



# Standard Practice for Assessment of White Blood Cell Morphology After Contact with Materials<sup>1</sup>

This standard is issued under the fixed designation F 2151; the number immediately following the designation indicates the year of original adoption or, in the case of revision, the year of last revision. A number in parentheses indicates the year of last reapproval. A superscript epsilon (ε) indicates an editorial change since the last revision or reapproval.

## 1. Scope

1.1 This practice provides a protocol for the assessment of the effect of materials used in the fabrication of medical devices, that will contact blood, on the morphology of white blood cells.

1.2 This practice is intended to evaluate the acute *in vitro* effects of materials intended for use in contact with blood.

1.3 This practice uses direct contact of the material with blood, and extracts of the material are not used.

1.4 This practice is one of several developed for the assessment of the biocompatibility of materials. Practice F 748 provides general guidance for the selection of appropriate methods for testing materials for a specific application.

1.5 *This standard does not purport to address all of the safety concerns, if any, associated with its use. It is the responsibility of the user of this standard to establish appropriate safety and health practices and determine the applicability of regulatory limitations prior to use.*

1.6 *Identification of a supplier of materials or reagents is for the convenience of the user and does not imply single source. Appropriate materials and reagents may be obtained from many commercial supply houses.*

## 2. Referenced Documents

### 2.1 ASTM Standards:

F 619 Practice For Extraction Of Medical Plastics<sup>2</sup>

F 748 Practice For Selecting Generic Biological Test Methods For Materials And Devices<sup>2</sup>

F 756 Practice for Assessment of Hemolytic Properties of Materials<sup>2</sup>

## 3. Terminology

### 3.1 Definitions:

3.1.1 *control material, n*—a material such as low density polyethylene (LDPE) which is expected to have minimal effect on the morphology of white blood cells.

3.1.2 *nuclear damage, n*—for the white blood cell morphology test, this term is used to describe the nucleus of a white blood cell appears to be condensed, fragmented or lysed; for the White Blood Cell Morphology Test. This includes nuclear damage that might be classified as karyolysis, karyorrhexis, pyknosis, or simply necrosis.

3.1.3 *positive control material, n*—a material such as latex (gloves, dental dam, or tubing) or TSV, tin-stabilized vinyl (slab), which is expected to have an adverse effect on the morphology of white blood cells.

### 3.2 Abbreviations:

3.2.1 *B*—basophil

3.2.2 *BR*—broken or lysed

3.2.3 *E*—eosinophil

3.2.4 *INDNM*—indistinct nuclear membrane: a degenerative change of the nucleus; for the White Blood Cell Morphology Test, this term is used to describe a nuclear membrane that is rough, ragged, or torn

3.2.5 *L*—lymphocyte

3.2.6 *M*—monocyte

3.2.7 *N*—neutrophil

3.2.8 *UNID*—unidentified

## 4. Summary of Practice

4.1 Test and control material specimens are exposed to contact with canine blood under defined static conditions and the effect on blood cell morphology is determined. The use of human blood is permissible if the laboratory is knowledgeable of precautions needed to handle human blood. If a source of blood other than canine or human is used, consideration should be given to the differences in hematologic values and morphology differences between that species and humans.

## 5. Significance and Use

5.1 The presence of material in contact with the blood may cause damage to white blood cells resulting in changes in function of these cells or changes in properties of the blood.

<sup>1</sup> This practice is under the jurisdiction of ASTM Committee F04 on Medical and Surgical Materials and Devices and is the direct responsibility of Subcommittee F04.16 on Biocompatibility Test Methods.

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<sup>2</sup> *Annual Book of ASTM Standards*, Vol 13.01.

5.2 This practice may not be predictive of events occurring during all types of blood contacting applications. The user is cautioned to consider the appropriateness of the method in view of the materials being tested, their potential applications, and the recommendations contained in Practice F 748. The propensity of a material to cause hemolysis should be addressed according to Practice F 756.

## 6. Preparation of Test and Control Specimens

6.1 Specimen samples should be prepared according to Practice F 619. Direct contact of the material with blood will be studied, the blood is the extractant, and other extracts of the material are not used. Prepare a sample size such that 1 mL of blood is used. If the sample size is such that larger volumes of blood are needed, this is permissible but note in the report. This scale up would be based on an expected sample size of 3 cm<sup>2</sup>/mL.

6.2 The final sample should be prepared with a surface finish consistent with end-use application.

6.3 The sample shall be sterilized by the method to be used for the final product.

6.4 Care should be taken that the specimens do not become contaminated during preparation, but aseptic technique is not required.

## 7. Preparation of Blood Sample

7.1 Trained personnel are required for the blood draw and the EDTA collection tubes should be used. If human blood is used, extra safety precautions may be needed. Fill the necessary numbers of EDTA blood collection tubes from the jugular vein or other appropriate vein using an appropriate size needle. The blood collection tube should be filled to capacity. Do not withdraw more than 5 mL/kg of blood per day and no more than 10 mL/kg/week from any one dog.

NOTE 1—EDTA is the anticoagulant of choice for morphology studies. Other anticoagulants (heparin, sodium citrate) may also be considered. However, it is not known at this time whether results using other anticoagulants are comparable to results using EDTA.

