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

REFERENCES

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**

A. Reagents/Materials
1. Human brain thromboplastin (or any commercial thromboplastin)
2. Veronal buffer in saline (VBIS)--see reagents
3. Normal pooled plasma--see reagents
4. Deficient substrate plasma that is deficient in the factor being tested. Many companies sell either lyophilized or frozen plasma from a human deficient donor.
5. 0.04M calcium chloride (CaCl2- prewarmed to 37°C
6. Fibrometer with attached heat block--BBL
7. Control plasma. Any one of several commercial plasmas that have been assayed for these factors.

B. Method — Fibrometer
1. A standard curve is established when each assay is performed: dilute normal pool plasma with VBIS to dilutions of 1:10, 1:20, 1:40, and 1:80
2. The patient and control plasmas are diluted 1:10 and 1:20
3. Into a fibrometer cup, pipet the following: 0.1 ml plasma dilution; 0.1 ml thromboplastin; 0.1 ml deficient substrate.
4. Mix well, place the mixture under the fibrometer probe and then add 0.1 ml CaCl2, while simultaneously starting the timer and fibrometer.
5. The tests can be performed on automated instruments also using the instructions provided by the manufacturer.

C. Calculations
1. Plot the standard curve using normal pooled plasma on 2 cycle log-log graph paper with the percentage plasma dilution on the abscissa where 1:10 dilution corresponds to100% and the time, in seconds, on the ordinate. The points should form a straight line.
2. Read the patient and control results from the ordinate to the abscissa by finding the point where seconds for the 1:10 dilution of the tested

plasma intersects the standard curve. Read the 1:20 dilution in the same manner, multiply that result by 2, and average the 1:10 and 1:20 dilution to give the final value for the factor concentration in percent of normal.

3. The control should come to within 15% of the value reported for the sample.

D. Normal Range: 60%-140% for Factors II, V, VII, and X

V. COMMENTS:

A. Isolated deficiency of Factor II may be congenital or occur as an acquired deficiency in patients with the lupus anticoagulant

B. Isolated deficiency of Factor V activity may be congenital, or it may occur through the effect of an inhibitor in rare cases of amyloidosis with concomitant Factor X inhibitor

C. Isolated deficiency of Factor VII may be congenital or may occur early in vitamin K deficiency

D. Isolated deficiency of Factor X may be congenital or may be acquired in patients with primary amyloidosis

REFERENCES

1. Babson AL, Flanagan ML: Quantitative one-stage assays for factors V and X. Am J Clin Pathol 64:817-819, 1975.
2. Kirkwood TBL, Snape TJ: Biometric principles in clotting and clot lysis assays. Clin Lab Haematol 2:155-158, 1980.

Clot Solubility (Screening Test for Factor XIII Deficiency)

I. **USE: As a screening test for congenital Factor XIII deficiency**

II. **PRINCIPLE OF TEST: Patient plasma is clotted with exogenous thrombin; the clot is incubated with 5 M urea for 24 hours or with 1% monochloroacetic acid for 2 hours at 37° C. The clot is observed for dissolution. If the patient has <2%-3% or less Factor XIII activity, the clot completely dissolves (positive test).**

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

IV. PROCEDURE

A. Reagents/Materials

1. 1% Monochloroacetic acid
2. Thrombin--2 units/ml in VBIS (see reagents).
3. 0.025M CaCl2
4. Factor XIII deficient plasma. This is obtained commercially (George King Biomedical) and is used as a positive control for deficiency.
5. Normal plasma is used as a negative control for no deficiency.

B. Method

1. Mix 3 ml of thrombin with 3ml CaCl2.
2. In a glass 12x75 mm test tube, add the following:
   0.2 ml plasma (patient sample, normal plasma, or Factor XIII deficient plasma)
   0.2 ml thrombin-calcium solution
3. Allow the mixture to clot.Then very gently dislodge the clot from the sides of the tube by tapping it lightly with a finger.
4. Add 1 ml of 1% monochloroacetic acid. Covertop of tube to limit evaporations.
5. Place in a 37°C water bath for 2 hours
6. Read for clot lysis. The factor XIII deficient plasma should be a clear solution with no detectable clot present. The normal plasma should still have the clot intact. Check the patient's sample to see if a clot is still present.

C. Calculations--there are no calculations in this test. In