B. Patients with severe liver disease may have elevated FDP due to reduced clearance by the liver; these patients may also have falsely elevated FDP on the basis of an acquired dysfibrinogenemia (as above)
C. Incomplete removal of fibrinogen during the preparation of the sample may yield falsely elevated FDP
D. Elevated FDP in the absence of DIC may occur with clot lysis, especially in:
   1. Deep venous thrombosis
   2. Pulmonary embolism
   3. Post-surgical state
   4. Portacaval shunts

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2. Carvalho ACA, Ellman LL, Colman RW: A comparison of the staphylococcal clumping test and an agglutination test for detection of fibrinogen degradation products. Am J Clin Pathol 62:107-112,1974.

Functional Assays for Factors VIII, IX, XI, XII, Prekallikrein, and High Molecular Weight Kininogen

I. **USED IN THE DIAGNOSIS OF: Congenital or acquired deficiencies of coagulation factors VIII, IX, XI, XII, prekallikrein (PK), or high molecular weight kininogen (HMWK)**

II. **PRINCIPLE OF TEST: The addition of patient plasma to plasma severely deficient in Factors VIII, IX, XI, XII, PK, or HMWK will correct the prolonged PTT of the factor deficient plasma if the patient plasma contains the factor in question. The level of factor activity in the patient plasma correlates with the extent of correction of the PTT. The activity is calculated by comparing the correction produced by the patient plasma to the correction produced by various dilutions of normal pooled plasma.**

III. **PATIENT PREPARATION; COLLECTION/HANDLING OF SPECIMEN: No patient preparation is needed. Venous blood is collected in citrate (blue-top Vacutainer tube). DO NOT COLLECT BLOOD THROUGH A HEPARIN LOCK OR OTHER HEPARINIZED LINE.**

IV. PROCEDURE

A. Reagents/Materials
   1. Factor deficient substrate can be obtained commercially from several different reagent vendors (Note: in the past, Factor VIII and Factor IX deficient plasmas were obtained directly from hemophiliac donors. Because of the prevalence of HIV in these donors, Factor VIII deficient plasma is now being made by immunodepletion by several manufacturers. The performance characteristics are identical.)
   2. General Diagnostics APTT reagent
   3. Veronal Buffer in saline (VBIS)- see reagents
   4. 0.025M CaCl2
   5. Normal pooled plasma
   6. Control plasma- any one of several commercial plasmas that have been assayed for these factors.

B. Method- tilt tube.
   1. Dilute the pooled plasma 1:10, 1:20, 1:40, and 1:80. Dilute the patient and control plasmas 1:10 and 1:20.
   2. Into a 10x75 mm glass test tube, pipet the following:
      0.1 ml diluted plasma (pooled normal, patient, or control)
      0.1 ml factor deficient plasma
      0.1 ml APTT reagent
   3. Mix well and incubate at 37°C for 5 minutes

4. To each tube, add 0.01 ml CaCl2 and begin timing immediately. Mix, then place the tube back to incubate until approximately 50 seconds has elapsed. The tube is then tilted slightly to mix and the plasma is allowed to slide down the side of the glass. The clot will appear as a small lump as the liquid ceases to run down the tube. Stop timer and record time when clot appears. Each dilution is run in duplicate.
5. This test can also be performed on the Coag-a-Mate X-2 (General Diagnostics). The method follows the manufacturer's recommendations for clot detection.

C. Calculations
1. Plot the standard curve using normal pool plasma on 2 cycle log-log graph paper. The 1:10 dilution corresponds to 100%, the 1:20 dilution to 50%, etc. The factor activity (expressed as a percentage of total rather than as the plasma dilution) is marked on the abscissa and the time, in seconds, on the ordinate. A straight line should be generated.
2. Obtain the patient and control values from the curve, by drawing a horizontal line from the time value of each 1:10 dilution sample (on the ordinate) to the point of interception on the standard curve. Then, from this point of interception, draw a vertical line to the abscissa. The point of interception on the abscissa represents the factor activity of the sample. Read the results of the 1:20 dilution in the same manner and multiply that result by 2. Average these 2 results to obtain the factor level in percent of normal.
3. The control value obtained should fall within 15% of the value reported for the sample

D. Notes
1. The prekallikrein assay is performed using the same procedure, but the dilution mixture should be preincubated for 1 minute instead of 5 minutes.

2. Factor VIII assays should be performed on fresh specimens only, usually within 2 hours after they have been drawn.

E. Normal Ranges (vary slightly between laboratories)
1. Factors VIII, XI, and XII: 50%-200%
2. Factor IX: 75%-125%
3. High Molecular Kininogen: 60%-140%
4. Prekallikrein: 65%-115%

V. COMMENTS:

A. A decreased level of Factor VIII is found in hemophilia A, hemophilia A carrier state, and some patients with von Willebrand's disease. (See hemophilia A, von Willebrand's Disease.)
B. An increased level of Factor VIII may occur in many inflammatory states, in pregnancy, and with estrogen supplementation
C. A decreased level of Factor IX is found in hemophilia B, hemophilia B carrier state, vitamin K deficiency, and severe liver disease. (See section on Hemophilia B.).
D. A decreased level of Factor XI is found in hemophilia C, and severe liver disease
E. A decreased level of Factor XII is found in congenital deficiency of Factor XII (an asymptomatic deficiency state which results in prolongation of the PTT).
F. Decreased levels of all of the above factors may be found in other circumstances such as massive transfusion with packed red blood cells without replacement of plasma.
G. The lupus inhibitor, which typically prolongs the PTT and gives a positive PTT inhibitor screen, decreases the levels of PTT factors as a group in vitro by its action as a phospholipid (coagulation cofactor) inhibitor.
H. A decreased level of prekallikrein is found in congenital deficiency of prekallikrein (an asymptomatic deficiency resulting in a prolonged PTT).

I. A decreased level of high molecular weight kininogen (HMWK) is found in congenital deficiency of HMWK, an asymptomatic deficiency resulting in a prolonged PTT

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1. Hardisty RH, McPherson JC: One-stage factor (AHG) assay and its uses, venous and capillary. Thromb et Diath Hemorr 7:215, 1962.
2. Egeberg O: Assay of antihemophilic A, B, and C factors by one-stage cephalin systems. Scand J Clin Lab Invest 13:140, 1961.

Functional Assays for Factors II, V, VII, and X

I. USED IN THE DIAGNOSIS OF: Congenital or acquired deficiencies in coagulation Factors II, V,VII, X (common pathway and extrinsic pathway factors).

II. PRINCIPLE OF TEST: The addition of test (patient) plasma to plasma which is severely deficient in Factor II, V, VII, or X will correct the prolonged PT of the factor-deficient plasma if the test plasma contains the factor in question. The level of factor activity in the test plasma correlates with the degree of correction of the PT. The activity is calculated by comparing the correction produced by the test plasma to the correction produced by various dilutions of normal pooled plasma.

III. PATIENT PREPARATION; COLLECTION/HANDLING OF SPECIMEN: No patient preparation is needed. Venous blood is collected in citrate (blue-top Vacutainer). DO NOT COLLECT BLOOD THROUGH A HEPARIN LOCK OR OTHER HEPARINIZED LINE.

IV. PROCEDURE