



Designation: F2382 – 04 (Reapproved 2010)

Standard Test Method for Assessment of Intravascular 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 cardiovascular 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 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 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 and health practices and determine the applicability of regulatory limitations prior to use.*

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. Referenced Documents

2.1 *ANSI Standard:*

ANSI/AAMI/ISO 10993-4 Biological Evaluation of Medical Devices—Part 4: Selection of Tests for Interactions with Blood²

2.2 *Other Document:*

Centers for Disease Control/National Institutes of Health Manual Biosafety in Microbiological Laboratories, 1999³

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.

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

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

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 Test samples that show a shortened PTT are activators of the 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 *Ice Bath*.
- 5.4 *Coagulation Analyzer (Automated Fibrometer)*.
- 5.5 *Agitating Water Bath*, $37 \pm 2^\circ\text{C}$, 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 *Reference Control Material*, see [Appendix XI](#).
- 6.5 *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 $37 \pm 2^\circ\text{C}$ 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 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) at $37 \pm 2^\circ\text{C}$.

8.7 Pre-warm calcium chloride at $37 \pm 2^\circ\text{C}$.

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 $37 \pm 2^\circ\text{C}$, at 60 rpm for 15 min to ensure complete rehydration of contents.

8.8.4 Vortex 15 s after rehydration is complete.

8.8.5 Place at $37 \pm 2^\circ\text{C}$.

8.9 If using frozen blood plasma, quick thaw the plasma at $37 \pm 2^\circ\text{C}$ 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 $37 \pm 2^\circ\text{C}$ 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 μL of the plasma into pre-warmed cuvettes and allow the plasma to equilibrate for 60 s at $37 \pm 2^\circ\text{C}$.

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.

$$\% \text{ negative control} = \quad (1)$$

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