7.2 Gently rock the collection tube back and forth three times to mix the anticoagulant with the blood. Record the time the blood was drawn. Testing should be initiated as soon as possible after the blood was drawn and definitely within 1 h. Pool the blood samples and mix well immediately before use. Adequate mixing (20 complete inversions by hand) to ensure suspension of all cellular components is necessary just before preparation of testing and time zero blood smear preparation. Do not refrigerate the blood before testing.

7.3 Transfer 1 mL of the blood into the vial containing the LDPE negative control and place into the 37°C ± 2 water bath. It is recommended that screw-capped borosilicate glass vials 11 by 48 mm with a 4-mL capacity be used. Dispense the appropriate volume of blood into the other vials containing the positive control and the test articles. This may be staggered to allow for processing time so that incubation times may be consistent. Place the vials into the 37°C ± 2 water bath immediately after dispensing the blood. Ensure that the test articles are covered with blood. It may be necessary to use a plastic pipette tip or wooden applicator stick to push the article

to the bottom of the vial. Incubate the sample vials for 120 ± 5 min without mixing or agitation. Do not sink floaters that rise from the bottom of the vial during the incubation.

7.4 At the end of the incubation time, remove the vials one at a time, remove the control or test article, and prepare the smears as described in Section 8.

## 8. Preparation and Staining of Smears

8.1 *Preparation of Time Zero Smears*—Prepare two acceptable smears from the anticoagulated blood within 1 h after the blood was drawn. An automated instrument may be used.

8.1.1 An acceptable smear has the following characteristics: smooth appearance, a feathered edge, and a slight margin on both sides of the length of the slide.

8.1.2 Quickly dry the smears by waving the slides rapidly in the air to prevent distortion of the cells. Label the two smears with the following: date of preparation, Time 0, A, or B.

8.1.3 When dry, stain the blood smears using Wright-Giemsa stain (or appropriate stain designated for blood smears) following the instructions. Purified water, which is neutral, rather than tap water, which may be alkaline, or distilled water, which may be acidic, should be used to control the pH in the rinse stage. Lean the slides in a vertical position to dry, draining from thick portion of smear to the thin area. Do not accelerate drying with heat, forced air, or other means. Do not coverslip the slides at this time.

8.1.4 *Evaluate the Staining Quality*—Microscopically scan the smear to locate an area with good white cell distribution. Using the highest magnification possible without the use of oil, assess the staining quality of individual white blood cells. There should be clear nuclear-cytoplasmic demarcations, distinct nuclear chromatin patterns, and cytoplasmic color differences.

8.1.5 If the staining quality is not acceptable, additional time zero smears can be made to correct the staining problem. Once the correct staining procedure is identified, this should be noted and then used on the control and test smears.

8.2 *Preparation of Control and Test Article Smears*:

8.2.1 After each vial has been incubated at 37°C for 120 ± 5 min, remove the negative control or test article from the vial with tweezers allowing as much blood as possible to drain from the article back into the vial.

8.2.2 Visually inspect the removed article for adherence of blood or blood clots and record the findings.

8.2.3 Immediately after the negative control or test article is removed and examined, swirl or rock the vial gently several times to mix the blood and prepare two acceptable blood smears as described in 8.1.1. Cells may become fragile after exposure to biomaterials and therefore should be handled gently. Appropriately label each pair of smears with the date, control or test article, and Smear A or B. All blood smears must be prepared within 4 h of the blood draw.

8.2.4 Stain the smears according to the protocol identified in 8.1.5.

8.3 *Preparation for Examination (Optional Procedure for Application of Cover Slip)*:

8.3.1 When all smears are dry, mount a cover slip by placing small drops or a thin line of mounting medium down the center of the smear and placing the coverslip on top. Keep the slide

flat allowing the medium to spread and cover the slide. Coverslipping is recommended if slides are to be archived.

8.3.2 Allow the slides to air dry for at least 6 h. Remove excess background color from the back of each slide by wiping with a paper towel or gauze pad dipped in methanol.

**8.4 Microscopic Examination of Blood Smears:**

8.4.1 Microscopically examine one blood smear from each set prepared keeping the second smear as an alternative or backup. Identify the smear examined on the worksheet.

8.4.2 Using an appropriate magnification, scan the blood smear for cell distribution. Find an area for examination with cells as a monolayer with cells lying adjacent to one another. This should be in the thin area and not in the feathered region. Place a drop of immersion oil onto the slide and examine the area with an oil immersion lens.

8.4.3 Perform a differential count on 100 intact white blood cells by moving across the width of the smear and then repeat

with the next adjacent but not overlapping area. Avoid the very edge of the smear and avoid thick streaks of cells. All white blood cells encountered must be included in the count. Intact cells should be scored on Table 1. The presence of broken or lysed cells should be recorded in Table 2. Refer to the referenced literature, Appendix X2 for additional information for cell identification, and Appendix X3 for examples.

**8.5 Performing a Differential:**

8.5.1 Count and record onto the test article scoring sheet for each number, the type of white blood cell observed, and any morphological changes. Refer to Appendix X2 and references for examples. Include in the count the number of altered white cells that are morphologically unidentifiable as to type. Those that are unidentifiable because they are broken or lysed are also counted but counted separately. Use the following codes for recording cell types.

