A TCT modification, the plasma-diluted TCT, provides a quantitative measure of dabigatran when used with calibrators of specific drug concentrations.[77]

The fibrinogen assay described in a subsequent section is a simple modification of the TCT. In the fibrinogen assay, the concentration of reagent thrombin is 50 NIH units/mL, or about 10 times that used in the TCT, and the patient specimen is diluted 1:10. This dilution minimizes the effects of heparin or antithrombotic proteins. The reptilase time procedure described below is identical to the TCT procedure, except that the reptilase reagent is insensitive to the effects of heparin.

Reptilase Time

Reptilase Time Reagent and Principle

Reptilase is a thrombin-like enzyme isolated from the venom of *Bothrops atrox* that catalyzes the conversion of fibrinogen to fibrin (Pefakit Reptilase Time; Pentapharm, Inc., Basel, Switzerland). In contrast to thrombin, this enzyme cleaves only fibrinopeptide A from the fibrinogen molecule, whereas thrombin cleaves both fibrinopeptides A and B.[78] The specimen requirements, procedure, and quality assurance protocol for the reptilase time test are the same as those for the TCT. The reagent is reconstituted with distilled water and is stable for 1 month when stored at 1° C to 6° C. Reptilase time reagent is a poison that may be fatal if it directly enters the bloodstream.

Reptilase Time Clinical Utility

Reptilase is insensitive to heparin but is sensitive to dysfibrinogenemia, which profoundly prolongs the assay time. The reptilase time test is also useful for detecting hypofibrinogenemia or dysfibrinogenemia in patients receiving heparin therapy. The reptilase time is prolonged in the presence of FDPs and paraproteins.

Russell Viper Venom

Russell viper venom (RVV) from the *Daboia russelii* viper, which triggers coagulation at the level of factor X, was once used as an alternative to the prothrombin time. The assay was named the Stypven time, but is now obsolete. Russell viper venom is used in a dilute form to detect and confirm lupus anticoagulant, an assay called the dilute Russell viper venom time described in Chapter 39.

COAGULATION FACTOR ASSAYS

Fibrinogen Assay

Fibrinogen Assay Principle

The clot-based method of Clauss, a modification of the TCT, is the recommended procedure for estimating the functional fibrinogen level.[79,80] The operator adds reagent bovine thrombin to dilute PPP, catalyzing the conversion of fibrinogen to fibrin polymer. In the fibrinogen assay, the thrombin reagent concentration is 50 NIH units/mL. The PPP to be tested is diluted 1:10 with Owren buffer. There is an inverse relationship between the interval to clot formation and the concentration of functional fibrinogen. Because the thrombin reagent is concentrated and the PPP is diluted, the relationship is linear when the fibrinogen concentration is 100 to 400 mg/dL. Diluting the PPP also minimizes the antithrombotic effects of heparin, FDPs, and paraproteins; heparin levels less than 0.6 units/mL and FDP levels less than 100 μg/dL do not affect the results of the fibrinogen assay provided the fibrinogen concentration is 150 mg/dL or greater.

The interval to clot formation is compared with the results for fibrinogen calibrators. A calibration curve is prepared in each laboratory and updated regularly.

Fibrinogen Assay Procedure

Fibrinogen Assay Thrombin Reagent. Most laboratory managers prefer commercially manufactured diagnostic lyophilized bovine thrombin reagent for fibrinogen assays. Pharmaceutical topical thrombin also may be used. The reagent is reconstituted according to manufacturer instructions and used immediately or aliquotted and frozen. If thrombin is to be frozen, it should be prepared in a stock solution of 1000 NIH units/mL and frozen at −70° C until it is ready for use. When thawed, the thrombin is diluted 1:2 with buffer, is stable for only a few hours, and cannot be refrozen.

Fibrinogen Assay Calibration Curve. The laboratory practitioner prepares a calibration curve every 6 months at a minimum and with each change of reagent lot numbers, with a shift in QC, and after major maintenance. The curve is prepared by reconstituting commercially available lyophilized fibrinogen calibration plasma. Using Owren buffer, five dilutions of the calibration plasma are prepared: 1:5, 1:10, 1:15, 1:20, and 1:40. An aliquot of each dilution, usually 200 μL, is transferred to each of three reaction tubes or cups, warmed to 37° C, and tested by adding 100 μL of working thrombin reagent at 50 NIH units/mL. Time from addition of thrombin to clot formation is recorded, results of duplicate tests are averaged, and the values in seconds are graphed against fibrinogen concentration (Figure 42-11). Because patient PPPs are diluted 1:10 before testing, the 1:10 calibration plasma dilution is assigned the same fibrinogen concentration value as that of the undiluted reconstituted calibration plasma.

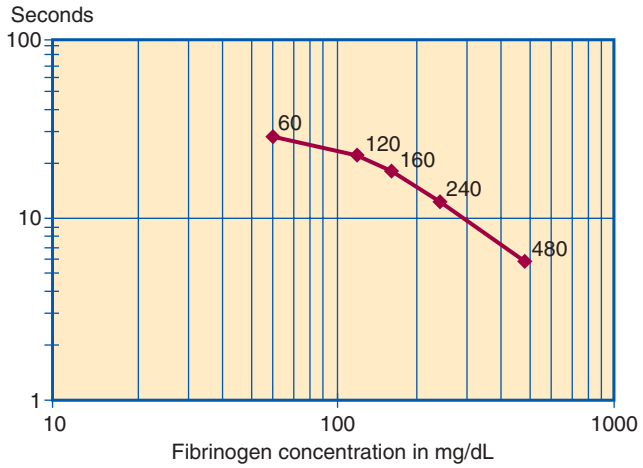

Figure 42-11 Fibrinogen calibrator curve plotted on log-log axes.

