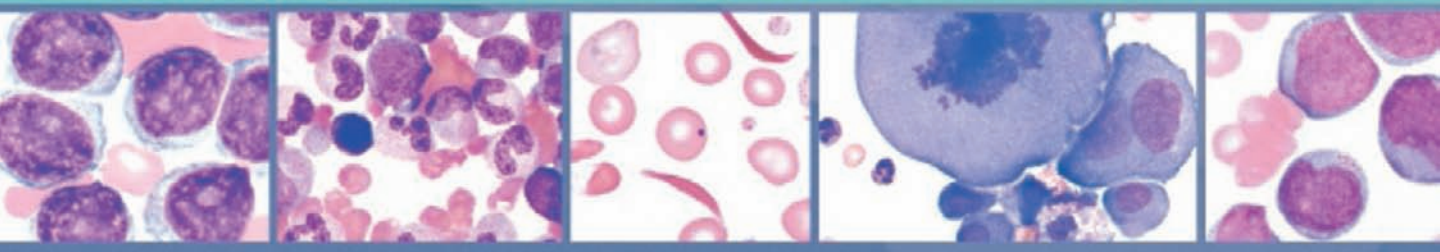
Fifth Edition

Clinical Hematology Atlas



Bernadette F. **Rodak** • Jacqueline H. **Carr**

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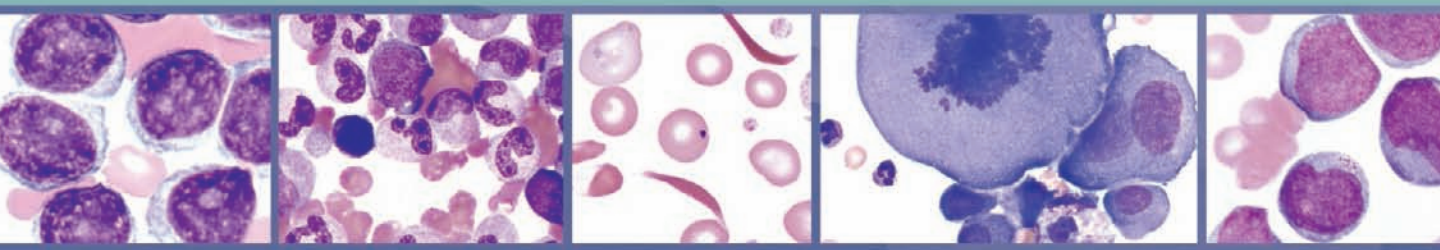

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CLINICAL HEMATOLOGY ATLAS, FIFTH EDITION ISBN: 978-0-323-32249-2

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International Standard Book Number: 978-0-323-32249-2

Library of Congress Cataloging-in-Publication Data
Rodak, Bernadette F., author.
Clinical hematology atlas / Bernadette F. Rodak, Jacqueline H. Carr.
–Fifth edition.
p. ; cm.
Includes index.
ISBN 978-0-323-32249-2 (pbk. : alk. paper)
I. Carr, Jacqueline H., author. II. Title.
[DNLM: 1. Hematologic Diseases–diagnosis–Atlases. 2. Hematologic Diseases–pathology–Atlases. WH 17]
RB145
616.1'5–dc23

2015036694

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Design Direction: Julia Dummitt

Printed in China
Last digit is the print number: 9 8 7 6 5 4 3 2 1

To our husbands,
Robert Hartman
and
Charles Carr,
daughters,
Kimberly Carr Mayrose
and
Alexis Carr,
and all of our colleagues who have encouraged us
to continue to publish this atlas in its concise format

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PREFACE

Because the emphasis of an atlas is morphology, the *Clinical Hematology Atlas* is intended to be used with a textbook, such as *Rodak's Hematology*, fifth edition, that addresses physiology and diagnosis along with morphology. This atlas is designed for a diverse audience that includes clinical laboratory science students, medical students, residents, and practitioners. It is also a valuable resource for clinical laboratory practitioners who are being retrained or cross-trained in hematology. It is not intended to be a detailed, comprehensive manual for diagnosis.

In this concise format, every photomicrograph and word has been evaluated for value to the microscopist. All superfluous information has been excluded in an attempt to maintain focus on significant microscopic findings while correlating this information with clinical diagnosis. What started as a primer for Clinical Laboratory Science students with no previous hematology education has evolved into an internationally recognized reference for multiple levels of expertise, from entry level to practicing professionals.

