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.

REAGENTS AND EQUIPMENT

1. Data-Fi thrombin reagent, approximately 100 NIH units. Reconstitute with 1.0 ml distilled water. (This reagent is good for 8 hours after reconstituting when it is stored at room temperature or 4°C.)
2. Data-Fi Fibrinogen Calibration Reference. Reconstitute with 1.0 ml of distilled water. (After reconstitution this reagent is good for 4 hours when it is stored at 4°C.) Do not shake this reagent.
3. Owren's Veronal Buffer (pH 7.35). This reagent is stable indefinitely at room temperature or at 4°C.
4. Dade Ci-trol Normal (citrated normal plasma) or SNP (standardized normal plasma). Reconstitute with 1.0 ml distilled water and mix gently. If citrated plasma is employed, use Ci-trol Normal; with oxalated plasma use SNP.
   Note: Reagent numbers 1, 2, 3, and 4 are available from Dade Diagnostics, Inc., American Hospital Supply Corporation, Miami, Florida.
5. Test tubes, 12 × 75 mm.
6. Pipets, 1.0 ml, 2.0 ml, 0.2 ml, and 0.1 ml.
7. Water bath, 37°C.
8. Stopwatch.
9. Wire loop (or fibrometer or Mechrolab Clot-Timer).
10. Double log graph paper.

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

A measured amount of thrombin is added to plasma and the clotting time noted. This result is compared with clotting times of plasmas containing known amounts of fibrinogen. From this information, the amount of fibrinogen in mg per dl in the unknown sample is determined.

PROCEDURE

1. The first time this procedure is set up, and each time a new lot number of thrombin is used, a new calibration curve must be set up as outlined below.
   A. Label two 12 × 75-mm test tubes, 1:5.
      Label two 12 × 75-mm test tubes, 1:15.
      Label two 12 × 75-mm test tubes, 1:40.
   B. Place 1.6 ml of Owren's Veronal Buffer in each of the preceding tubes labeled 1:5; 0.8 ml of Owren's Veronal Buffer into each of the 1:15 labeled tubes; 2.8 ml of Owren's Veronal Buffer into the two tubes labeled 1:40.
   C. Add 0.4 ml of the Fibrinogen Calibration Reference to both of the tubes labeled 1:5. Mix both tubes. Transfer 0.4 ml from the 1:5 dilution into each of the tubes labeled 1:15. Mix the 1:15 dilutions. Again, transfer 0.4 ml of the 1:5 dilution into each of the tubes labeled 1:40. Mix the 1:40 dilutions.
   D. Reconstitute Thrombin Reagent with 1.0 ml of distilled water. Mix well. Keep at room temperature.
   E. Pipet 0.2 ml of the 1:5 diluted Fibrinogen Calibration Reference into each of two 12 x 75-mm test tubes. (One 0.2 ml sample from each of the 1:5 dilutions.)
   F. Incubate the two tubes at 37°C for at least 2 minutes but no longer than 5 minutes.
   G. Pipet 0.1 ml of Thrombin Reagent into the first tube and immediately start the stopwatch.
   H. While keeping the tube in the water bath, immediately begin running the wire loop through the

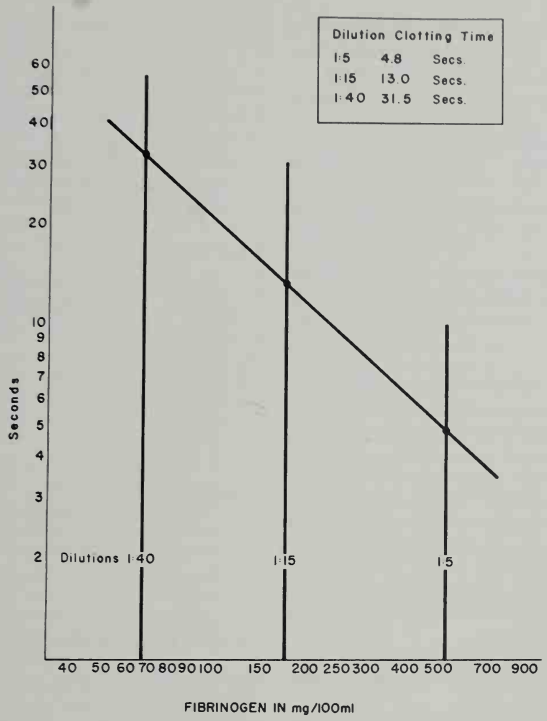

FIG. 129. Curve for quantitative fibrinogen procedure.

mixture. At the first sign of fibrin formation, stop the stopwatch and record the result. Repeat steps E through H for the duplicate specimen. Average the two results. If the clotting times are too far apart, however, repeat steps E through H again, or set up two more dilutions and repeat.

