Comparison of Rapid Clottable Fibrinogen Assays

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Measurements of fibrinogen concentration by use of two rapid methods (clot-weight method and thrombin-turbidity method) were compared with the reference thrombin-coagulation method of Ratnoff and Menzie [J. Lab. Clin. Med. 37, 316 (1951)]. Both rapid methods gave results (in the high, middle, and low ranges) that correlated well with those of the thrombin-coagulation method. However, the thrombin-turbidity method was more precise, easier, and quicker than the clot-weight method.

Additional Keyphrases evaluation of disorders of bleeding • normal values • clot-weight method • thrombin-turbidity method • thrombin-coagulation method

Measurement of plasma fibrinogen concentration is an essential diagnostic determination in evaluating many patients with acute bleeding disorders. Therefore, a method is needed that is accurate, precise, rapid, and simple.

Methods that involve precipitation of fibrinogen with different concentrations of various salts are imprecise and nonspecific (1, 2, 3). Early fibrinogen breakdown products are largely thermolabile and are measured with fibrinogen when a heat-precipitation technique is used (4). Fibrinogen concentrations of less than 100 mg/100 ml of plasma are difficult to measure by either the heat- or salt-precipitation methods. Immunochemical techniques also have limitations in that results are not available for many hours and nonclottable fibrinogen and fibrin degradation products possess antigenic determinants similar to fibrinogen (4). Fibrinogen and fibrin degradation products react similarly to fibrinogen antibody, and thus a distinction between fibrinogen and its degradation products cannot be made by use of routine immunodiffusion techniques.

Fibrinogen assays that depend on the clot-forming ability of fibrinogen are the most specific and usually most accurate (2, 4–7). The reference method of Ratnoff and Menzie (8) is accurate though time consuming. In this report we compare the results of two rapid fibrinogen methods to those obtained with the thrombin-coagulation method of Ratnoff and Menzie.

Materials and Methods

Specimen: Human blood, 4.5 ml, was collected in Vacutainer tubes (Becton-Dickinson Co., Rutherford, N.J. 07070) (No. 4656) containing 0.5 ml of buffered sodium citrate (0.3 ml of 0.1 mol/liter citrate, 0.2 ml of 0.1 mol/liter citric acid), and centrifuged at 2500 rpm for 10 min. The plasma was immediately separated from the packed cells and stored at room temperature until ready to use. Assays were performed within 4 h after the blood was collected.

Clot-Weight Method (8)

Variations of this method have been described and evaluated by several investigators (9–15). Thrombin (0.1 ml, 1000 NIH units/ml; Parke-Davis Co., Detroit, Mich. 48232) is added to 1 ml of plasma. The plasma is incubated at 37°C for 10 min and the clot harvested with an applicator stick. The clot is blotted firmly on filter paper and then immersed in acetone to harden and dehydrate it. After 10 min, the clot is removed, and air-dried until it becomes dull white. It is then weighed to the nearest milligram.

Fifteen percent of the clot weight is first subtracted from the total as an empirical correction for acetone and (or) moisture remaining, and then the fibrinogen concentration is calculated by the formula:

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\text{Fibrinogen, mg/100 ml} = \frac{\text{clot weight (mg)}}{\text{vol of plasma (ml)}} \times 100
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Thrombin-Turbidity Method (16)

This method was originally described by Ellis and Stransky (6) and has been modified by other investigators (7, 16). Plasma, 0.5 ml, is added to each of two 20-ml test tubes containing 10 ml of barbital-saline buffer (0.1 mol/liter, pH 7.2) and mixed. Six milliliters of the resulting solution is placed into Coleman cuvets (19 × 109 mm). To one cuvet two drops of the calcium thrombin solution are added and mixed for 15 s; the other sample without the calcium-thrombin solution is used as the blank. After 20 min the absorbance is read at 470 nm in a Coleman Junior Spectrophotometer (Model 6/35; Coleman Instruments Corp., Maywood, Ill. 60153). The calcium-thrombin solution is composed of equal parts of dilute thrombin (33.8 NIH units per milliliter, diluted with NaCl solution, 8.5 g/liter) and 3.38 molar calcium chloride solution. From six randomized blood samples, absorbance by the thrombin-turbidity method is plotted on linear graph paper against fibrinogen concentration as determined the Ratnoff–Menzie (8) method. Fibrinogen concentrations can then be determined by the thrombin-turbidity method alone by referring to this calibration curve. A new graph must be constructed with each new batch of calcium-thrombin solution made.

Thrombin-Coagulation Method (8)

Five-tenths milliliter of citrated plasma is diluted with 10 ml of isotonic saline and clotted with thrombin. We modified the method by omitting the glass beads and harvesting the clot with applicator sticks after it had been washed three times with saline. It is hydrolyzed in sodium hydroxide solution (100 g/liter) in a bath of boiling water, and the tyrosine equivalent is determined by the use of Folin–Ciocalteau phenol reagent. The fibrinogen concentration is corrected for dilution with citrate buffer and the results expressed as mg/100 ml of plasma.

