anti-factor VIII from the patient PPP neutralizes a percentage of the reagent normal plasma factor VIII activity. The degree of factor VIII activity neutralized is proportional to the level of inhibitor activity. After incubation, residual factor VIII activity in the patient PPP–reagent normal plasma mixture is measured using the specific factor activity assay as described in the section on factor assays using the PTT system.

The titer of inhibitor is expressed as a percentage of the control. If the patient PPP-reagent normal plasma mixture retains 75% of the residual factor VIII activity of the control, no factor VIII inhibitor is present. If the residual factor VIII level is 25% that of the control, the patient PPP factor VIII inhibitor level is titered using several dilutions of the patient specimen in reagent normal PPP. One Bethesda unit of activity is the amount of antibody that leaves 50% residual factor VIII activity in the mixture.

Single-Factor Assays Using the Prothrombin Time Test

If the PTT and the PT are both prolonged, the TCT is normal, and there is no ready explanation for the prolonged test results, such as liver disease, vitamin K deficiency, DIC, or Coumadin therapy, the medical laboratory practitioner may suspect a congenital single-factor deficiency of the common pathway (Chapter 37). Three relatively rare factor deficiencies that give this reaction pattern and cause hemorrhage are prothrombin deficiency, factor V deficiency, and factor X deficiency. If the PT is prolonged and all other test results are normal, factor VII deficiency is suspected. The next step is the performance of a one-stage single-factor assay based on the PT test system, which is a relatively rare event. The principles and procedure described in the section on single-factor assay using the PTT system may be applied except that PT reagent replaces the PTT reagent in the test system, and the PT protocol is followed. Factor II (prothrombin)-depleted, factor V-depleted, factor VII-depleted, and factor X-depleted plasmas are available (Table 42-8).

Factor XIII Assay

Coagulation factor XIII is a transglutaminase that catalyzes covalent cross-links between the α and γ chains of fibrin polymer.[89] Cross-linking strengthens the fibrin clot and renders it resistant to proteases. This is the final event in coagulation, and it is essential for normal hemostasis and normal wound healing. Factor XIII from plasma, platelets, and tissue function identically. Neither the PT nor the PTT is prolonged by factor XIII deficiency.

Inherited factor XIII deficiency, an autosomal recessive disorder, affects both sexes in all races. The first report of the deficiency appeared in 1960, and the frequency is estimated at 1 in 2 million. Factor XIII levels also may be low in chronic DIC secondary to Crohn disease, leukemias, ulcerative colitis, sepsis, inflammatory bowel disease, surgery, and Henoch-Schönlein purpura. In these cases, the factor XIII level decreases to 50% of normal, not low enough to create symptoms, although occasionally acquired factor XIII deficiencies produce low enough levels to cause mild bleeding. Acquired factor XIII inhibitors have been described in patients treated with isoniazid, penicillin, valproate, and phenytoin.[90] These drugs may cause complete absence of factor XIII.

Factor XIII activity levels lower than 5% result in hemorrhage. In congenital factor XIII deficiency, bleeding is evident in infants, with seepage at the umbilical stump.[91] In adults, bleeding is slow but progressive, accompanied by poor wound healing and slowly resolving hematomas. Recurrent spontaneous abortion and posttraumatic hemorrhage are common. Acquired factor XIII inhibitors cause severe bleeding that does not respond to therapy.

When a patient comes for treatment of bleeding and poor wound healing and the PTT, PT, platelet count, and fibrinogen level are normal, the laboratory practitioner may recommend a factor XIII assay such as the Technochrom Factor XIII (DiaPharma Group, Inc., West Chester, OH).[92] In this representative assay, quantitation of factor XIII activity is based on the measurement of ammonia released during an in vitro transglutaminase reaction. Plasma factor XIII is first activated by reagent thrombin. The resultant factor XIIIa then cross-links the fibrin amine substrate glycine ethyl ester to the glutamine residue of a peptide substrate, releasing ammonia. The concentration of ammonia is monitored in a glutamate dehydrogenase–catalyzed reaction that depends on NADPH, the reduced form of nicotinamide adenine dinucleotide phosphate. NADPH consumption is measured by the decrease of absorbance at 340 nm. The absorbance is inversely proportional to factor XIII activity. Several manufacturers market immunoassays for factor XIII, which provide factor XIII concentration but do not identify functional factor XIII abnormalities.

TABLE 42-8 Factor Assays Using the TCT, PT, and PTT Test Systems

Factor	System
Fibrinogen (I)	Clauss method: modified thrombin clotting time
Prothrombin (II)	Prothrombin time
Factor V	Prothrombin time
Factor VII	Prothrombin time
Factor VIII	Partial thromboplastin time
Factor IX	Partial thromboplastin time
Factor X	Prothrombin time
Factor XI	Partial thromboplastin time
Factor XIII	Chromogenic assay

PT, prothrombin time; *PTT*, partial thromboplastin time; *TCT*, thrombin clotting time.

TESTS OF FIBRINOLYSIS

Quantitative D-Dimer Immunoassay

Physiology of Fibrin Degradation Products and D-Dimers

During coagulation, fibrin polymers become cross-linked by factor XIIIa and simultaneously bind plasma plasminogen and tissue plasminogen activator (TPA) (Chapter 37). Over several hours, bound TPA activates nearby plasminogen to form plasmin. The bound plasmin cleaves fibrin and yields the FDPs D, E, X, and Y and D-dimer. The FDPs represent