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LABORATORY ASSAYS

Prothrombin Time (PT)

I. USES:

A. Monitoring warfarin (Coumadin) therapy
B. Screening test for the diagnosis of vitamin K deficiency
C. The diagnosis of disseminated intravascular coagulation (DIC)
D. The diagnosis of congenital and acquired deficiencies of coagulation proteins in the extrinsic (Factor VII) and common (Factors I, II, V, X) pathways.
E. The diagnosis of inhibitors to Factors II, V, and X.

II. PRINCIPLE OF TEST: **A mixture of thromboplastin (which contains phospholipid and tissue factor [Factor III] from rabbit brain and calcium) is added to citrated patient plasma and the time to clot formation is determined. This procedure may be performed manually using a Fibrometer (BBL), or in an automated instrument which prewarms and incubates the reagents as required and then measures the time to clot formation.**

III. PATIENT PREPARATION; COLLECTION/HANDLING OF SPECIMEN: **No patient preparation needed. Venous blood is collected in citrate (blue top Vacutainer tube). Specimens should be centrifuged at 1500 x g, the plasma transferred to a plastic test tube, and then preserved on ice. DO NOT COLLECT BLOOD THROUGH A HEPARIN LOCK OR OTHER HEPARINIZED LINE. The presence of clot in the specimen is cause for rejection.**

IV. PROCEDURE

A. Reagents/Materials
1. General Diagnostics Simplastin Excel- with accompanying diluent. There are several different thromboplastins currently on the market and their sensitivities vary as far as their ability to detect factor deficiencies and their sensitivity to fibrin degradation products as an interfering factor in the assay. Since thromboplastins also vary from lot to lot, it is a generally accepted practice to establish normal ranges using a single lot number and to purchase enough to last until a new normal range can be established.
2. Normal control plasma. One such plasma is General Diagnostics Verify Normal.
3. Abnormal control plasmas. Two examples are General Diagnostics Verify Abnormal Level I and Verify Abnormal Level II, which differ in their degree of abnormality.
4. Patient plasma
5. Fibrometer with attached 37°C heat block or any instrument used for automatic clot detection, such as the Coag-a-Mate X-2, by General Diagnostics

B. Method—Fibrometer
(Method for automated testing is instrument dependent)
1. Add diluent to the bottle of Simplastin as noted in the instructions and warm the Simplastin in a test tube in the fibrometer well for 5 minutes

2. Pipet 0.1 ml of patient or control plasma into a fibrometer cup and incubate in the fibrometer at 37°C for 3 minutes.
3. Pipet 0.2 ml of the prewarmed Simplastin into the cup and start timing simultaneously. Record time until clot formation.
4. Each test should be performed in duplicate.

C. Calculations: Average the time in seconds for the duplicate specimens and report this average as the patient's prothrombin time

D. Normal Range: Each laboratory should establish its own normal range and report this along with the patient's result

V. COMMENTS

A. Common causes of acquired deficiency of one or more extrinsic or common pathway factors include:
1. Severe liver disease
2. Vitamin K deficiency

B. Inhibitors of Factors V and X are rare, occurring most commonly in patients with primary amyloidosis.

C. To distinguish a factor deficiency from an inhibitor in a patient with a prolonged PT, an inhibitor screen ("1:1 mixing study") is performed, which consists of a PT using equal volumes of normal plasma and patient plasma (see inhibitor screen assays on page 199). If the PT corrects completely, a deficiency of an extrinsic or common pathway factor should be suspected; if the PT does not correct, an inhibitor should be suspected

D. Ingestion of warfarin results in production of non-carboxylated vitamin K-dependent clotting factors, which have no procoagulant activity, resulting in a prolonged PT.

E. The PT of a patient on a stable dose of Coumadin should not vary significantly over time. Causes of variation in the PT in such patients include:

1. Ingestion of drugs that either promote or interfere with the absorption or action of warfarin
2. Marked changes in dietary ingestion of vitamin K
3. Exacerbation of underlying hepatic disease
4. Biliary obstruction
5. Profuse diarrhea or vomiting

F. A prothrombin time >40 seconds (with a mean of normal range of approximately 10 seconds) resulting from warfarin overdose may be critical enough to warrant immediate treatment with fresh frozen plasma or vitamin K. (See section on warfarin therapy.)

REFERENCES

1. Quick AJ, Stanley-Brown M, Bancroft FW: A study of the coagulation defect in hemophilia and in jaundice. Am J Med Sci 190:501-511, 1935.
2. Koepke JA, Gilmer PR, Triplett DA, O'Sullivan MB: The prediction of prothrombin time system performance using secondary standards. Am J Clin Pathol 68:191-194, 1977.

Partial Thromboplastin Time (PTT)

I. USEFUL IN:

A. Screening for the diagnosis of congenital or acquired deficiencies of coagulation proteins of the intrinsic pathway (Factors VIII, IX, XI, XII, prekallikrein, and high molecular weight kininogen)

B. Monitoring heparin therapy

C. Screening for inhibitors of Factors VIII, IX, or XI

D. Screening for the presence of a lupus anticoagulant

E. Diagnosis of disseminated intravascular coagulation (DIC), although it is a less sensitive test for DIC than the prothrombin time.

II. **PRINCIPLE OF TEST: A mixture of phospholipid (in partial thromboplastin) and calcium is incubated with citrated plasma and the time until clot formation is measured. The PTT may be performed as a one-**