**TABLE 1 Test Article Scoring Sheet**

Cell	Type	Ind NM	N. Damage	Other	Cell	Type	Ind NM	N. Damage	Other
1					51				
2					52				
3					53				
4					54				
5					55				
6					56				
7					57				
8					58				
9					59				
10					60				
11					61				
12					62				
13					63				
14					64				
15					65				
16					66				
17					67				
18					68				
19					69				
20					70				
21					71				
22					72				
23					73				
24					74				
25					75				
26					76				
27					77				
28					78				
29					79				
30					80				
31					81				
32					82				
33					83				
34					84				
35					85				
36					86				
37					87				
38					88				
39					89				
40					90				
41					91				
42					92				
43					93				
44					94				
45					95				
46					96				
47					97				
48					98				
49					99				
50					100				

**TABLE 2 Differential**

Cell Identification	Expected Range (Canine)	Expected Range (Human)	% Total WBCs
Neutrophil	60–77 %	45–75 %	
Lymphocyte	12–30 %	16–46 %	
Monocyte	3–10 %	4–11 %	
Eosinophil	2–10 %	0–8 %	
Basophil	rare <1 %	0–3 %	
Unidentified			
Number of WBCs scored	100	100	
Lysed cells			

N = neutrophil,  
M = monocyte,  
L = lymphocyte,  
E = eosinophil,  
B = basophil,  
UNID = unidentified, and  
BR = broken or lysed.

8.5.2 Evaluate the morphological changes while performing the differential count as “INDNM” for indistinct nuclear membrane, “N. damage” for any type of nuclear damage, and under the column other list the observation such as inclusions, foamy, etc.

8.5.3 A minimum of 100 cells must be counted. If 100 intact white blood cells cannot be counted on the first slide, record the results for Slide A and continue the counting using Slide B until 100 cells are reached. Record the results for each slide separately and then the combined count. Record the data in Tables 2 and 3.

#### 8.6 Summarizing the Data:

8.6.1 The smears will be evaluated with respect to literature values for the differential count. The numbers expected for canine blood are: neutrophils 60 to 77 %, lymphocytes 12 to 30 %, monocytes 3 to 10 %, eosinophils 2 to 10 %, basophils, rare <1 %. The numbers expected for human blood are: neutrophils 45 to 75 %, lymphocytes 16 to 46 %, monocytes 4 to 11 %, eosinophils 0 to 8 %, basophils, 0 to 3 %.

**TABLE 3 Morphology**

Cell Type	Indistinct NM	N. Damage	Other	% Abnormal
Neutrophils				
Lymphocytes				
Monocytes				
Eosinophils				
Basophils				
Unidentified				

#### 8.6.2 Interpretation:

8.6.2.1 If the number of intact white cells, including intact unidentifiable cells, with morphological changes is 4 % or less, the test article is considered to not have had an adverse effect upon white blood cell morphology (1).<sup>3</sup>

8.6.2.2 If the number of intact white cells, including intact unidentifiable cells, with morphological changes is greater than 4 %, the test article is considered to have had an adverse effect upon white blood cell morphology (1).

8.6.2.3 If the white blood cell differential of the blood in contact with the material differs from that expected as described in 8.6.1, and the number of intact white cells, including intact unidentifiable cells, with morphological changes is 4 % or less, count an additional 100 cells to verify.

8.6.2.4 If the number of intact white cells, including intact unidentifiable cells, with morphological changes is greater than 4 % for the negative control, and/or if the white blood cell differential of the blood in contact with the negative control material differs from that expected as described in 8.6.1, count an additional 100 cells to verify. If the results remain the same, evaluate the results of the time zero control. If the number of abnormal cells is greater than 4 % for the time zero control, then it is recommended that the test be repeated.

8.6.2.5 The number of intact white cells, including intact unidentifiable white cells, with morphological changes must be greater than 4 % for the blood exposed to the positive control material. Approximate ranges are 9 to 17 % for latex and 22 to 33 % for TSV.

## 9. Precision and Bias

9.1 *Precision*—The precision of this test method is being established.

9.2 *Bias*—The bias of this test method includes the quantitative estimates of the uncertainties of the calibration of the test equipment and the skill of the operators. At this time, statements of bias should be limited to the documented performance of particular laboratories.

## 10. Keywords

10.1 biocompatibility; blood compatibility; direct contact; white blood cells

<sup>3</sup> The boldface numbers in parentheses refer to the list of references at the end of this practice.

## APPENDIXES

### (Nonmandatory Information)

#### X1. RATIONALE

X1.1 The interaction of blood with a material may result in changes in the morphology of the white blood cells or cause destruction of these cells as indicated by the presence of lysed cells or both. A high number of lysed cells may be found on smears that have abnormal differential counts. Lysed cells may also be caused by applying too much pressure when preparing the smear.

X1.2 This standard practice describes a screening method to evaluate the effects following interaction *in vitro*. It does not attempt to address the issue of alteration of production of

specific cell types. Any change in differential with respect to the expected normal negative or the zero time control is a result of the direct effect of the material on the blood cells and does not address or reflect systemic effects.

X1.3 The results obtained with this procedure are intended to be used in conjunction with the results of other tests assessing blood compatibility and should also consider the duration of contact and nature of tissue contact during intended use.