ORGANIZATION

As is frequently expounded, morphology on a peripheral blood film is only as good as the quality of the smear and the stain. Chapter 1 reviews smear preparation, staining, and the appropriate area in which to evaluate cell distribution and morphology. A table that summarizes the morphology of leukocytes found in a normal differential, along with multiple examples of each cell type, facilitates early instruction in blood smear review.

Chapter 2 schematically presents hematopoietic features of cell maturation. General cell maturation, along with an electron micrograph with labeled organelles, will help readers correlate the substructures with the appearance of cells under light microscopy. Visualizing normal cellular maturation is essential to the understanding of disease processes. This correlation of schematic, electron micrograph, and Wright-stained morphology is carried throughout the maturation chapters. Figure 2-1 has been formatted to reflect recent hematopoietic theory. In addition, the chart aids readers in recognizing the anatomical sites at which each stage of maturation normally occurs.

Chapters 3 to 9 present the maturation of each cell line individually, repeating the respective segment of the overall hematopoietic scheme from Chapter 2, to assist the student in seeing the relationship of each cell line to the whole. In these chapters, each maturation stage is presented as a color print, a schematic, and an electron micrograph. A description of each cell, including overall size, nuclear-to-cytoplasmic ratio, morphologic features, and reference ranges in peripheral blood and bone marrow, serves as a convenient summary. The final figure in each of these chapters summarizes lineage maturation by repeating the hematopoietic segment with the corresponding photomicrographs. Multiple nomenclatures for erythrocyte maturation are used to accommodate use in multiple settings and demographic groups.

Chapters 10 to 12 present discrete cellular abnormalities of erythrocytes, that is, variations in size, color, shape, and distribution, as well as inclusions found in erythrocytes. Each variation is presented along with a description of the abnormality, or composition of the inclusion, and associated disorders.

Because diseases are often combinations of the cellular alterations, Chapter 13 integrates morphologic findings into the diagnostic features of disorders primarily affecting erythrocytes.

In Chapter 14, nuclear and cytoplasmic changes in leukocytes are displayed and correlated with non-malignant leukocyte disorders.

Diseases of excessive or altered production of cells may be caused by maturation arrest, asynchronous development, or proliferation of one cell line, as presented in Chapters 15 to 19. Cytochemical stains are presented with disorders in which they are useful.

The therapeutic use of myeloid growth factors causes morphologic changes that mimic severe infections or malignancies. Chapter 20 presents examples of peripheral blood morphology following G-CSF or GM-CSF. It is the authors' design that the cellular defects in leukocyte disorders be visually compared with the process of normal hematopoiesis for a more thorough comprehension of normal and altered development. Readers are encouraged to refer to the normal hematopoiesis illustration, Figure 2-1, for comparison of normal and abnormal cells and the progression of diseases.

Microorganisms, including parasites, may be seen on peripheral blood smears. A brief photographic overview is given in Chapter 21. Readers are encouraged to consult a microbiology reference, such as Mahon CM, Lehman DC, Manuselis G: *Textbook of Diagnostic Microbiology*, fifth edition, for a more detailed presentation.

Chapter 22 includes photomicrographs that are not categorized into any one particular area, such as fat cells, mitotic figures, metastatic tumor cells, and artifacts.

Chapter 23 describes findings expected in the peripheral blood of neonates, including anticipated variations in morphology and cellular distribution. Comparison of the hematogone, normal for newborns, with the blast cell of acute leukemia is included.

Chapter 24 is intended to be an overview of the most frequent microscopic findings in body fluids. It is not proposed as a comprehensive review of the cytology of human body fluids, but rather a quick reference for the beginning microscopist as well as the seasoned professional.

As with the third edition and fourth editions, the fifth edition features spiral binding, making the atlas more convenient when used at the microscope bench.

All of these chapters combine into what we believe is a comprehensive and valuable resource for any clinical laboratory. The quality of the schematic illustrations, electron micrographs, and color photographs stand for themselves. We hope that this atlas will enrich the learning process for the student and serve as an important reference tool for the practitioner.

EVOLVE

The Evolve website provides free materials for both students and instructors. Instructors have access to an electronic image collection featuring all of the images from the atlas. Students and instructors have access to summary tables, student review exercises, and additional photos for identification.

