Designation: F2382 – 17

Standard Test Method for Assessment of Circulating Blood-Contacting Medical Device Materials on Partial Thromboplastin Time (PTT)[1]

This standard is issued under the fixed designation F2382; 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 covers the screening of circulating blood-contacting device materials for their ability to induce blood coagulation via the intrinsic coagulation pathway. This assay should be part of the hemocompatibility evaluation for devices and materials contacting human blood, as per ANSI/AAMI/ISO 10993-4.

1.2 All safety policies and practices shall be observed during the performance of this test method.

1.3 All plasma and any materials that had contact with plasma will be bagged in a biohazard bag, properly labelled with the contents, and disposed of by appropriate means. The plasma should be handled at the Biosafety Level 2 as recommended in the Centers for Disease Control/National Institutes of Health Manual Biosafety in Microbiological Laboratories.

1.4 The normal pooled human plasma must have tested negative for Hepatitis B (HBV) or Human Immunodeficiency (HIV) viruses. The plasmas should be treated like any patient plasma using standard precautions. The plasma should be handled at the Biosafety Level 2 as recommended in the Centers for Disease Control/National Institutes of Health Manual Biosafety in Microbiological Laboratories.

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

1.6 *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.*

1.7 *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 *ANSI/AAMI Standard:*

ANSI/AAMI/ISO 10993-4 Biological Evaluation of Medical Devices—Part 4: Selection of Tests for Interactions with Blood[2]

2.2 *Other Document:*

U.S. Department of Health and Human Services Biosafety in Microbiological and Biomedical Laboratories (BMBL), 5th ed., 1999[3]

3. Terminology

3.1 *Definitions of Terms Specific to This Standard:*

3.1.1 *activator*—a medical material which demonstrates a shortened clotting time; an initiator of the intrinsic coagulation pathway.

3.1.2 *partial thromboplastin time (PTT) assay*—a modification of the Activated Partial Thromboplastin Time (APTT) assay; unlike the APTT test, the PTT assay uses reagent (rabbit brain cephalin) without activating substances such as silica, kaolin, elagic acid. The material being tested acts as the activator.

3.1.3 *read time*—the time during which data is collected to detect a clot.

3.1.4 *blank time*—a period at the beginning of an assay when no data is taken. This is done to eliminate interference from premixing reagents, bubbles, and so forth.

3.1.5 *equilibration time*—the time allowed for the plasma samples to warm to 37°C. The coagulation analyzer can be set to zero if samples are pre-warmed to this temperature.

3.1.6 *duplicate flag*—the agreement between the results of duplicate samples in percent. For example, if set to "15," the difference between the two channels must be less than or equal

[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.

Current edition approved Sept. 1, 2017. Published September 2017. Originally approved in 2004. Last previous edition approved in 2004 as F2382 – 04. DOI: 10.1520/F2382-17.

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

[3] The BMBL 5th Edition (December 2009) is available from the Government Printing Office or https://www.cdc.gov/biosafety/publications/bmbl5/bmbl.pdf

to 15 %. If the variance in clot times exceeds this percentage, an asterisk "*" will be printed by the average results on the report.

4. Significance and Use

4.1 The purpose of this test method is to determine the time citrated plasma exposed to medical materials takes to form a clot when exposed to a suspension of phospholipid particles and calcium chloride. In this test method, the test article is the activator. The PTT assay is a general screening test for a medical material's ability to activate the intrinsic coagulation pathway. Material samples that show a shortened PTT are activators of the intrinsic coagulation pathway.

4.2 The test article, reference materials, and controls are exposed to human plasma. The plasma is tested on a coagulation device. Each sample tube is assayed in duplicate. The results are reported as a percentage of the negative control.

5. Apparatus

5.1 *Polypropylene Test Tubes with Caps,* 12 by 75 mm.

5.2 *Automatic Pipets and Tips,* 100 and 1000 µL.