#### X2. ADDITIONAL INFORMATION AND AN ATLAS OF WHITE BLOOD CELL MORPHOLOGY

##### X2.1 Normal Canine White Blood Cell Morphology (2, 3)

X2.1.1 *Neutrophils*—Normal range is 60 to 77 %.

X2.1.1.1 *Cytoplasm*—Stains a faint pinkish gray with indistinct, diffuse pinkish granulations. The granules appear as fine, dust-like particles. The cytoplasm is sometimes transparent.

X2.1.1.2 *Nucleus*—Irregularly lobed with rounded prominences. Most lobes are separated by a narrowing of the nucleus between lobes. An occasional filament may be seen connecting the lobes. The chromatin is clumped and coarse and stains deeply. Bands are rarely found; the nucleus is horseshoe in shape and the ends are often round and larger than the midportion. The nuclear membrane is smooth. If the nuclear membrane is irregular or a nuclear indentation occurs, the cell is classified as segmented. The presence of more than five lobes of the nucleus indicates aging of the cell. Hypersegmentation may occur as an artifact in stored blood. Cytoplasmic vacuolization in neutrophils may also result in stored blood (usually over 4 h) when EDTA is used. Clear, discrete cytoplasmic vacuoles are indicative of a delay in smear preparation and not inflammation.

X2.1.2 *Lymphocytes*—Normal range is 12 to 30 %.

X2.1.2.1 *Cytoplasm*—Usually pale blue and may contain azurophilic granules.

X2.1.2.2 *Nucleus*—The chromatin is clumped and deeply stained. The size of lymphocytes varies from small (around 10  $\mu\text{m}$ ) to large ( up to 15  $\mu\text{m}$ ). Canine lymphs are commonly of the small type. Smudge cells (bare nuclei) sometimes occur.

X2.1.2.3 *Small Cells*—The diameter of the nucleus is equal to or slightly greater than that of the canine RBC (7 to 10  $\mu\text{m}$ ). The nucleus is round to slightly indented with heavy coarse clumps of chromatin which stain dark purple. No nucleolus is present. The nucleus nearly fills the cell, leaving a narrow rim of (usually) sky blue cytoplasm. The cytoplasm can occasionally be an intense blue.

X2.1.2.4 *Large Cells*—Large lymphs vary greatly in size. Both the nucleus and the cytoplasm are more abundant than in the small lymph. The nucleus is round to slightly indented or

kidney shaped. The chromatin is more reticular, stains less intensely, and forms clumps. Nucleoli are absent. The nucleus is eccentrically placed in the cytoplasm, which is abundant and pale blue. Well-defined large pinkish granules (lysosomes) may be present in the cytoplasm. Occasional reactive lymphs are commonly found. The cytoplasm becomes more intensely basophilic, and the nucleus may be large with light chromatin and a nucleolus. The nucleus may be convoluted. Antigenic stimulation is responsible for the altered appearance. Lymphocytes are fragile, easily molded cells and the cytoplasmic margins are often indented by RBCs. When this occurs, the cytoplasmic margins may stain more intensely blue than the rest of the cytoplasm.

X2.1.3 *Monocytes*—Normal range is 3 to 10 %.

X2.1.3.1 *Cytoplasm*—Blue-gray (characteristically) ground glass appearance, foamy, lacy, or stringy. Vacuoles are sometimes found at one end and vary in size, giving the cytoplasm a frothy appearance. Dust-like pinkish granules may be observed in some cells.

X2.1.3.2 *Nucleus*—The nucleus is variable; if band-like, the ends are knob-like but rarely round. The chromatin is diffuse or mesh-like (characteristic) and more loose than the clumped chromatin of the lymphocyte. Any clumping is not uniform. Nuclear folds are characteristic. The monocyte is the largest leukocyte. If cell identification is in doubt, compare its cytoplasmic features to the nearest neutrophil: neutrophilic metamyelocytes and bands have a nearly colorless cytoplasm with barely visible neutrophilic granules and a regular nuclear outline. The cytoplasm of the mature neutrophil is usually pinky gray and may be granulated, as opposed to the more basophilic cytoplasm of the monocyte. Monocytes that have phagocytosed RBCs or nuclear debris may occasionally be found at the feather edge of the smear.

X2.1.4 *Eosinophils*—Range from 2 to 10 %.

X2.1.4.1 *Cytoplasm*—The cytoplasm is light blue with red or pink granules that are loosely packed in the cell. Canine eosinophils are unique because of the variable granulation.

Granules range from small and regular to few and large. They may be uniform in size or vary considerably with large and small granules present in the same cell. Frequently eosinophilic granules stain less intensely in the dog than in other species and the cells can be missed if not carefully inspected.

X2.1.4.2 *Nucleus*—Usually has two lobes that are darkly stained and polymorphic, yet smoother and less segmented than the mature neutrophil.

X2.1.5 *Basophils (Rare)*—Normal range is 0 %.

X2.1.5.1 *Cytoplasm*—The canine basophil is rarely recognized because of its unusual morphological features. The cytoplasm is gray blue with red-violet granules or purplish to black granules. In some cells, the granules are gray. The granules are evenly distributed, vary in number and size, and may appear as partially vacuolated. They have a regular round shape and fill the cytoplasm, masking the nucleus.