Bernadette F. Rodak
Jacqueline H. Carr

ACKNOWLEDGMENTS

From inception to completion we have had a great deal of assistance and encouragement from the faculty and staff of the Department of Pathology and Laboratory Medicine, Indiana University School of Medicine. The following individuals have "gone the extra mile" to help us continue to realize our dream: **George Girgis**, MT(ASCP), for sharing his incredible collection of body fluid slides and his expertise in both blood cell and body fluid morphology; **Linda Marler** and **Jean Siders** for their technical assistance with digital photography and digital editing; and **Linda Marler** and **Carla Clem**, faculty members in the Clinical Laboratory Science program, for their support and patience during this endeavor. Carla also provided authoritative comments on images and helped us determine which images were classic examples. A particular thank you goes out to our families for their understanding during the many hours that we spent away from them while pursuing this goal.

A special thank you goes to the professionals at Elsevier who navigated us through the production of this atlas. We would especially like to thank **Laurie Gower,** Content Development Manager, **Karen Turner,** Content Development Specialist, **Rebecca Corradetti,** Developmental Editor at Spring Hollow Press, **Abigail Bradberry**, Project Manager, and **Julie Eddy**, Publishing Services Manager.

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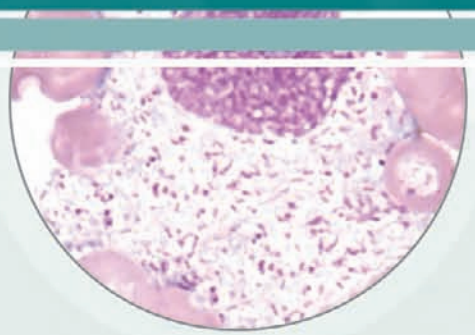
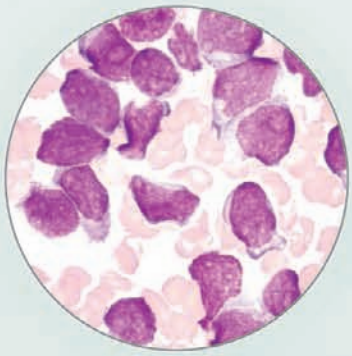
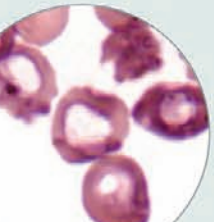
CHAPTER 1

INTRODUCTION TO PERIPHERAL BLOOD FILM EXAMINATION







A properly prepared blood film is essential to accurate assessment of cellular morphology. A variety of methods are available for preparing and staining blood films, the most common of which are discussed in this atlas. It is beyond the scope of this atlas to discuss other methodologies; however, detailed descriptions of these procedures can be found in textbooks on hematology, such as Keohane, Smith, and Walenga's *Rodak's Hematology: Clinical Principles and Applications*.

WEDGE FILM PREPARATION

MAKING THE PERIPHERAL BLOOD FILM

Although some automated analyzers prepare and stain blood films according to established criteria, manual blood film preparation is still used in many places. The wedge film is a convenient and commonly used technique for making peripheral blood films. This technique requires at least two 3 × 1-inch (75 × 25-mm) clean glass slides. High-quality, beveled-edge microscope slides are recommended. One slide serves as the blood film slide, and the other as the spreader slide. These can then be reversed to prepare a second film. A drop of ethylenediaminetetraacetic acid (EDTA) anticoagulated blood about 3 mm in diameter is placed at one end of the slide. Alternatively, a similar size drop of blood directly from a finger or heel puncture is acceptable. The size of the drop of blood is important. Too large a drop creates a long or thick film, and too small a drop often makes a short or thin film. In preparing the film, the technician holds the pusher slide securely in front of the drop of blood at a 30- to 45-degree angle to the film slide (Figure 1-1, *A*). The pusher slide is pulled back into the drop of blood and held in that position until the blood spreads across the width of the slide (Figure 1-1, *B*). It is then quickly and smoothly pushed forward to the end of the film slide, creating a wedge film (Figure 1-1, *C*). It is important that the whole drop of blood is picked up and spread. Moving the pusher slide forward too slowly accentuates poor leukocyte distribution by pushing larger cells, such as monocytes and granulocytes, to the very ends and sides of the film. Maintaining a consistent angle between the slides and an even, gentle pressure is essential. It is frequently necessary to adjust the angle between the slides to produce a satisfactory film. For higher than normal hematocrit, the angle between the slides must be lowered so that the film is not too short and thick. For extremely low hematocrit, the angle must be raised. A well-made peripheral blood film (Figure 1-2) has the following characteristics:

1. About two-thirds to three-fourths of the length of the slide is covered by the film.
2. It is slightly rounded at the feather edge (thin portion), not bullet shaped.
3. Lateral edges of the film should be visible. The use of slides with chamfered (beveled) corners may facilitate this appearance.
4. It is smooth without irregularities, holes, or streaks.
5. When the slide is held up to light, the feather edge of the film should have a "rainbow" appearance.
6. The whole drop is picked up and spread.

