Designation: F2888 – 13

Standard Test Method for Platelet Leukocyte Count—An *In-Vitro* Measure for Hemocompatibility Assessment of Cardiovascular Materials[1]

This standard is issued under the fixed designation F2888; 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 test method assists in the evaluation of cardiovascular device materials for their ability to induce thrombus formation. Thrombus formation is assessed by means of a reduction in human platelets and leukocytes when consumed by thrombus after activation on the material surface. This assay may be part of the hemocompatibility evaluation for devices and materials contacting human blood, as per ANSI/AAMI/ISO 10993–4. See also Test Method F2382.

1.2 All safety policies and practices shall be observed during the performance of this test method. All human blood and any materials that had contact with human blood shall be bagged in a biohazard bag, properly labeled as the contents, and disposed of by appropriate means.

1.3 The human blood should be handled at Biosafety Level 2 as recommended in the Centers for Disease Control/National Institutes of Health Manual Biosafety in Microbiological Laboratories. The human blood donor must have tested negative for Hepatitis B (HBV) and Human Immunodeficiency (HIV) viruses. The blood should be treated like any patient blood in using universal precautions.

1.4 The values stated in SI units are to be regarded as standard. No other units of measurement are included in this standard.

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. Some specific hazards statements are given in Section 7 on Hazards.*

2. Referenced Documents

2.1 *ASTM Standards:*[2]

F2382 Test Method for Assessment of Intravascular Medical Device Materials on Partial Thromboplastin Time (PTT)

2.2 *Other Standards:*

ANSI/AAMI/ISO 10993–4 Biological Evaluation of Medical Devices—Part 4: Selection of Tests for Interactions with Blood[3]

Centers for Disease Control /National Institutes of Health Manual Biosafety in Microbiological Laboratories, 1993

3. Summary of Test Method

3.1 This test method identifies materials which are capable of activating blood platelets and leukocytes on their surface when exposed to freshly drawn human blood and causing the formation of thrombi on the material surface. A significant decrease in the number of platelets and leukocytes when counted by a blood analyzer is an indication of these cells being entrapped in thrombi. The materials are exposed to blood immediately after the blood is drawn with anticoagulant. Another anticoagulant is added at the appropriate time (one hour) to stop the reaction. Different blood analyzers may be used.

4. Significance and Use

4.1 The purpose of this test method is to determine if medical materials exposed to human whole blood will adversely affect the platelet and leukocyte counts in whole blood. A large number of platelets and leukocytes as part of thrombi adhering to the material will be reflected by a decrease in their counts in blood. Thrombogenic materials should not be used

[1] This test method 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] For referenced ASTM standards, visit the ASTM website, www.astm.org, or contact ASTM Customer Service at service@astm.org. For *Annual Book of ASTM Standards* volume information, refer to the standard's Document Summary page on the ASTM website.

[3] Available from American National Standards Institute (ANSI), 25 W. 43rd St., 4th Floor, New York, NY 10036, http://www.ansi.org.

for cardiovascular medical devices, unless the purpose of the device is to promote thrombosis.

5. Interferences

5.1 There is potential for interference if the materials of the test tubes used are thrombogenic (for example, glass tubes). Therefore, polyethylene or polypropylene tubes should be used.

6. Apparatus

6.1 *Hematology analyzer* capable of determination of a complete blood count.

6.2 *Polypropylene test tubes* with caps.

6.3 *Commercial blood collection tubes* containing 3.2 %, 0.105 M sodium citrate.

6.4 *Agitating water bath/incubator,* 37 ± 2ºC.

6.5 *Pipettes and tips (non-glass).*

7. Reagents and Materials

7.1 Cell count controls suitable for hematology analyzer.

7.2 Fresh whole human blood.

7.3 EDTA (ethylenediaminetetraacetic acid), 500 mM.

7.4 Saline, optional.

7.5 Positive reference control material (for example, natural rubber latex, black rubber), optional.

7.6 Positive control material (for example, black rubber, natural rubber latex).

7.7 Negative reference control material (for example, high density polyethylene (HDPE)).

8. Hazards

8.1 Human blood should be treated with the proper universal precautions, including eye wear and laboratory gloves.

9. Sampling, Test Specimens, and Test Units

9.1 Prepare each test sample, reference material, negative control, and positive control in triplicate. All material samples are prepared based on a ratio of 12 cm^2 of material to 1 mL of blood and placed into polypropylene tubes.

9.2 Thirty-six square centimeters of the test sample, positive and reference controls are divided into three 12 cm^2 samples, cut to maximize blood exposure, for triplicate testing.

NOTE 1—If other volumes of blood are used, the ratio of total surface area to blood volume should remain at 12:1.

9.3 For each test sample, the percentage of test value (platelet count or leukocyte count) per negative control (blood not exposed to a material) is calculated as follows:

$$A/B \times 100 = C \quad (1)$$

where:

A = average count (platelets or leukocytes),
B = average count (platelets or leukocytes) of negative control, and
C = percentage (%) of negative control.

When applicable, a comparison article should be used in the formula in place of the negative control.

10. Preparation of Apparatus

10.1 Initialize the hematology analyzer and allow it to perform internal self-checks. If no errors are noted, the analyzer is ready for use.

10.2 To verify the analyzer is functioning properly, prior to analyzing samples, cell count controls shall be run to conform that the results fall within the allowable ranges.

10.3 Fresh human blood is drawn for the test system. Blood should be from donors who have not taken aspirin, acetaminophen, naproxen, warfarin, heparin, or ibuprofen for ten days. Blood should be collected in a tube containing 3.2 %, 0.105 M sodium citrate (at a ratio of 9:1 blood to sodium citrate as per commercial blood collection tubes), pooled, gently mixed by inversion, and stored on ice until use.

10.4 For each analysis, a single donor's blood will be exposed to the test sample, reference material, negative control, and positive control (when applicable) to provide a consistent test system to evaluate all reference and test materials. It is recommended to pre-screen the blood to ensure the blood parameters fall within the normal expected range (normal leukocyte count 3.4 to 8.37 × 10^3/uL, normal platelet count 116 to 329 × 10^3/uL). If the donor blood parameters fall outside of the normal expected ranges, blood from another donor should be used.

11. Calibration and Standardization

11.1 Perform daily calibration procedures as per instrument instructions. (Typically the instrument self-calibrates upon initiation.)

12. Procedure

12.1 The test sample(s), reference material, and positive control materials are placed into polypropylene tubes and exposed to the appropriate quantity of blood, based on a ratio of 12 cm^2 of material to 1 mL blood. The negative control is blood only. Optionally, the test sample(s), reference materials(s), and positive control material may be pre-wetted with saline prior to exposure to blood by adding the same ratio of saline to each tube, allowed to sit for 30 s at room temperature, then all saline is removed prior to addition of blood.

12.2 The samples are exposed to blood for 1 h ± 5 min in a 37 ± 2ºC agitating water bath or incubator at approximately 60 r/min.

12.3 After the 1 h incubation period, EDTA is added to all tubes for a final concentration of 5 mM to stop any further reaction from occurring. Typically, 0.01 mL of EDTA (500 mM) is added per 1.0 mL of human whole blood to achieve the correct final concentration.

12.4 All tubes are gently mixed, blood removed as completely as possible from each sample, and transferred to new,