5.3 *Ice Bath.*

5.4 *Coagulation Analyzer* (Siemens BFT II analyzer or other).

5.5 *Agitating Water Bath,* 37 ± 2°C, capable of 60 rpm.

5.6 *Coagulation Analyzer Cuvettes,* or equivalent for specific analyzer.

6. Reagents and Materials

6.1 *Calcium Chloride,* 25 mM.

6.2 *Citrated Human Blood Plasma,* fresh (less than 4 h from draw) or freshly-frozen, maintained at minus 80°C, pooled.

6.3 *Lyophilized Rabbit Brain Cephalin (RBC).*

6.4 *Positive Reference Material (Optional),* see Appendix X1.

6.5 *Positive Control,* glass (Pasteur pipette tips or glass beads).

6.6 *Negative Reference Material* (e.g. High Density Polyethylene, HDPE).

6.7 *Marketed Comparator Device (Optional).* A legally marketed, clinically acceptable device that has similar blood contact nature and clinical use as the material/device being investigated.

NOTE 1—It may be helpful to use a positive reference control material (n=1) per assay to assure continuity between runs.

7. Hazards

7.1 The human blood plasma should be treated like any patient plasma using standard precautions. The plasma should be handled at the Biosafety Level 2 as recommended in the US Department of Health and Human Services Biosafety in Microbiological and Biomedical Laboratories.

8. Preparation of Apparatus

8.1 Prepare each test article, the negative reference materials, marketed comparator device (if used) and controls in triplicate. If a positive reference control material is used, a single replicate is acceptable. All samples are prepared based on a ratio of either 4 or, preferably 6 cm^2 of material to 1 mL plasma and placed into polypropylene tubes. For device testing, if test sample quantity allows, use three separate devices; otherwise, take three representative samples from one device.

8.2 Label duplicate polypropylene tubes and place in the ice bath.

8.3 Turn on the coagulation analyzer and allow it to warm up to 37 ± 2°C and equilibrate for at least 10 min.

8.4 Program the analyzer to test under the APTT function with an equilibration time of 60 s, activation time of 120 s, a blank time of 14 s, and a read time of 286 s.

8.5 Pre-warm analysis cuvettes (or cups, depending on analyzer selected) at 37 ± 2°C.

8.6 Pre-warm calcium chloride at 37 ± 2°C.

8.7 *Rabbit Brain Cephalin (RBC) Preparation:*

8.7.1 Allow the RBC to come to room temperature.

8.7.2 Reconstitute the RBC as indicated by the RBC reagent manufacturer.

8.7.3 Place in an agitating water bath set at 37 ± 2°C and 60 rpm for 15 min to ensure complete rehydration of contents.

8.7.4 Vortex 15 s after rehydration is complete.

8.7.5 Place at 37 ± 2°C.

8.8 If using frozen blood plasma, quick thaw the plasma at 37 ± 2°C and place on ice immediately.

9. Procedure

9.1 The test material(s), reference material(s), marketed comparator device (if used) and controls are placed into polypropylene tubes and exposed to the appropriate quantity of plasma, based on a ratio of 4 cm^2, or preferably 6 cm^2 of material to 1 mL plasma. The negative control is a polypropylene tube with 1 mL of plasma, without additional material.

9.2 The samples are exposed to the plasma for 15 ± 1 min in a 37 ± 2°C agitating water bath at 60 rpm.

9.3 After 15 min of incubation, the tubes are immediately placed into the ice bath and immediately transferred into pre chilled new polypropylene tubes.

9.4 Vortex each sample 15 s before each use/run.

9.5 Avoiding bubbles, transfer 100 pL of the plasma into pre-warmed cuvettes and allow the plasma to equilibrate for 60 s at 37 ± 2°C.

9.6 To each cuvette/cup, add 100 pL warmed RBC preparation, initiating the 2 min activation step. (Invert RBC to mix prior to each use.)

9.7 After activation, add 100 pL warmed 25 mM calcium chloride to each cuvette.