Designation: F2888 – 19

Standard Practice 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 practice 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 in accordance with 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 practice. All human blood and any materials that had contact with human blood shall be bagged in a biohazard bag, properly labeled with the contents, and disposed of by appropriate means.

1.3 The human blood should be handled at Biosafety Level 2 (BSL-2) as recommended in the Centers for Disease Control/National Institutes of Health publication, Biosafety in Microbiological and Biomedical Laboratories (BMBL). 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 and handled/manipulated using standard precautions.

NOTE 1—The results of this *in-vitro* test may not correspond to actual human response.

1.4 The values stated in SI (International System of Units) 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, health, and environmental practices and determine the applicability of regulatory limitations prior to use.* Some specific hazards statements are given in Section 8 on Hazards.

1.6 *This international standard was developed in accordance with internationally recognized principles on standardization established in the Decision on Principles for the Development of International Standards, Guides and Recommendations issued by the World Trade Organization Technical Barriers to Trade (TBT) Committee.*

2. Referenced Documents

2.1 *ASTM Standards:*[2]

F2382 Test Method for Assessment of Circulating Blood-Contacting 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]

BMBL Biosafety in Microbiological and Biomedical Laboratories, 5th ed., 2009[4]

3. Summary of Practice

3.1 This practice 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 fresh whole blood that is drawn with anticoagulant. Another anticoagulant is added at the appropriate time (one hour) to stop further thrombus formation. Different blood analyzers may be used.

4. Significance and Use

4.1 The purpose of this practice is to determine if thrombus formation has occurred by comparing platelet and leukocyte

[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] 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.

[4] Available from U.S. Department of Health and Human Services, Centers for Disease Control and Prevention (CDC), 1600 Clifton Rd., Atlanta, GA 30329-4027, http://www.cdc.gov.

counts in the blood exposed to the test material relative to the blood cell counts in the control blood that has not been exposed to the test material. A large number of platelets and leukocytes becoming entrapped/incorporated in thrombi adhering to the material will be reflected by a decrease in their counts in blood. Thrombogenic materials should not be used 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 human whole blood.

7.3 EDTA (ethylenediaminetetraacetic acid), 500 mM.

7.4 Saline, optional.

7.5 Positive control material (for example, black rubber, natural rubber latex, and glass).

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

7.7 Marketed comparator device (a legally marketed device that has similar blood contact and clinical use as the material/device being investigated), optional.

8. Hazards

8.1 Human blood should be handled according to standard microbiological practices and techniques (for example, use of personal protective equipment)[5] and specific required precautions (for example, OSHA Bloodborne Pathogen Standard[6]).

9. Sampling, Test Specimens, and Test Units

9.1 Each test sample, negative reference control material, positive control material, and marketed comparator device (if applicable) shall be prepared in triplicate. All test samples and controls, with the exception of, the negative control which is blood only, shall be prepared based on a ratio of 12 cm^2 of material to 1 mL of blood and placed into polypropylene tubes. Also, three empty test tubes shall be prepared for the negative control (blood in test tubes without test materials).

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

NOTE 2—If this surface area cannot be achieved with the device, or other volumes of blood are used, the ratio of total surface area to blood volume should remain at 12 cm^2:1 mL.

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 confirm that the results fall within the allowable ranges.

10.3 Fresh human blood from at least 3 donors should be used for the test to account for blood variability among donors. Testing with blood from each donor separately or testing with pooled blood is acceptable. Blood should be from donors who have not taken nonsteroidal, anti-inflammatory drugs (NSAIDs; for example, ibuprofen, aspirin, naproxen), acetaminophen, or antithrombotic drugs (for example, heparin, warfarin) for ten days prior to blood collection. Blood should be collected in a tube containing 3.2 %, 0.105 M sodium citrate (at a ratio of 9:1 v/v blood to sodium citrate), gently mixed by inversion, and stored at room temperature until use. Alternatively, blood should be collected in a tube containing a low concentration of heparin (final concentration approximately 1 unit/mL). Other blood collection tubes/anticoagulant concentrations may be used if validated for use in this practice, using two or more moderately thrombogenic positive control materials (for example, black rubber, natural rubber latex, and glass) for the validation.

NOTE 3—Blood from an individual donor could be used directly to perform the test, as long as the test is repeated separately with blood collected from 2 additional donors. Alternatively, blood from 3 donors may be pooled for analyses, but in this case, blood type mismatch between donors should be avoided. The test report should indicate the type and concentration of anticoagulant used, and whether the blood samples were pooled or not.

10.4 For each analysis, a single donor's blood (or pooled blood, if applicable) will be exposed to the test sample, negative control reference material, positive control material, and marketed comparator device (when applicable) to evaluate their impact on platelet and leukocyte counts. 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/μL, normal platelet count 116 to 329 × 10^3/μL). 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 in accordance with instrument instructions. (Typically the instrument self-calibrates upon initiation.)

[5] U.S. Department of Health and Human Services, Centers for Disease Control and Prevention, and National Institutes of Health, "Section III—Principles of Biosafety," Biosafety in Microbiological and Biomedical Laboratories (BMBL), L. C. Chosewood and D. E. Wilson, eds. 2009.

[6] U.S. Department of Labor, "Occupational Exposure to Bloodborne Pathogens," Final Rule, 29 CFR, 1910.1030, 1991.