

Figure 1.15 Cell volumes assigned to size bins. In the case of erythrocytes, a cell volume scale of approximately 30–250 fl is divided into a large number of discrete size bins (e.g., 60–61 fl, 61–62 fl). As the cells are counted, they are assigned to size bins (circles). Accumulation of many cells allows 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.

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 (Figure 1.16), 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 of 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 (Figure 1.16). 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

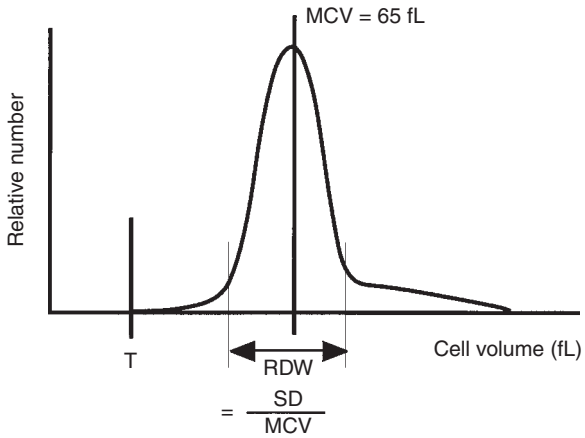

Figure 1.16 Histogram of erythrocyte size distribution. The *x* axis is the cell volume, and the *y* axis is the relative number of cells at each volume. Only cells above a specified volume or threshold are included in the analysis; this is indicated by the vertical bar (T). The mean cell volume (MCV) 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.

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 7.

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:

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

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. Microclots are also a common contributor to some fraction of platelets not being counted. 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 as described above 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 1.17. 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

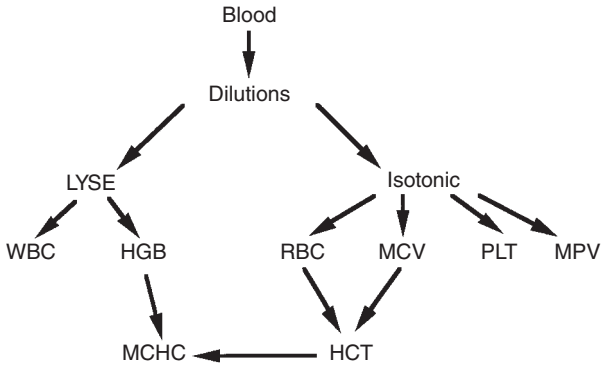

Figure 1.17 Summary of blood analysis pathways in an automated instrument. Two major dilutions are made (see text). In the left pathway, a lytic agent is added, and leukocytes are counted and the hemoglobin concentration measured. In the right pathway, erythrocytes and platelets are counted and sized. 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).

bone marrow is the best assessment regarding the function of the erythroid component of bone marrow. (See Chapters 7–9 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 maturity, the nucleus undergoes degeneration and is extruded from the cell, and the organelles supporting the synthetic and metabolic events are removed. After denucleation of the metarubricyte, the remaining erythrocyte undergoes its final maturation, which involves the loss of ribosomes and mitochondria during a period of 1–2 days. To enumerate reticulocytes, a stain is applied to erythrocytes, thereby causing aggregation of these residual organelles. This results in visible, clumped granular material that can be seen microscopically (Figure 1.18). The aggregation is referred to as reticulum, hence the name reticulocyte. Reticulocytes are equivalent to the polychromatophilic cells observed on the Wright-stained blood films (Figure 1.18). Evaluation of polychromatophilic cells on the Wright-stained blood film can provide an assessment of the bone marrow response to anemia. The appearance of these cells, however, is more subjective, and they are more difficult to quantitate than counting the corresponding cells on the reticulocyte stain.

Stains that can be used are new methylene blue (liquid) and brilliant cresyl blue, which is available in disposable tubes that facilitate the procedure (Figure 1.19). First, several drops of blood are added to the stain in a tube. The tube then is mixed and incubated for 10 minutes. From this mixture, a conventional blood film is made and air-dried. A total of 1000 erythrocytes are counted and categorized as either reticulocytes or normal cells. From this, the percentage

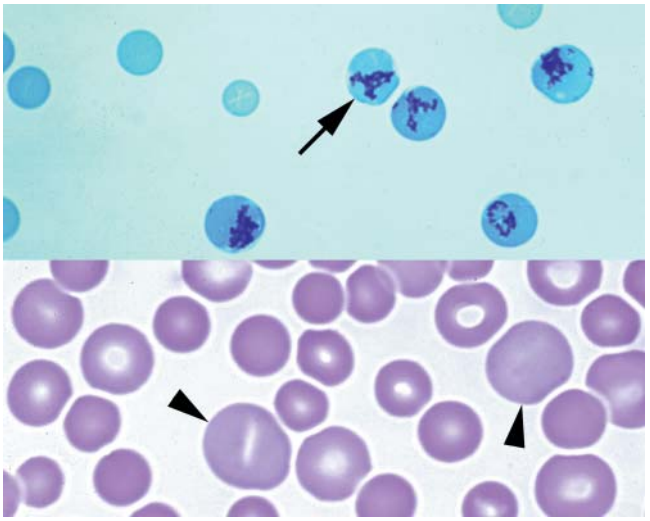

Figure 1.18 Reticulocytes. Top. Representative reticulocyte (arrow) using new methylene blue stain. Note the dark-staining, aggregated organelles in several reticulocytes. Bottom. Blood film stained with Wright-Giemsa stain. Polychromatophilic cells (arrowheads) are roughly equivalent to reticulocytes on the counterpart stain.

