Designation: F 2382 – 04

Standard Test Method for Assessment of Intravascular Medical Device Materials on Partial Thromboplastin Time (PTT)[1]

This standard is issued under the fixed designation F 2382; 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 cardiovascular device materials for their ability to induce blood coagulation. This assay should be part of the hemocompatibility evaluation for devices and materials contacting human blood.

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 labeled with the contents, and disposed 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 universal 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 *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.*

2. Referenced Documents

2.1 *ANSI 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:*

Centers for Disease Control/National Institutes of Health Manual Biosafety in Microbiological Laboratories, 1999[3]

3. Terminology

3.1 *Definitions:*

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 (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 fibrometer 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 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 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 In this test method, the test sample is the activator. Test samples that show a shortened PTT are activators of the

[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 May 1, 2004. Published June 2004.

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

[3] Available from National Institute of Health (NIH), 9000 Rockville Pike, Bethesda, MD 20892.

intrinsic coagulation pathway. The results are reported as a percent of the negative control. 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.

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 *Lyophilized Rabbit Brain Cephalin (RBC)*.
5.4 *Ice Bath*.
5.5 *Coagulation Analyzer (Automated Fibrometer)*.
5.6 *Agitating Water Bath*, 37 ± 2°C, capable of 60 rpm.
5.7 *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 *Reference Control Material*, see Appendix X1.
6.4 *Positive Control Material*, glass (Pasteur pipette tips or glass beads).

7. Hazards

7.1 The human blood plasma should be treated like any patient plasma using universal 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.

8. Preparation of Apparatus

8.1 Prepare each test article in triplicate. The reference material(s), and the controls are prepared as singles. All samples are prepared based on a ratio of 4 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 ice bath.
8.3 Initialize coagulation analyzer and allow it to warm up to 37 ± 2°C and equilibrate for a minimum at least 10 min.
8.4 Program the analyzer to test under the APTT function with a 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 Print out test parameters and verify changes. Photocopy printout and attach to original data.
8.6 Pre-warm analysis cuvettes (or cups, dependent on analyzer selected).
8.7 Pre-warm calcium chloride at 37 ± 2°C.
8.8 *Rabbit Brain Cephalin (RBC) Preparation*:
8.8.1 Allow the RBC to come to room temperature.
8.8.2 Reconstitute RBC with 10 mL reagent grade water/distilled water.
8.8.3 Place in agitating water bath set at 37 ± 2°C, at 60 rpm for 15 min to ensure complete rehydration of contents.
8.8.4 Vortex 15 s after rehydration completed.
8.8.5 Place at 37 ± 2°C.
8.9 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), and controls are placed into polypropylene tubes and exposed to the appropriate quantity of plasma, based on a ratio of 4 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 incubation of samples, 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 µL 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 µL warmed RBC preparation initiating the 2 min activation step. (Invert RBC to mix prior to each use.)
9.7 After activation, add 100 µL warmed 25 mm calcium chloride to each cuvette.
9.8 Allow the analyzer to read the sample for the formation of clots (up to 5 min).
9.9 Record the clotting time (seconds) for each sample, as well as the average clotting time of the duplicate samples.

10. Calculation or Interpretation of Results

10.1 Calculate the test sample result (% negative control) for test material, reference, and positive control sample mean.

$$\%\ \text{negative control} = \frac{\text{Average clotting time (s) of sample}}{\text{Average clotting time (s) of negative control}} \times 100 \quad (1)$$

10.2 *Test Sample Acceptance Criteria*:

% Negative Control	Thrombogenicity
_____ >100	Non-activator of intrinsic coagulation pathway
_____ 75 to 100	Minimal activator
_____ 50 to 74	Mild activator
_____ 25 to 49	Moderate activator
_____ <25	Activator

% Negative Control	Interpretation
_____ <25	Activator
_____ 25 to 50	Retest
_____ >50	Pass

10.3 As a comparison, the reference material(s) results are reported using the formula 15.1.
10.4 The positive control result % negative control must be <50 %. If the assay does not meet this specification, the experiment is to be repeated until the controls are within range. Reference material and positive control results should be equivalent (within the same thrombogenicity category) run to run.
10.5 The variance between the duplicates for each sample must be ≤15 %. The duplicates of each test article sample are averaged and one value is reported as the clotting time. This results in three clotting time values for each test article. The three values are then averaged to report a final clotting time of the test article. The values for each test article sample must be

within ±25 % of this average. If the values are greater than 25 % of the average of the run, the experiment needs to be repeated.

11. Precision and Bias

11.1 The precision and bias of this test method has not yet been determined.

12. Keywords

12.1 blood coagulation; blood compatibility; partial thromboplastin time; PTT

ANNEX

(Mandatory Information)

A1. VENDOR INFORMATION

A1.1 *Rabbit Brain Cephalin (RBC)*—Bio/Data,[4] or equivalent vendor.

A1.2 *Coagulation Analyzer*—Cascade M-4, Helena Laboratories[5] or equivalent instrument.

A1.3 *Reference Control Material*—Natural latex tubing (Small Parts, Inc.,[6] or equivalent vendor) or black rubber stopper (Fisher[7] or equivalent vendor). Alternate reference materials may be selected, once they have demonstrated a consistent, ideally a mildly or moderately thrombogenic response. More than one reference material may be used.

[4] Available from Bio/Data, 155 Gilbraltar Rd., Horsham, PA 19044.
[5] Available from Helena Laboratories, P.O. Box 752, Beaumont, TX 77704.
[6] Available from Small Parts, Inc., 13980 NW 58th Ct., P.O. Box 4650, Miami Lakes, FL 33014.
[7] Available from Fisher Scientific, 600 Business Center Dr., Pittsburgh, PA 15205.

APPENDIX

(Nonmandatory Information)

X1. RATIONALE

X1.1 This test method allows assessment of intravascular medical device materials' ability to induce blood coagulation. It should be part of the hemocompatibility evaluation for devices and materials contacting human blood.

REFERENCES

(**1**) Sawyer, A., "In Vitro Hemocompatibility Screening Method for Biomaterials," World Congress for Biomaterials Meeting Transactions, 1992, p. 669.
(**2**) U.S. Department of Health and Human Services, Public Health Service, Centers for Disease Control and Prevention and National Institutes of Health, "Biosafety in Microbiological and Biomedical Laboratories", Fourth Edition, April 1999, p. 21-27.