Figure 1-3 shows examples of unacceptable films.

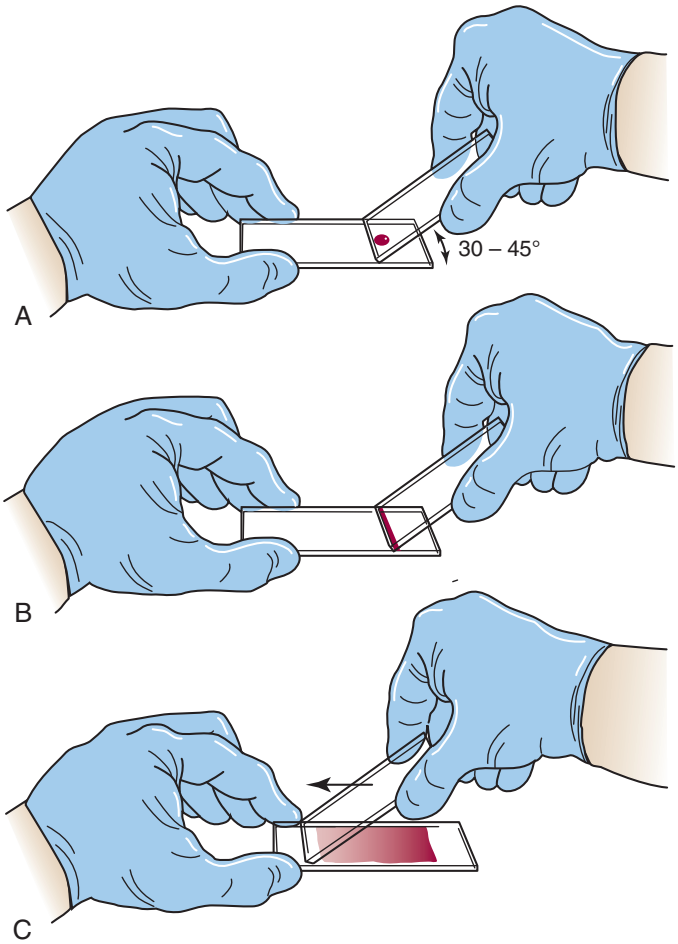


FIGURE 1–1 Wedge technique of making a peripheral blood film. **A**, Correct angle to hold spreader slide. **B**, Blood spread across width of slide. **C**, Completed wedge film.
(From Keohane E.A., Smith L., Walenga J. (Eds.) (2016). *Rodak's hematology: clinical principles and applications*. (5th ed.). St. Louis: Saunders Elsevier.)

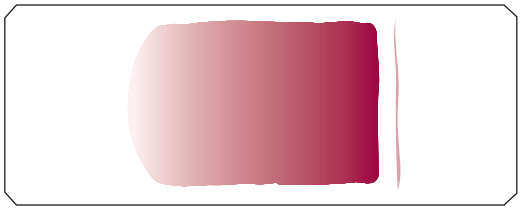


FIGURE 1–2 Well-made peripheral blood film.
(From Keohane E.A., Smith L., Walenga J. (Eds.) (2016). *Rodak's hematology: clinical principles and applications*. (5th ed.). St. Louis: Saunders Elsevier.)