X2.1.5.2 *Nucleus*—The nucleus is characteristically long and polymorphonuclear (two to three lobes), longer than a neutrophil nucleus, thinner than most monocyte nuclei, and usually masked by granules. The basophil is a small round cell. Basophils are rarely seen in canine blood, and if present, they are usually associated with eosinophilia or canine heartworm disease.

## X2.2 Abnormal Canine White Blood Cell Morphology

X2.2.1 *Karyolysis and Karyorrhexis*—The nucleus of a white blood cell appears to be condensed, fragmented, or lysed (4). For the White Blood Cell Morphology Test, karyolysis is

used to describe a fragmented nucleus or one that coalesces to form one large drop or scatters into several droplets (5, p. 198-199, Fig. 6), and (3, p. 54-55, for photographs of white blood cells that would be scored as karyolytic).

X2.2.2 *Indistinct Nuclear Membrane*—A degenerative change of the nucleus (1). For the White Blood Cell Morphology Test, this term is used to describe a nuclear membrane that is rough, ragged, or torn.

X2.2.3 *Inclusions*—An inclusion body is a characteristic stainable particle in the nucleus or cytoplasm of the cell (3, p. 302) (6, Plate 1E (facing p. 2) for a photograph of neutrophil with an inclusion body in the cytoplasm).

X2.2.4 *Smudge Cell*—The bare nucleus of a ruptured white cell. A few may be found in a normal blood smear. They may be caused by heavy pressure on the cells during the smearing process. They should not be counted as part of the 100-cell differential (7). For further description and accompanying photograph, see (3, p. 166-167).

X2.2.5 *Basket Cell*—A net-like nucleus from a ruptured white cell. They are probably older forms of the smudge cell, and a few may be found in a normal blood smear. They are not counted as part of the 100-cell differential (7). For further description and accompanying photograph, see (3, p. 166-167).

X2.2.6 *Disintegrated Cell*—A ruptured cell. Both the nucleus and cytoplasm are visible. They are recorded as lysed cells. Excessive pressure during smearing may cause the cells to rupture. Disintegrated cells may also be found in anticoagulated blood that is over 2 h old (7).

