IV. PROCEDURE

A. Reagents/Materials
1. APTT reagent containing a phospholipid and an activator — such as General Diagnostics APTT reagent
2. Veronal buffer in saline (VBIS) — see reagents
3. Normal pooled plasma — (NPP)
4. Prekallikrein deficient plasma (George King Biomedical)
5. 0.025 M CaCl2

B. Method
1. Into a 12 x 75 mm plastic test tube, add the following:
0.4 ml APTT reagent
0.4 ml test plasma
2. Mix gently and begin timing immediately. Incubate at 37°C.
3. At 5 minutes and at 30 minutes, remove 0.2 ml into a 10 x 75 mm glass test tube and add 0.1 ml CaCl2. Measure time until clot formation.

C. Normal Range: Correction of the PTT to near normal is suggestive of a prekallikrein deficiency.

V. COMMENTS: **A negative screen (no correction of the PTT upon prolonged incubation) obviates the need for a quantitative test for prekallikrein.**

REFERENCE

1. Hattersley PG, Hayse D: The effect of increased contact activation time on the activated partial thromboplastin time. Am J Clin Pathol 66:479-482, 1976.

Fibrinogen Assays

I. USEFUL IN:

A. The diagnosis of afibrinogenemia, congenital or acquired hypofibrinogenemia, or dysfibrinogenemia.

B. The diagnosis and monitoring of DIC
C. Monitoring thrombolytic therapy
D. The diagnosis of primary fibrinogenolysis

II. **PRINCIPLE OF TEST: Varies with method used**

A. Clauss method (dilute thrombin time)-measures the time to clot formation after the addition of thrombin to dilute patient plasma; fibrinogen value determined from standard curve.
B. Ellis method — measures change in turbidity of undiluted patient plasma following the addition of thrombin.
C. Clottable protein method-measures protein mass of clot formed in plasma following the addition of thrombin.
D. Immunologic method — measures immunologically reactive fibrinogen
E. Salt precipitation method — measures turbidity of plasma following precipitation of fibrinogen by sodium sulfite.
F. DuPont aca method — measures rate of change in turbidity of dilute plasma as fibrin is formed from fibrinogen following the addition of thrombin.

III. **PATIENT PREPARATION; COLLECTION/HANDLING OF SPECIMEN: No patient preparation needed. Venous blood is collected in citrate (blue top Vacutainer tube). DO NOT COLLECT BLOOD THROUGH HEPARIN LOCK OR OTHER HEPARINIZED LINE. Any clots in the specimen are cause for rejection.**

IV. **PROCEDURE — Clauss Method**

A. Reagents/Materials
1. Fibrometer (BBL) with attached heat block
2. Thrombin — 100 units/ml (General Diagnostics-Fibriquik)
3. Fibrinogen control with known concentration of fibrinogen (General Diagnostics Fibriquik Fibrinogen Calibration Reference)

rinogen Calibration Reference)
4. Veronal Buffer in saline (VBIS)- see reagent list

B. Method
1. Dilute control plasma with known fibrinogen concentration 1:5, 1:10, 1:20, 1:40 with VBIS
2. Add 0.2 ml control plasma to Fibrometer cup and incubate in Fibrometer at 37°C for 3 minutes
3. Add 0.1 ml thrombin, start timer, and determine time until clot formation
4. On 2x2 cycle log paper, graph the seconds until clot formation vs. the different fibrinogen concentrations. This curve may be used for every fibrinogen assay using the same lot of thrombin.
5. To determine the fibinogen level of a patient sample, dilute the plasma 1:10 with VBIS. Repeat step #3 and determine value from standard curve.

C. Calculations — The patient's plasma fibrinogen concentration can be determined directly from the graph .

D. Normal Range: 170-410 mg/dl

V. PROCEDURE- Ellis and Stransky Method

A. Reagents/Materials
1. Barbital buffer- see reagent list
2. 0.025 M calcium chloride (CaCl2)
3. Thrombin — 100 units/ml (General Diagnostics-Fibriquik)
4. Spectrophotometer which reads at ultraviolet wavelengths
5. Quartz cuvettes with covers
6. Saline
7. Control plasma (see above)

B. Method
1. Prepare thrombin solution of 10 units/ml in the following manner:
0.1 ml thrombin, 100 units/ml
0.1 ml NaCl
0.8 ml 0.025M CaCl2

2. Pipette 0.8 ml barbital buffer into each of 3 cuvettes
3. For each sample and control, 3 matched cuvettes are needed. One of the three serves as a blank, to which no thrombin-calcium should be added; it is used to blank the spectrophotometer for the sample or the control
4. Add 0.04 ml plasma (patient sample or control) to each of the 3 matched cuvettes
5. Cover cuvette and invert 3 times to mix
6. Pipet 0.04 ml of thrombin — CaCl2 mixture into the first cuvette. Immediately cover and invert to mix. Repeat with the second cuvette — add 0.04 ml of thrombin — CaCl2, cover and mix. Start timer for 15 minutes.
7. Pipet 40 microliters saline into the third cuvette . This is the blank for the sample.
8. At the end of 15 minutes, zero each sample using the blank cuvette and record the absorbance of the duplicate cuvettes.

C. Calculations
1. Average the duplicate absorbance readings for each sample.
2. Read the absorbance from a standard curve made in the same manner as the one described for the Clauss method and convert into mg/dl plasma fibrinogen.

D. Normal Range 170-410 mg/dl

VIII. COMMENTS

A. Clauss and aca methods may yield falsely low values if specimen contains fibrin degradation products (as in patients with DIC or on thrombolytic therapy) or structurally abnormal fibrinogen

B. Immunologic and salt precipitation methods do not reflect functional (clotting) ability of the fibrinogen molecule and may yield discrepant values when compared to other methods in cases of dysfibrinogenemia (immunologic > functional)

C. Some modifications of the Ellis method are sensitive to heparin and may yield falsely low values in specimens containing heparin

REFERENCES

1. Clauss A: Gerinnungs physiologische schell methode zur bestimmung des fibrinogens. Acta Hematol 17:237-246, 1957.
2. Ellis BC, Stransky A: A quick and accurate method for the determination of fibrinogen in plasma. J Lab Clin Med 58:477-488, 1961.
3. Goodwin JF: An evaluation of technics for the separation and estimation of plasma fibrinogen. Clin Chem 11:63-73, 1965.
4. Ratnoff OD, Menzie C: A new method for the determination of fibrinogen in small samples of plasma. J Lab Clin Med 37:316-320, 1951.

Thrombin Time (TT)

I. USES:

A. To document the presence of heparin:
1. A prolonged TT which corrects with the additon of protamine sulfate
2. A prolonged TT in a sample with a normal reptilase time

B. The diagnosis of dysfibrinogenemia when performed in combination with a reptilase time

C. Monitoring thrombolytic therapy (in some protocols)

D. The diagnosis of afibrinogenemia or hypofibrinogenemia (congenital or acquired), when performed in conjunction with fibrinogen assays

II. **PRINCIPLE OF TEST: Thrombin is incubated with the patient's plasma and the time to clot is measured.**

III. **PATIENT PREPARATION; COLLECTION/HANDLING OF SPECIMEN: No patient preparation need-**