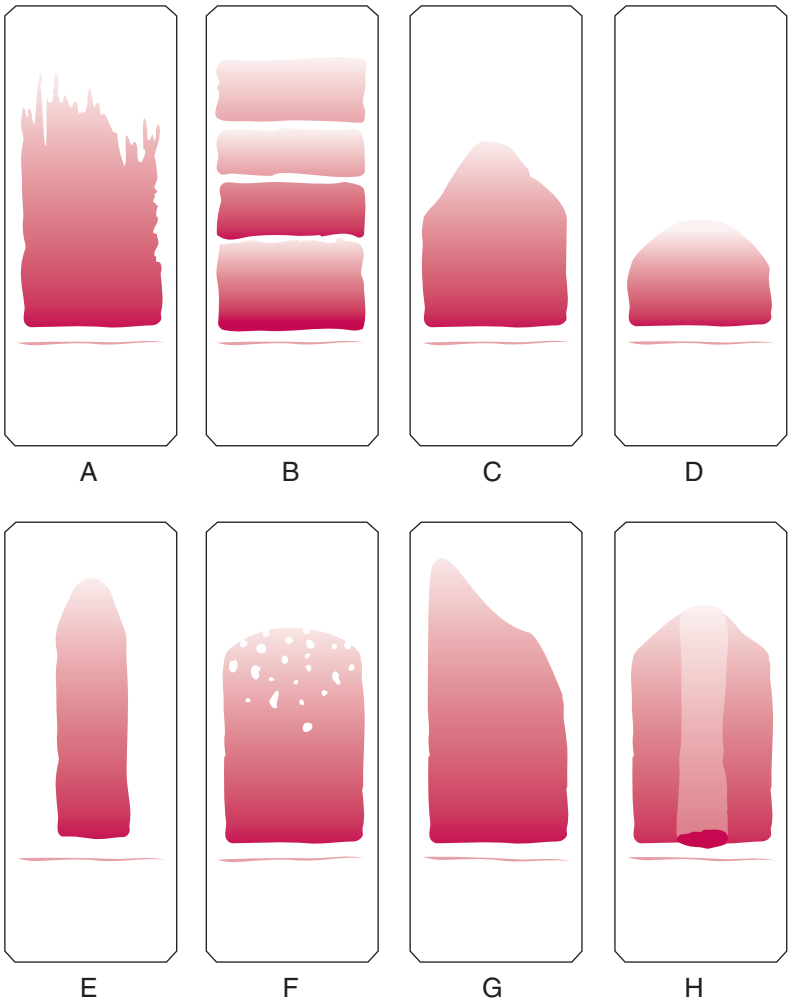


FIGURE 1–3 Unacceptable peripheral blood films. Slide appearances associated with the most common errors are shown, but note that a combination of causes may be responsible for unacceptable films. **A**, Chipped or rough edge on spreader slide. **B**, Hesitation in forward motion of spreader slide. **C**, Spreader slide pushed too quickly. **D**, Drop of blood too small. **E**, Drop of blood not allowed to spread across the width of the slide. **F**, Dirt or grease on the slide; may also be caused by elevated lipids in the blood specimen. **G**, Uneven pressure on the spreader slide. **H**, Time delay; drop of blood began to dry. (From Keohane E.A., Smith L., Walenga J. (Eds.) (2016). *Rodak's hematology: clinical principles and applications*. (5th ed.). St. Louis: Saunders Elsevier.)

STAINING OF PERIPHERAL BLOOD FILMS

The purpose of staining blood films is to identify cells and recognize morphology easily through the microscope. Wright or Wright-Giemsa stains are the most commonly used for peripheral blood and bone marrow films. These stains contain both eosin and methylene blue and are therefore termed *polychrome stains*. The colors vary slightly from laboratory to laboratory, depending on the method of staining.

Slides must be allowed to dry thoroughly before staining. The cells are fixed to the glass slide by the methanol in the stain. Staining reactions are pH dependent, and the actual staining of the cellular components occurs when a buffer (pH 6.4) is added to the stain. Free methylene blue is basic and stains acidic cellular components, such as RNA, blue. Free eosin is acidic and stains basic components, such as hemoglobin or eosinophilic granules, red. Neutrophils have cytoplasmic granules that have a neutral pH and accept some characteristics from both stains. Details for specific methods of staining peripheral blood and bone marrow films, including automated methods, may be found in a standard textbook of hematology.

An optimally stained film (Figure 1-4) has the following characteristics:

1. The red blood cells (RBCs) should be pink to salmon.
2. Nuclei are dark blue to purple.
3. Cytoplasmic granules of neutrophils are lavender to lilac.
4. Cytoplasmic granules of basophils are dark blue to black.
5. Cytoplasmic granules of eosinophils are red to orange.
6. The area between the cells should be colorless, clean, and free of precipitated stain.

A well-stained slide is necessary for accurate interpretation of cellular morphology. The best staining results are obtained from freshly made slides that have been prepared within 2 to 3 hours of blood collection. Box 1-1 lists common reasons for poorly stained slides and may be used as a guide when troubleshooting.