Fibrinogen Assay Test Protocol. The laboratory practitioner prepares a 1:10 dilution of each patient PPP and control with Owren buffer. Then 200 μL of each of the diluted PPPs is warmed to 37° C in each of two reaction tubes or cups for 3 minutes. After incubation, 100 μL of thrombin reagent is added, a timer is started, and the mixture is observed until a clot forms. The timer is stopped, values for duplicate runs are averaged, and the interval in seconds is compared with the graph. Results are reported in mg/dL of fibrinogen.

If the clotting time of the patient PPP dilution is short, indicating a fibrinogen level greater than 480 mg/dL, a 1:20 dilution is prepared and tested. The resulting fibrinogen concentration from the graph must be multiplied by 2 to compensate for the dilution. If the clotting time of the original 1:10 patient PPP dilution is prolonged, indicating less than 200 mg/dL of fibrinogen, a 1:5 dilution is prepared. The operator divides the resulting concentration reading from the graph by 2 to compensate for the greater concentration of the specimen.

Fibrinogen Assay Quality Control

All results for duplicate tests must agree within a coefficient of variation of less than 7%. The medical laboratory practitioner tests a normal control sample and an abnormal control sample with each batch of specimens for which fibrinogen levels are measured and records the results. The normal control results should be within the laboratory's reference interval. The abnormal control results should be less than 100 mg/dL. If either control result falls outside the control limits, the reagents, control, and equipment are checked; the problem is corrected; and the control is retested. The actions taken to correct out-of-limit tests are recorded. Control results are analyzed at regular intervals (weekly is typical) to determine the longitudinal validity of the procedure.

Specimen errors that affect the PTT likewise affect the fibrinogen assay and all factor assays (Table 42-7).

Fibrinogen Assay Results and Clinical Utility

One institution's reference interval for fibrinogen concentration is 220 to 498 mg/dL, although each local institution prepares its own interval. Hypofibrinogenemia, a fibrinogen level of less than 220 mg/dL, is associated with DIC and severe liver disease. Moderately severe liver disease, pregnancy, and a chronic inflammatory condition may cause an elevated fibrinogen level, greater than 498 mg/dL. Congenital afibrinogenemia leads to prolonged clotting times and is associated with a variable hemorrhagic disorder. Dysfibrinogenemia may give the same results as hypofibrinogenemia by this test method, because some abnormal fibrinogen species are hydrolyzed more slowly by thrombin than is normal fibrinogen. Some forms of dysfibrinogenemia may be associated with thrombosis.[81]

Fibrinogen values measured using immunologic assays and turbidimetric methods (Ellis-Stransky technique; PT-Fibrinogen HS Plus, Instrumentation Laboratory, Bedford, MA) are normal in dysfibrinogenemia. The fibrinogen concentration is estimated from reaction mixture turbidity and reported with each PT.

Fibrinogen Assay Limitations

Although antithrombotic effects are minimized by the dilution of PPP specimens, heparin levels greater than 0.6 units/mL and FDP levels greater than 100 μg/mL prolong the results and give falsely lowered fibrinogen results. The operator ensures that the thrombin reagent is pure and has not degenerated. Exposure to sunlight or oxidation results in rapid breakdown. The working dilution lasts only 1 hour at 1° C to 6° C and should remain cold until just before testing.

Single-Factor Assays Using the Partial Thromboplastin Time Test

Principle of Single-Factor Assays Based on Partial Thromboplastin Time

If the PTT is prolonged and the PT and TCT are normal, and there is no ready explanation for the prolonged PTT such as heparin therapy, LA, or a factor-specific inhibitor, the medical laboratory practitioner may suspect a congenital single-factor deficiency. Three factor deficiencies that give this reaction pattern and cause hemorrhage are factor VIII deficiency (hemophilia A), factor IX deficiency (hemophilia B), and factor XI deficiency, which causes a mild intermittent bleeding disorder called *Rosenthal syndrome* found primarily in Ashkenazi Jews.[82,83] These deficiencies are most often detected in childhood. The next step in diagnosis of a congenital single-factor deficiency is the performance of a one-stage single-factor assay based on the PTT system.

Although necessary for diagnosis, PTT-based single-factor assays are most often performed on specimens from patients with previously identified single-factor deficiencies. Their purpose is to monitor supportive therapy during bleeding episodes or invasive procedures. Because hemophilia A is the most common single-factor deficiency disorder, this discussion is confined to the factor VIII assay; however, the protocol may be generalized to the assays for factors IX and XI.

The medical laboratory practitioner uses the PTT system to estimate the concentration of functional factor VIII by incorporating commercially prepared factor VIII-depleted PPP in the test system (Cryo*check* Factor VIII Deficient Plasma; Precision BioLogic Inc, Dartmouth, Nova Scotia). Distributors collect plasma from normal donors and employ *immunodepletion*, relying on a monoclonal anti-factor VIII antibody bound to a separatory column, to prepare factor VIII-depleted plasma.[84]

In the PTT-based factor assay system, factor VIII-depleted PPP provides normal activity of all procoagulants except factor VIII. Tested alone, factor VIII-depleted PPP has a prolonged PTT, but when normal PPP is added, the PTT reverts to normal. In contrast, a prolonged result for a mixture of patient PPP and factor VIII-depleted PPP implies that the patient PPP is factor VIII deficient. The clotting time interval for the mixture of patient PPP and factor VIII-depleted PPP may be compared with a previously prepared reference curve to estimate the level of factor VIII activity in the patient PPP. The quantitative factor assay is typically performed on three or four dilutions of patient PPP—for instance, 1:10, 1:20, 1:40, and 1:80—and the results compared with mathematical manipulation. Multiple dilutions contribute to the accuracy of the results.