7. When sodium oxalate is used as an anticoagulant, the test must be performed within 1 hour of blood collection. Citrated blood should be spun down within 30 minutes of blood collection and may be stored on ice up to 1½ hours. Plasma allowed to sit longer than the recommended times gives prolonged and abnormal results.

PLASMA RECALCIFICATION TIME

(Plasma Clotting Time)

The plasma recalcification time is a measure of the overall intrinsic coagulation process. In the procedure outlined here, a deficiency in platelets, or platelet activity, is not detected. The normal plasma recalcification time on platelet-poor plasma is 90 to 250 seconds. A decrease in any of the clotting factors present in the intrinsic system will cause a prolonged clotting time.

REFERENCES

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Miale, J.B.: *Laboratory Medicine: Hematology*, 5th Edition, C. V. Mosby Company, St. Louis, 1977.

REAGENTS AND EQUIPMENT

1. Water bath, 37°C.
2. Calcium chloride, 0.025 M.

Anhydrous calcium chloride	1.38 g
Distilled water	500 ml

3. Sodium chloride, 0.85% (w/v).
4. Normal platelet-poor control plasma.
5. Test tubes, 13 × 100 mm.
6. Stopwatch.

SPECIMEN

Citrated plasma: one part 0.11 M sodium citrate to nine parts whole blood; or oxalated plasma: one part 0.1 M sodium oxalate to nine parts whole blood.

PRINCIPLE

Platelet-poor plasma is mixed with sufficient calcium chloride to neutralize the effects of the anticoagulant, and the clotting time is then recorded.

PROCEDURE

1. Immediately after collection, centrifuge blood at 2500 RPM for at least 20 minutes in order to obtain a platelet-poor plasma.
2. Incubate, at 37°C for 2 to 3 minutes prior to each test, each of the following, in separate test tubes:
   A. Patient's platelet-poor plasma
   B. Normal platelet-poor control plasma
   C. Calcium chloride, 0.025 M
   D. Sodium chloride, 0.85%
3. Into a 13 × 100 mm test tube, in the 37°C water bath, pipet 0.1 ml 0.85% sodium chloride and 0.1 ml of patient's plasma. Mix.
4. Blow in 0.1 ml 0.025 M calcium chloride and simultaneously start a stopwatch.
5. Allow the tube to remain in the 37°C water bath for 90 seconds, tilting the tube gently every 30 seconds.
6. After 90 seconds, remove the tube from the water bath and gently tilt. Stop the watch as soon as a clot forms, and record the results.

DISCUSSION

1. The plasma recalcification time varies according to the number of platelets present in the plasma. As the number of platelets increases, the plasma recalcification time shortens. Therefore, it is important to centrifuge the blood in the prescribed manner.
2. The plasma recalcification time may be performed on platelet-rich plasma, in which case the normal range is 90

to 120 seconds. However, plasmas containing a standard number of platelets are difficult to obtain.

3. A modification of the plasma clotting time is called the activated recalcification time, and employs the use of 0.1 ml of platelet-rich plasma, 0.1 ml of 0.03 M calcium chloride, and 0.1 ml of 1% celite as an activator. The normal clotting time in this procedure is less than 50 seconds.

THROMBIN TIME

The thrombin time tests the third stage of coagulation, the conversion of fibrinogen to fibrin. It measures the availability of functional fibrinogen. The normal thrombin time for this procedure is 15 to 20 seconds. Prolonged times are found when the fibrinogen level is below 100 mg per dl, when the function of fibrinogen is impaired, and in the presence of thrombin-inhibitors, such as heparin or fibrin-split products. The thrombin time is a most sensitive test in detecting heparin inhibition. The thrombin time may be normally prolonged in the newborn and in multiple myeloma (the abnormal globulin interferes with the polymerization of fibrin).

REFERENCES

Rapaport, S.I., and Ames, S.B.: Clotting factor assay on plasma from patients receiving intramuscular or subcutaneous heparin, Am. J. Med. Sci., *234*, 678, 1957.

Wintrobe, M.M.: *Clinical Hematology*, 7th Edition, Lea & Febiger, Philadelphia, 1974.

REAGENTS AND EQUIPMENT

1. Stock thrombin (100 units per 1 ml). Reconstitute one vial of Bovine Thrombin, Topical, 5000 NIH units (Parke, Davis & Company, Detroit, Mich.) with 5 ml saline diluent. Add 100 mg barium sulfate, and incubate at 37°C for 20 minutes. Centrifuge at 2500 RPM for 5 minutes. Carefully remove the supernatant and add it to 20 ml 0.85% sodium chloride and 25 ml glycerin. This mixture, stored at 0°C, is stable for several months.
2. Tris buffer, pH 7.35.

Sigma 121 Primary Standard Biochemical Buffer	6 g
Sodium chloride	6.6 g
Hydrochloric acid, 0.1 N	440 ml

   Dilute to 1000 ml with distilled water. Store at 4°C.
3. Normal control plasma.
4. Water bath, 37°C.
5. Nichrome wire loop.
6. Stopwatch.
7. Test tubes, 13 × 100 mm.

SPECIMEN

Plasma obtained from whole blood collected in sodium oxalate, sodium citrate, or EDTA.

PRINCIPLE

A measured amount of thrombin is added to plasma. The length of time for a fibrin clot to form is recorded as the thrombin time.

PROCEDURE

1. Centrifuge blood at 2500 RPM for 10 minutes to obtain platelet-poor plasma.
2. Immediately before use, prepare working thrombin solution by diluting 0.1 ml of stock thrombin with 0.9 ml Tris buffer. Incubate at 37°C. (This solution is stable for 20 minutes at 37°C.)
3. Incubate a sufficient amount of Tris buffer at 37°C.
4. Place 0.2 ml of patient's plasma, or normal control, into a 13 × 100-mm test tube.
5. Add 0.2 ml Tris buffer to the tube, mix, and allow to incubate for 1 minute.
6. At the end of 1 minute, pipet 0.2 ml of working thrombin solution into the tube, simultaneously starting the stopwatch.
7. With a nichrome wire loop, sweep through the mixture, two times per