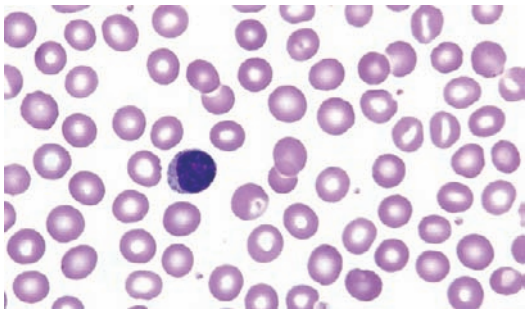


FIGURE 1–4 Optimally stained peripheral blood film demonstrating the appropriate area in which to perform the white blood cell differential and morphology assessment and the platelet estimate. Only the center of the field is shown; an entire field would contain 200 to 250 red blood cells (original ×1000).

BOX 1-1

Troubleshooting Poorly Stained Blood Films

First Scenario

Problems

- Red blood cells appear gray
- White blood cells are too dark
- Eosinophil granules are gray, not orange

Causes

- Stain or buffer too alkaline (most common)
- Inadequate rinsing
- Prolonged staining
- Heparinized blood sample

Second Scenario

Problems

- Red blood cells too pale or red color
- White blood cells barely visible

Causes

- Stain or buffer too acidic (most common)
- Underbuffering (time too short)
- Over-rinsing

From Keohane E.A., Smith L., Walenga J. (Eds.) (2016). *Rodak's hematology: clinical principles and applications.* (5th ed.). St. Louis: Saunders Elsevier.

PERIPHERAL FILM EXAMINATION

10× EXAMINATION

Examination of the blood film is a multistep process. Begin the film examination with a scan of the slide using the 10× or low-power objective (total magnification = 100×). This step is necessary to assess the overall quality of the film, including abnormal distribution of RBCs, suggesting the presence of rouleaux or autoagglutination, and/or the presence of a disproportionate number of large nucleated cells such as monocytes or neutrophils at the edges of the film. If the latter exists, another film should be prepared. In addition, the 10× film examination allows for the rapid detection of large abnormal cells such as blasts, reactive lymphocytes, and parasites.

40× OR 50× EXAMINATION

Using the 40× (high dry) objective or the 50× oil objective (400× and 500× total magnification, respectively), find an area of the film in which the RBCs are evenly distributed and barely touching one another (two or three cells may overlap; Figure 1-5). Scan eight to ten fields in this area of the film, and determine the average number of white blood cells (WBCs) per field. Although an exact factor varies with the make and model of microscope, in general, an approximate WBC count per cubic millimeter can be determined by multiplying the average number of WBCs per high-power field by 2000 (if 40× is used), or 2500 (if 50× is used).

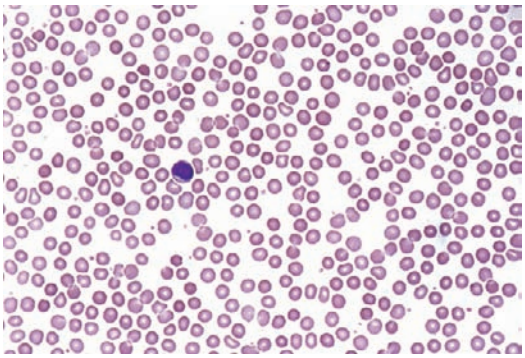


FIGURE 1–5 Correct area of blood film in which to evaluate cellular distribution and perform white blood cell estimate (×400).

This estimate is a useful quality-control tool for validating WBC counts from hematology analyzers. Any discrepancy between the instrument WBC count and the slide estimate must be resolved. Some reasons for discrepancies include the presence of WBC or platelet clumps, fibrin strands, severe RBC agglutination, cryoprecipitate, and giant platelets, in addition to a mislabeled film, a film made from the wrong patient's sample, and an instrument malfunction.

100× EXAMINATION

The next step in film evaluation is to perform the WBC differential. This is done in the same area of the film as the WBC estimate but using the 100 × oil immersion objective (1000 × total magnification). When the correct area of the film from a patient with a normal RBC count is viewed, about 200 to 250 RBCs per oil immersion field are seen (see Figure 1-4). Characteristically, the differential count includes counting and classifying 100 consecutive WBCs and reporting these classes as percentages. The differential count is performed in a systematic manner using the "battlement" track (Figure 1-6), which minimizes WBC distribution errors. The results are reported as percentages of each type of WBC seen during the count. An example of a WBC differential count is 3% bands, 55% segmented neutrophils, 30% lymphocytes, 6% monocytes, 4% eosinophils, and 2% basophils (Table 1-1). Any WBC abnormalities, such as toxic changes, Döhle bodies, reactive lymphocytes, and Aüer rods, are also reported. When present, nucleated red blood cells (NRBCs) are counted and reported as number of NRBCs per 100 WBCs. The RBC, WBC, platelet morphology evaluation, and platelet estimates are also performed under the 100 × oil immersion objective. RBC inclusions, such as Howell-Jolly bodies, and WBC