Evaluation of the Methods

Fibrinogen concentrations in a normal population and in unselected clinic and hospitalized patients. Plasma fibrinogen concentrations were determined on: (a) 42 normal people by the clot-weight and thrombin-coagulation methods; (b) 18 normal people by the thrombin-turbidity method; (c) 37 unselected hospitalized and clinic patients by the clot-weight and thrombin-coagulation methods; and (d) 42 unselected hospitalized and clinic patients by the thrombin-turbidity and thrombin-coagulation methods.

Evaluation of precision. Twenty fibrinogen determinations were performed sequentially on pooled normal plasma by the clot-weight, thrombin-turbidity, and thrombin-coagulation methods.

Evaluation of fibrinogen at various dilutions of plasma with saline. Two plasma samples were each diluted 0, 3:4, 1:2, 1:4, and 1:8 with saline (8.5 g/liter), resulting in a plasma concentration of 100%, 75%, 50%, 25%, and 12.5%. Fibrinogen was determined at each dilution by the clot-weight, thrombin-turbidity and thrombin-coagulation methods.

Statistical analysis. The data were analyzed by the paired t-test, and the coefficient of correlation was calculated for the thrombin-turbidity method vs. the thrombin-coagulation method and also for the clot-weight vs. the thrombin-coagulation method.

Results

The mean fibrinogen concentration in plasma from 42 normal people by the clot-weight method was 304 ± 59 mg/100 ml of plasma, and by the thrombin-coagulation method was 274 ± 46.0 mg/100 ml plasma. The mean fibrinogen concentration in the plasma of 18 different normal people by the thrombin-turbidity method was 249 ± 45 mg/100 ml. The comparison of fibrinogen determinations in another 37 unselected cases by the clot-weight method and the thrombin-coagulation method is shown in Figure 1. The coefficient of correlation is 0.888 and the paired t-test indicates that the results of the two determinations are not significantly different (P > 0.2).

A comparison of fibrinogen concentrations in 42 unselected plasma specimens by the thrombin-turbidity method and the thrombin-coagulation
The coefficient of correlation between the two methods is 0.986. The paired t-test indicates that the two measurements are not significantly different ($P > 0.5$).

**Evaluation of precision.** The precision of the three methods is shown in Table 1. The coefficient of variation is essentially the same for the thrombin-coagulation method and the thrombin-turbidity method. The clot-weight method has the largest variation.

**Evaluation of fibrinogen at various plasma dilutions of saline.** Measurement of fibrinogen concentration of two plasma aliquots at various saline dilutions is shown in Figure 3. In each method there is a linear relationship between the plasma dilution and concentration of fibrinogen. All three methods appear to measure fibrinogen values of less than 100 mg/100 ml accurately.

**Discussion**

In general, the normal values demonstrated by the three methods were within a range suggested by other investigators (2). The clot-weight method tends to give higher values. This phenomenon has been attributed in part to incomplete dehydration of clot specimen and the attachment of extraneous proteins, lipids, and salts to the clot (9–15). A more accurate measurement can be obtained with this method by placing the specimen in hot air ($90°-110°$C) for 3 h after immersion of the clot specimen in acetone (11) or diluting 1 ml of plasma with 10 ml of physiological saline (12).

The coefficient of correlation (0.986) between the thrombin-turbidity method and the thrombin-coagulation method is excellent. Likewise, the coefficient of correlation (0.888) between the clot-weight method and the thrombin-coagulation method is also acceptable. More importantly, there is no significant difference between the values obtained by the two methods and the reference Ratnoff–Menzie (8) procedure.

The precision of the thrombin-turbidity method and the thrombin-coagulation method is excellent. The coefficient of variation in the clot-weight method is larger than that observed in the other two methods, but is acceptable for a rapid semi-quantitative procedure.

Both the thrombin-turbidity methods and clot-weight methods are straightforward, uncomplicated, rapid (30 min), and reasonably accurate, especially at low concentration ranges of fibrinogen. Furthermore, the equipment and reagents necessary to perform these determinations are available in most clinical pathology laboratories.

Elevated plasma concentrations of bilirubin or other pigments have not been shown to interfere with the determination of fibrinogen concentration in the clot-weight method. However, markedly elevated concentrations of plasma bilirubin, hemoglobin, or urea will marginally decrease the value of fibrinogen in the thrombin-turbidity method (6).
The thrombin-turbidity method is easier and more rapid than the clot-weight method and is a satisfactory measurement for studying emergency and routine bleeding disorders. The thrombin-coagulation method should be periodically performed as a quality control procedure.

When more accurate quantitative fibrinogen determinations are necessary, the more laborious thrombin-coagulation method of Ratnoff and Menzie (8) or the clot-weight method of Fearnley and Chakrabarti (17) is suggested.

References

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