second, until a clot is formed. Stop the watch and record the thrombin time.

8. Run a normal control with each series of thrombin times. Each specimen must be tested in duplicate.

DISCUSSION

1. Duplicate tests performed on the same plasma sample should check within ±1.5 seconds of each other.
2. Whenever thrombin is used, plastic or siliconized pipets should be employed to pipet the thrombin.
3. The concentration of thrombin in the working thrombin solution should be at a concentration that gives a clotting time of 15 to 20 seconds on normal plasma. When the stock thrombin solution is first prepared, it may be necessary to use a 1:12 or greater dilution when preparing the working thrombin mixture. As the stock solution ages, the reverse is true, and a dilution of 1:8 or less with Tris buffer may be required.
4. If the thrombin time is greater than 25 seconds, repeat the procedure, using a 1:1 mixture of the patient's plasma and normal control to test for inhibitors. If inhibitors are present, the thrombin time will not be shortened.

FIBRINOGEN TITER

A deficiency in fibrinogen is a rare occurrence. However, when it does occur, it may produce severe hemorrhage, and little time should be lost in diagnosing the problem. A lack of fibrinogen may be caused by a congenital defect, and it may also be found in certain obstetric and surgical cases. A chronic deficiency of fibrinogen may occur in such cases as liver disease, where production may be defective. Elevated fibrinogen levels are normally found in pregnancy, near term or after delivery. The fibrinogen titer is useful in detecting a deficiency in fibrinogen and in detecting an alteration in the conversion of fibrinogen to fibrin. The normal fibrinogen titer is 1:128 to 1:256. A titer below 1:64 is abnormal.

REFERENCES

Biggs, R., and MacFarlane, R.G.: *Human Blood Coagulation and Its Disorders*, Blackwell Scientific Publications, Oxford, 1962.

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REAGENTS AND EQUIPMENT

1. Water bath, 37°C.
2. Glass test tubes, 13 × 100 mm.
3. Sodium chloride, 0.85% (w/v).
4. Pipets, 1 ml, plastic.
5. Thrombin (100 units per 1 ml). Reconstitute one vial of Bovine Thrombin, Topical, 5000 NIH units (Parke, Davis & Company, Detroit, Mich.) with 5 ml of saline diluent. Add 100 mg of 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, will be stable for several months. Alternatively 50 units of human thrombin (Fibrindex, Ortho Diagnostics, Raritan, N.J.) may be dissolved in 0.5 ml 0.85% sodium chloride (use this mixture only as a fresh preparation).
6. Control plasma from a normal individual.

SPECIMEN

Citrated plasma: one part 0.11 M sodium citrate to nine parts whole blood, or oxa-

lated plasma: one part 0.1 M sodium oxalate to nine parts whole blood. Citrated blood is recommended, since oxalated plasma may yield lower titration results.

PRINCIPLE

Thrombin is added to serial dilutions of the patient's plasma. The fibrinogen titer is the highest plasma dilution in which a visible fibrin clot forms.

PROCEDURE

1. Centrifuge the control and patient's blood at 2500 RPM for 10 minutes and remove the plasma.
2. Number consecutively two sets of eight, 13 × 100-mm test tubes, and place in two rows in a test tube rack. One set of tubes is for the control plasma and one set for the patient's plasma.
3. Pipet 0.5 ml 0.85% sodium chloride into each of the 16 test tubes.
4. Pipet 0.5 ml of normal plasma into tube #1 of the control set. Serially dilute the control plasma by mixing tube #1 and transferring 0.5 ml of the diluted plasma to tube #2. Mix tube #2, and pipet 0.5 ml to tube #3, and so on. Discard 0.5 ml of the dilution from tube #8.
5. Pipet 0.5 ml of the patient's plasma into tube #1 of the patient's tubes, and serially dilute as described in step 4.
6. Add 0.1 ml of thrombin solution to each of the 16 tubes and mix.
7. Place the tubes in a 37°C water bath for 15 minutes.
8. At the end of 15 minutes, observe each tube for the presence of a clot.
9. The highest dilution in which a clot is visible is reported as the fibrinogen titer (Table 5).

TABLE 5. PLASMA DILUTIONS FOR THE FIBRINOGEN TITER

TUBE NUMBER	DILUTION
1	1:2
2	1:4
3	1:8
4	1:16
5	1:32
6	1:64
7	1:128
8	1:256

DISCUSSION

1. When checking tubes for clot formation, tilt the tubes gently.
2. The results of the normal control should fall within the normal range. If not, the thrombin may be unsatisfactory. The test should be repeated with a new mixture of thrombin.
3. Whenever thrombin is used, plastic or siliconized pipets should be employed to pipet the thrombin.
4. It is advisable to keep the tubes in the 37°C water bath, and check for lysis of the clots at 1, 2, and 24 hours after the fibrinogen titer has been read. Lysis of the clots is indicative of increased fibrinolysis.
5. When reading the fibrinogen titer, if the first few tubes (lower dilutions) contain no clot, but there is clot formation in the last tube(s) (higher dilutions), the presence of a circulating anticoagulant is indicated.

QUANTITATIVE FIBRINOGEN

The following procedure is a method for determining the amount of fibrinogen in a plasma. Although it is a quantitative method, it can be included in the coagulation screen in place of the thrombin time. This procedure is quick and easy to perform.

The normal value for this test is 200 to 400 mg per dl.

REFERENCE

Dade Diagnostics, Inc., American Hospital Supply Corporation: *Fibrinogen Determination* Set, Dade Division, American Hospital Supply Corporation, Miami, Florida, 1976.