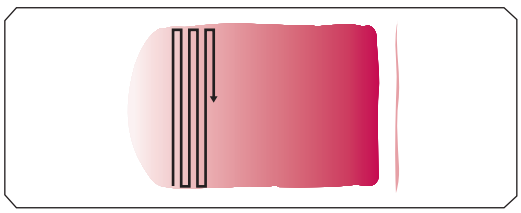


FIGURE 1–6 "Battlement" pattern for performing a white blood cell differential. (From Keohane E.A., Smith L., Walenga J. (Eds.) (2016). *Rodak's hematology: clinical principles and applications*. (5th ed.). St. Louis: Saunders Elsevier.)

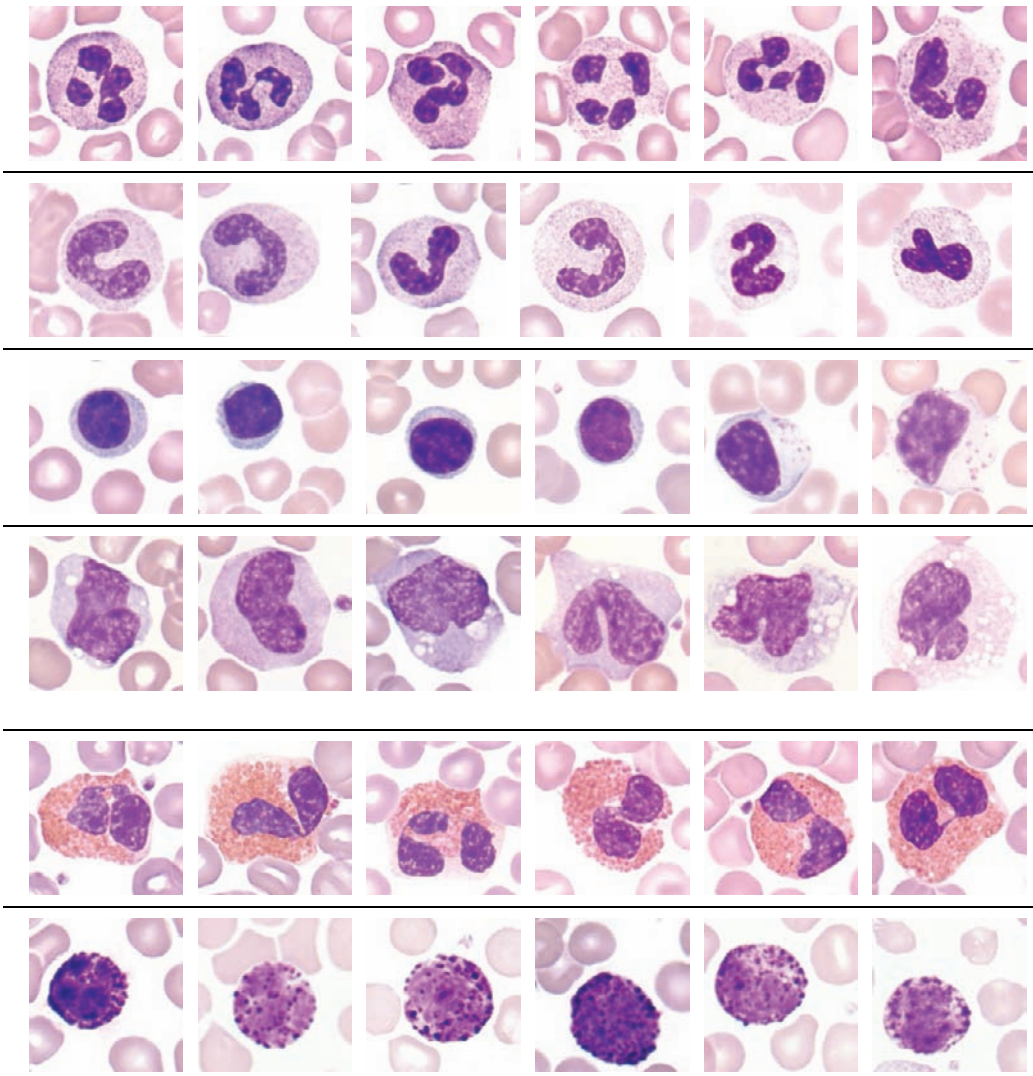
TABLE 1-1

Cells Found in a Normal White Blood Cell Differential

Cell Type	Cell Size (μm)	Nucleus	Chromatin	Cytoplasm	Granules	Adult Reference Range: Peripheral Blood (%)	Adult Reference Range: Cells × 10^9/L
Segmented neutrophil (Seg), polymorphonuclear neutrophil (Poly, PMN)	10 to 15	2 to 5 lobes connected by thin filaments without visible chromatin	Coarsely clumped	Pale pink, cream colored, or colorless	1°: Rare 2°: Abundant	50 to 70	2.3 to 8.1
Band neutrophil (Band)	10 to 15	Constricted, but chromatin must be visible within the thinnest part	Coarsely clumped	Pale blue to pink	1°: Few 2°: Abundant	0 to 5	0.0 to 0.6
Lymphocyte (Lymph)	7 to 18*	Round to oval; may be slightly indented; occasional nucleoli	Condensed to deeply condensed	Scant to moderate; sky blue	± Few azurophilic	20 to 40	0.8 to 4.8
Monocyte (Mono)	12 to 20	Variable; may be round, horseshoe, or kidney shaped; often has folds producing "brainlike" convolutions	Moderately clumped; lacy	Blue-gray; may have pseudopods; vacuoles may be absent or numerous	Many fine granules, frequently giving the appearance of ground glass	3 to 11	0.5 to 1.3
Eosinophil (Eos)	12 to 17	2 to 3 lobes connected by thin filaments without visible chromatin	Coarsely clumped	Cream to pink; may have irregular borders	1°: Rare 2°: Abundant red to orange, round	0 to 5	0.0 to 0.4
Basophil (Baso)	10 to 14	Usually two lobes connected by thin filaments without visible chromatin	Coarsely clumped	Lavender to colorless	1°: Rare 2°: Lavender to dark purple; variable in number with uneven distribution; may obscure nucleus or wash out during staining, giving the appearance of empty areas in cytoplasm	0 to 1	0.0 to 0.1

*The difference in size from small to large lymphocyte is primarily a result of a larger amount of cytoplasm. See Chapter 9 for more detailed information on lymphocyte size.

1°, primary; *2°*, secondary.



inclusions, such as Döhle bodies, can be seen at this magnification. Each laboratory should have established protocols for standardized reporting of abnormalities.