## X3. ATLAS FOR SAMPLES OF CELL MORPHOLOGY

### X3.1 Normal Canine White Blood Cells

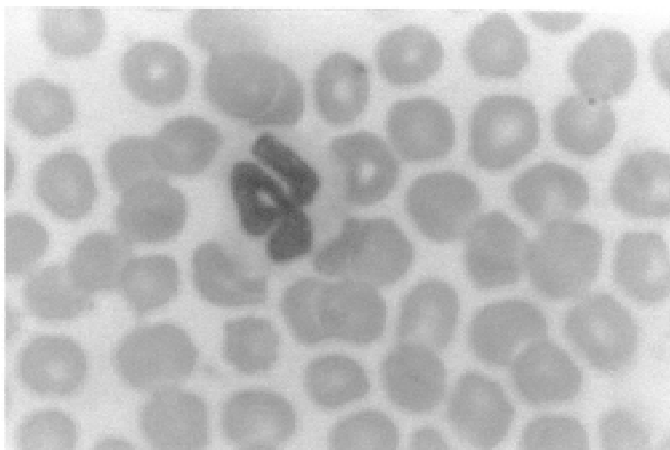


FIG. X3.1 Neutrophil

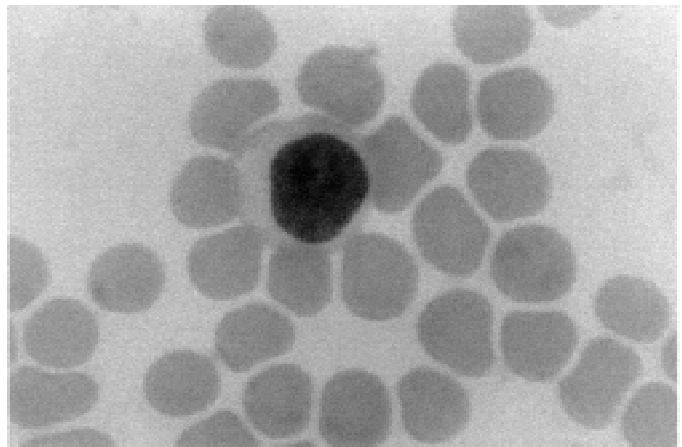


FIG. X3.2 Lymphocyte

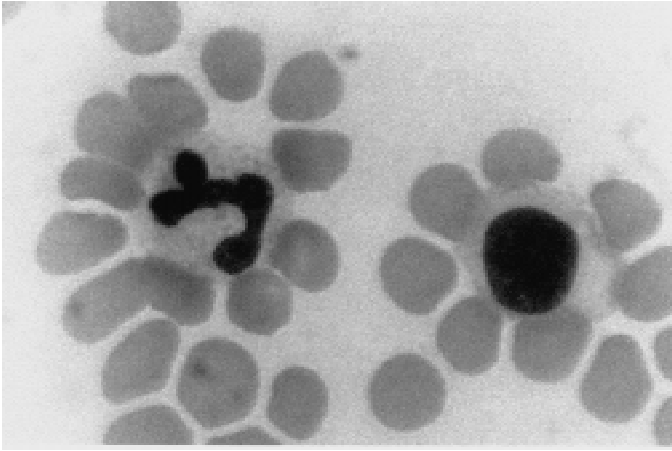


FIG. X3.3 Neutrophil and Lymphocyte

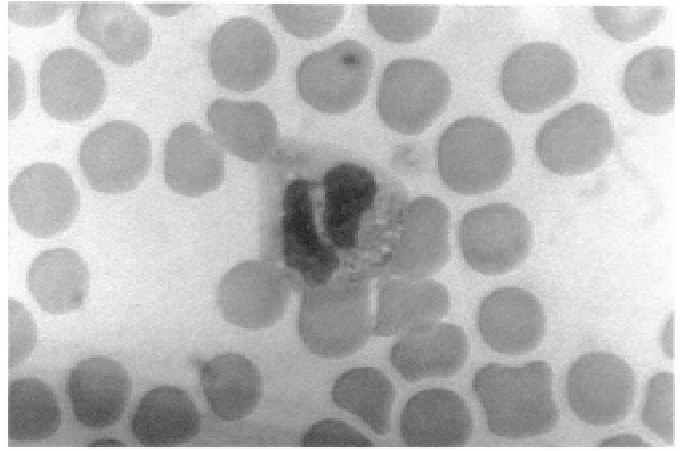


FIG. X3.5 Eosinophil

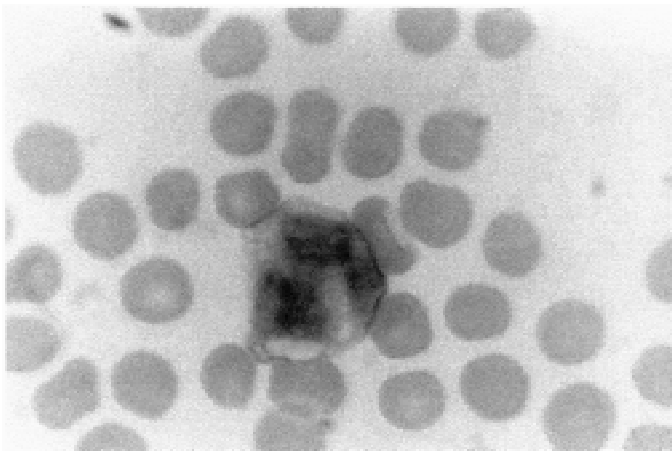


FIG. X3.4 Monocyte

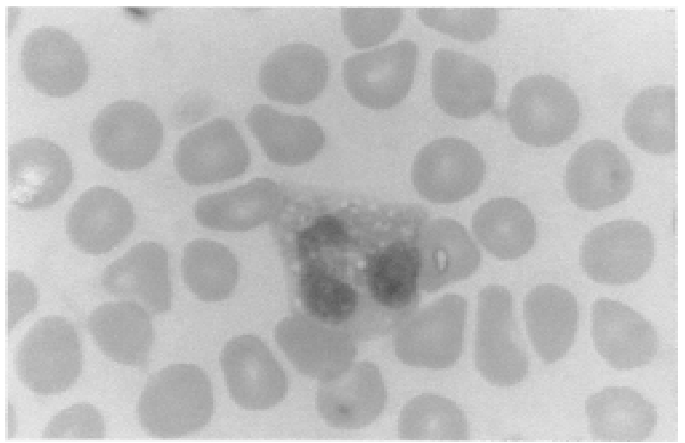
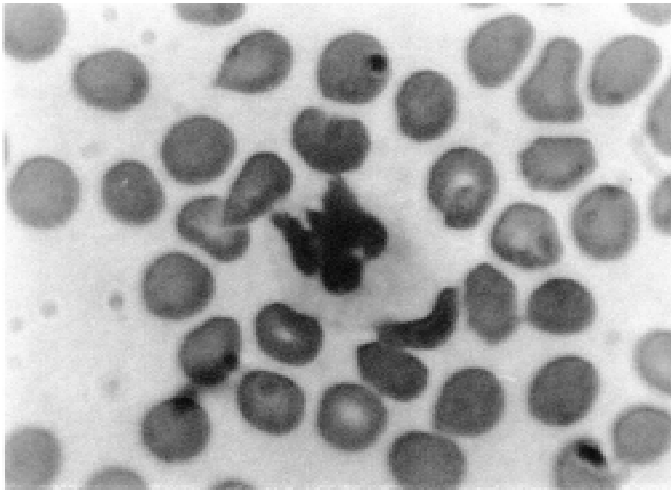
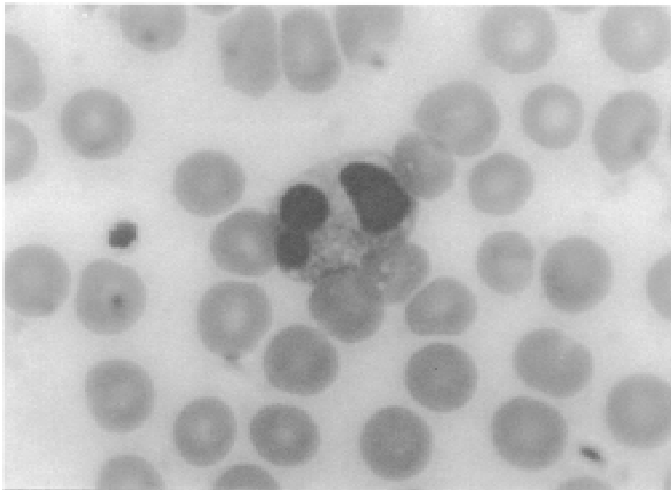


