



Designation: F 2151 – 01

Standard Practice for Assessment of White Blood Cell Morphology After Contact with Materials¹

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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 Plastics²

F 748 Practice For Selecting Generic Biological Test Methods For Materials And Devices²

F 756 Practice for Assessment of Hemolytic Properties of Materials²

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.

¹ 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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² *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 cm²/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				