Evaluation of the RBC morphology is an important aspect of the film evaluation and is used in conjunction with the RBC indices to describe cells as normal or abnormal in size, shape, and color. Each laboratory should establish a standard reporting protocol. Most laboratories use concise statements describing overall RBC morphology that is consistent with the RBC indices. The microscopic evaluation of RBC morphology must be congruent with the information given by the automated hematology analyzer. If not, discrepancies must be resolved before reporting patient results.

The final step in the performance of the differential count is the estimation of the platelet number. This is done under the 100× oil immersion objective. In an area of the film where RBCs barely touch, the number of platelets in five to ten oil immersion fields is counted. The average number of platelets is multiplied by 20,000 to provide an estimate of the total number of platelets per cubic millimeter. This estimate is reported as adequate if the estimate is consistent with a normal platelet count, decreased if below the lower limit of normal for that laboratory, and increased if above the upper limit of normal. A general reference range is 150,000 to 450,000/mm^3 (150–450 × 10^9/L). When a patient is extremely anemic or has erythrocytosis, a more involved formula for platelet estimates may be used.

The estimate can be compared with an automated platelet count as an additional quality-control measure. If the estimate and the instrument platelet count do not agree, discrepancies must be resolved. Some causes for discrepancies include the presence of giant platelets, many schistocytes (red blood cell fragments), and platelet satellitism. Notably, high-quality 40× or 50× oil immersion objectives can be used by the experienced technologist to perform the differential analysis of the blood film. However, all abnormal findings must be verified under the 100× objective.

SUMMARY

A considerable amount of valuable information can be obtained from properly prepared, stained, and evaluated peripheral blood films. Many laboratories use films made by the wedge technique from EDTA anticoagulated blood and stained with Wright or Wright-Giemsa stain. The films should be evaluated in a systematic manner using first the 10×, then 40× high dry or 50× oil, and finally the 100× oil immersion objectives on the microscope. WBC differential and morphology and the RBC morphology and platelet estimate are included in the film evaluation.

CHAPTER 2

HEMATOPOIESIS