FIG. X3.6 Eosinophil

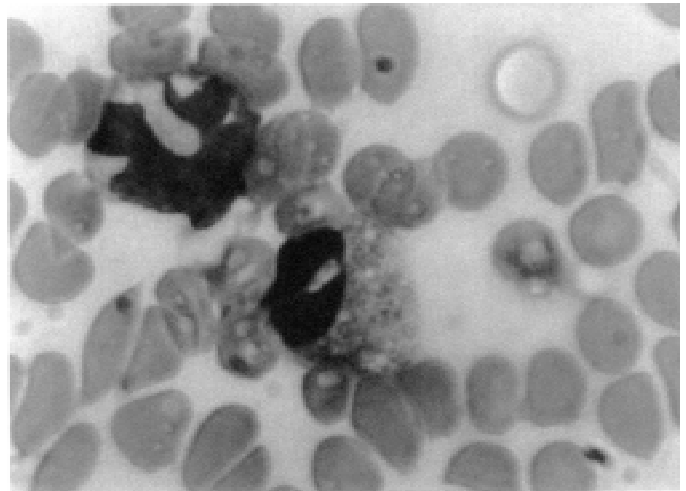
**X3.2 Abnormal Cells**



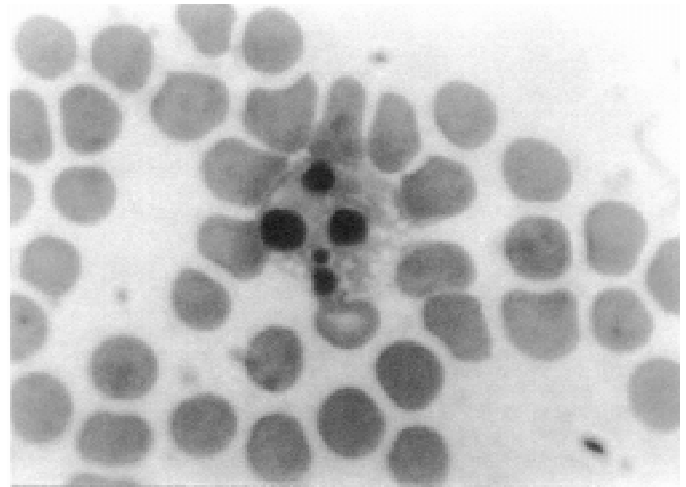
NOTE—The nuclear membrane is indistinct and appears to be torn.  
**FIG. X3.7 Abnormal Neutrophil Morphology**



NOTE—The nucleus is fragmenting into degenerative pieces. Karyolysis.  
**FIG. X3.8 Abnormal Neutrophil**

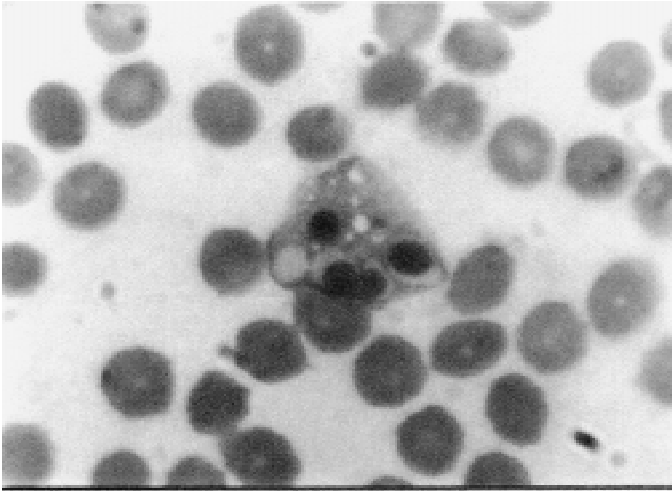


NOTE—The nucleus in each cell has sharp angular ends (as opposed to smooth and rounded) and areas of indistinct nuclear membrane.  
**FIG. X3.9 Abnormal Monocyte (top left) and Abnormal Neutrophil (center)**



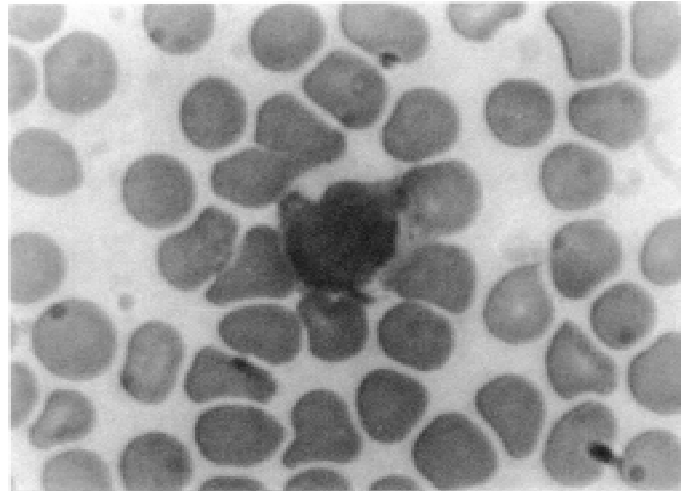
NOTE—Identification based on cell size, cytoplasmic color. Fragmented nucleus. Nuclear damage.  
**FIG. X3.10 Unidentified Cell: Probably Abnormal Monocyte**