I. Repeat steps E through H for the 1:15 and 1:40 dilutions.

J. Using double log graph paper draw a fibrinogen curve as shown in Figure 129.

K. Use Ci-trol Normal (or SNP) to check the preceding curve. Reconstitute the Ci-trol Normal with 1.0 ml distilled water. Allow to sit for 10 to 15 minutes. Mix gently. Dilute the Ci-trol Normal 1:10; place 0.9 ml of Owren's Veronal Buffer into a 12 × 75-mm test tube and add 0.1 ml of Ci-trol Normal. Mix well. Determine the clotting time on duplicate samples of the diluted Ci-trol Normal, following steps E through H. Using the previously drawn fibrinogen curve,

determine the fibrinogen value in mg per dl for the Ci-trol Normal. This result should fall within the ranges given for that lot number of Ci-trol Normal.

2. Centrifuge blood at 2500 RPM for 10 minutes to obtain platelet-poor plasma.
3. Reconstitute Thrombin Reagent with 1.0 ml of distilled water. Mix carefully.
4. Reconstitute Ci-trol Normal (or SNP) with 1.0 ml of distilled water. Allow to sit for 10 to 15 minutes. Mix gently.
5. Make a 1:10 dilution of both the plasma and Ci-trol Normal: place 0.9 ml of Owren's Veronal Buffer into appropriately labeled 12 × 75-mm test tubes (one tube for the Ci-trol Normal and one tube for each specimen to be tested). Add 0.1 ml of Ci-trol Normal to the control tube and 0.1 ml of patient plasma to each of the appropriately labeled patient tubes. Mix carefully.
6. Pipet 0.2 ml of the diluted Ci-trol Normal into each of two 12 × 75-mm test tubes.
7. Incubate foregoing tubes at 37°C for at least 2 minutes, but no longer than 5 minutes.
8. Pipet 0.1 ml of Thrombin Reagent (kept at room temperature) into the first tube and immediately start the stopwatch.
9. While keeping the tube in the water bath, immediately begin running the wire loop through the mixture. At the first sign of fibrin formation, stop the stopwatch and record the result.
10. Repeat steps 7 through 9 for the duplicate specimen. Average the two results. Both clotting times should agree with each other within 1.5 seconds. If they do not, repeat steps 7 through 9.
11. Repeat steps 7 through 10 for each patient specimen.
12. Determine the fibrinogen concentration for the Ci-trol Normal and each patient specimen by referring to the previously prepared fibrinogen curve. The Ci-trol Normal result should agree within the ranges given for that lot number of Ci-trol Normal. If it does not, the cause must be found, and the entire test repeated on all of the specimens.

DISCUSSION

1. The preceding procedure may be performed on both the Fibrometer and Mechrolab Clot-Timer with excellent results.
2. When the fibrinogen value is below 50 mg per dl, the plasma should be diluted 1:5 (0.2 ml of plasma added to 0.8 ml of Owren's Veronal Buffer), or 1:2 (0.4 ml of plasma added to 0.4 ml of Owren's Veronal Buffer). Perform the fibrinogen in duplicate as just outlined, average the results, and determine the fibrinogen value from the curve. Divide these results by 2 (for the 1:5 dilution) or 5 (for the 1:2 dilution). If there is no clot formed with the 1:2 dilution, report a result of less than 15 mg per dl.
3. When the fibrinogen value is above 800 mg per dl, the plasma should be diluted 1:20 (0.1 ml of plasma added to 1.9 ml of Owren's Veronal Buffer). Perform the fibrinogen in duplicate as just outlined, average the results, and determine the fibrinogen value from the curve. Multiply these results by 2.
4. In the presence of significant levels of fibrin degradation products (above 100 μg/ml) or heparin (above 0.6 USP units/ml), the test results will be invalidly low.

PROTHROMBIN CONSUMPTION TEST

The prothrombin consumption test is merely a prothrombin time carried out on serum. It tests mainly for the coagulation