Figure 1.19 Examples of reticulocyte stains. Left. New methylene blue in a liquid dropper bottle. Right. Commercial preparation of brilliant cresyl blue. The stain is coated on the bottom of disposable tubes.

of reticulocytes is derived. Interpretation of the percentage reticulocytes is somewhat misleading, however, because it does not account for the degree of anemia. Thus, for purposes of interpretation, the absolute reticulocyte concentration should be calculated by multiplying the erythrocyte concentration (RBC) by the percentage of erythrocytes that are reticulocytes:

$$\text{RBC}/\mu\text{L} \times \%\ \text{Reticulocyte} = \text{Reticulocytes}/\mu\text{L}$$

Some instrument systems are also capable of reticulocyte enumeration. The method involves staining erythrocytes with a fluorescent dye that binds to residual RNA in the reticulocyte that is not present in the mature erythrocyte. RNA content, proportional to fluorochrome per cell, is measured and gated to differentiate reticulocytes from mature erythrocytes and other nonerythroid cell types. The percent and absolute values are presented as described above.

Interpretation of the Reticulocyte Concentration

The reticulocyte concentration is most useful in dogs and cats, and it also has some application in cows. It is not used in horses, however. Reticulocyte maturation is confined to the marrow space in the horse, and reticulocytes almost never are released into their circulation. Reticulocyte concentration guidelines for domestic mammals are the concentrations to be expected when the hematocrit is normal:

Dogs and cats	0–60,000 cells/μL
Cows	0 cells/μL
Horses	Do not release reticulocytes

When anemia is present, a greater degree of release from the marrow is to be expected if the marrow can respond to the anemia. This gives rise to the following guidelines for the interpretation of reticulocyte concentrations with respect to the type of anemia present:

Nonregenerative anemia to very poor regeneration	0–10,000 cells/μL
Nonregenerative to poorly regenerative anemia	10,000–60,000 cells/μL
Regenerative anemia with mild to moderate output	60,000–200,000 cells/μL
Maximal regeneration	200,000–500,000 cells/μL

Reticulocyte maturation

In dogs, reticulocyte maturation occurs in 24–48 hours. Maturation involves a continuum of progressive loss of the visible organelles (Figure 1.20).

Cats are unique in that more than one kind of reticulocyte may be present. These reticulocytes are of the aggregate and the punctate forms (Figure 1.21). The aggregate reticulocyte has a clumped reticulum that appears to be identical to that of other species. In the punctate reticulocyte, discrete dots are seen without any clumping; other species do not have this reticulocyte counterpart. Only aggregate reticulocytes appear to be polychromatophilic with Wright stain. Punctate reticulocytes are indistinguishable from normal, mature erythrocytes with Wright stain.

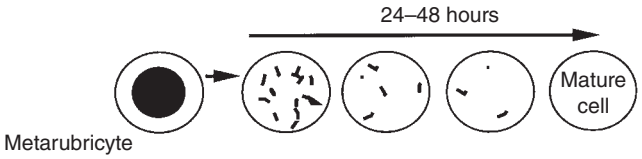

Figure 1.20 Sequential erythroid maturation as related to the reticulocyte stain and interpreted in dogs. The metarubricyte denucleates on leaving the reticulocyte. Reticulum is progressively lost during a 24–48-hour period, resulting in a mature erythrocyte.

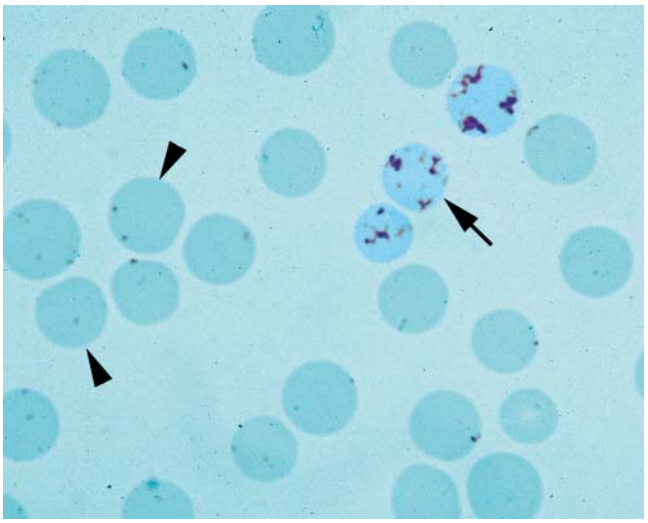

Figure 1.21 Feline reticulocyte morphology with new methylene blue stain. Three aggregate reticulocytes are in the field; note the representative one (arrow). The remainder of the cells are punctate reticulocytes; note the representative cells (arrowheads).

Reticulocyte maturation in cats also may be viewed as a continuum (Figure 1.22). Aggregate reticulocytes mature to the punctate form in approximately 12 hours; the punctate cells may continue to mature for another 10–12 days. Because of the short maturation time of aggregate reticulocytes, these cells are the best indicator of active marrow release. Therefore, only aggregate cells are counted in cats, and interpretive guidelines apply to this cell type only. Experience is required to exclude punctate cells when performing the reticulocyte count.

Organization of the complete blood count (hemogram)

It is useful to summarize the described basic and advanced determinations in a way that shows the organization of how they are performed and interpreted. This provides a mental framework for simplifying the complexity of this information into an everyday, intuitive tool: the hemogram. The techniques for generating data may be organized conceptually as