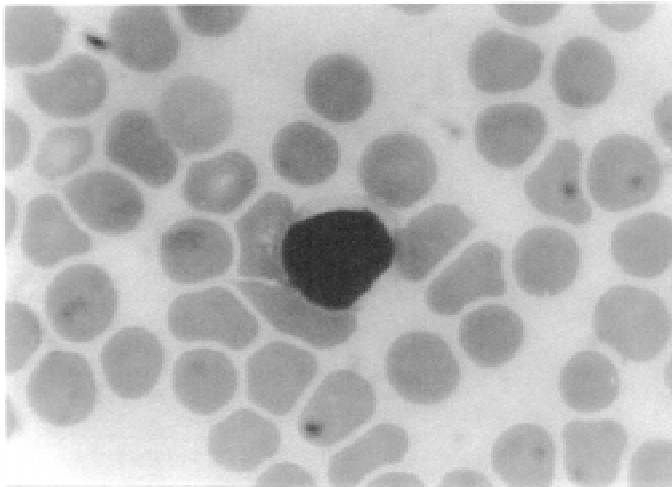
NOTE—Identification based on cell size, cytoplasmic color. Fragmented nucleus, nuclear damage.

**FIG. X3.11 Unidentified Cell: Probably Abnormal Monocyte**



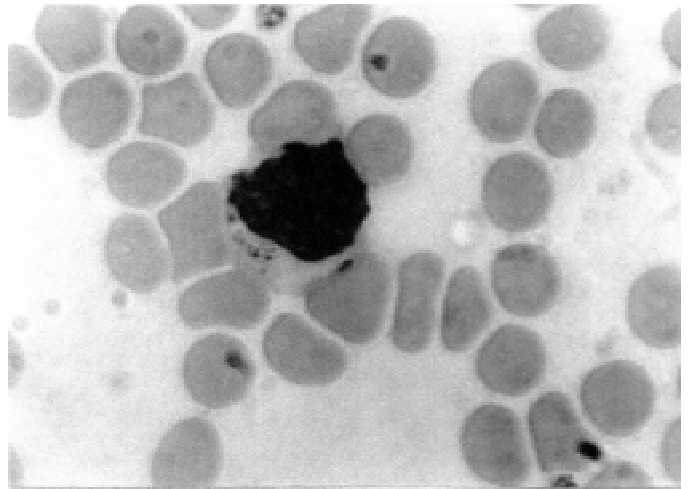
NOTE—The nucleus is irregular and the outline indistinct.

**FIG. X3.13 Abnormal Lymphocyte Morphology**



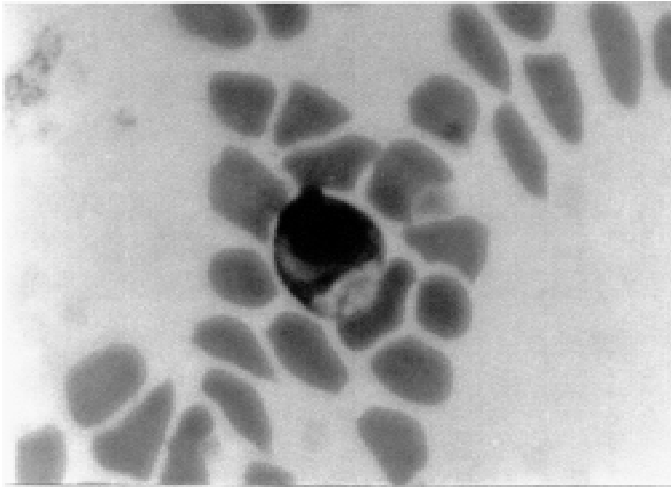
NOTE—Indistinct nuclear membrane.

**FIG. X3.12 Abnormal Lymphocyte Morphology**



NOTE—Nuclear membrane irregularities. The membrane is torn, indistinct areas, ragged.

**FIG. X3.14 Abnormal Lymphocyte Morphology**



NOTE—Appears to be a lymphocyte based on cell size, nuclear shape, and cytoplasmic color. The cell is degenerating.

**FIG. X3.15 Abnormal Cell**

## REFERENCES

- (1) Weiss, D.J., "Uniform Evaluation and Semiquantitative Reporting of Hematologic Data in Veterinary Laboratories," *Vet. Clin. Path.*, 13: 27-31, 1984.
- (2) Schalm, O.W., *Manual of Feline and Canine Hematology*, Southern California Graphics, 1980.
- (3) O'Connor, B.H., *A Color Atlas and Instruction Manual of Peripheral Blood Cell Morphology*, Williams & Wilkins, 1984.
- (4) Sirois, M., "Mammalian Blood Cell Morphology, Part II, Leukocytes and Thrombocytes," *Vet. Tech.*, 11: 465-470, 1990.
- (5) *Schalm's Veterinary Hematology*, 4th ed., N.C. Jain, 1986.
- (6) Willard, M.D., Tvedten, H., Turnwald, G.H., *Small Animal Clinical Diagnosis by Laboratory Methods*, 2nd ed., W. B. Saunders Co., 1994.
- (7) Seiverd, C.E., *Hematology for Medical Technologists*, 5th ed., 1983.

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