

# Prothrombin Time and INR

## Performing the PT

**Synonyms:** PT, Prottime, Quick's time.

**Patient Preparation:** No specific patient preparation is required. However, specimens should not be obtained after a meal since lipemia may interfere with photo-electric measurements of clot formation.

**Specimen Requirements:** Citrated, platelet-poor plasma is used for the PT. The quality of the blood specimen is the most critical determinant of an accurate PT/INR result. A non-traumatic venipuncture must be performed, with the blood specimen collected into the proper anticoagulant at the correct concentration. To avoid contamination with tissue thromboplastin, specimen tubes for the PT should be drawn last if multiple tubes of blood are being obtained.

The PT should be performed within 24 hours after sample procurement if the unopened, centrifuged or uncentrifuged specimen tube is refrigerated (2°C - 4°C) or stored at room temperature (18°C - 24°C). Frozen plasma stored at -20°C can be used within two weeks, or plasma stored at -70°C can be used within 6 months if the blood specimen is spun properly to remove all platelets and promptly frozen. Platelet-poor plasma (platelet count <10 X 10<sup>9</sup>/L) should be prepared by centrifuging the capped specimen tube at 1500 g for a minimum of 15 minutes at room temperature.

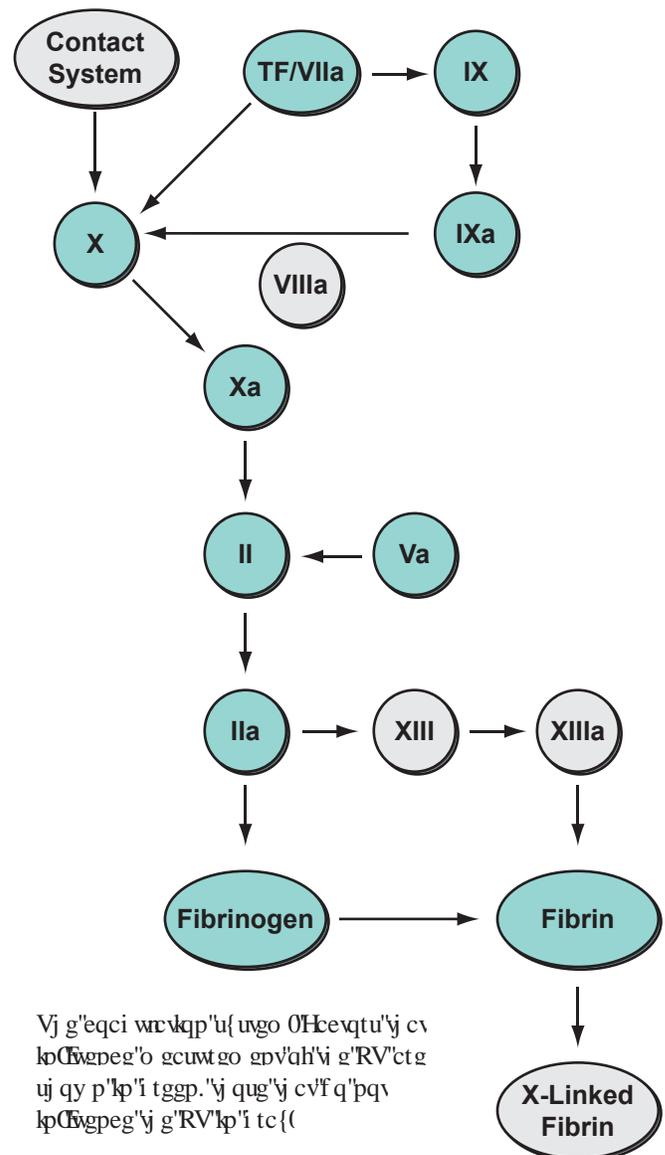
**Test Methodology:** The PT is performed on platelet-poor plasma prepared from blood collected into citrate anticoagulant. In the PT an aliquot of plasma is incubated at 37°C with a reagent containing a phospholipid-protein extract of tissue (thromboplastin). CaCl<sub>2</sub> is then added and the time required for clot formation is measured by one of a variety of techniques (photo-optical, electromechanical, etc.). The result is reported in seconds (prothrombin time), or as a ratio compared to the laboratory mean normal control (prothrombin ratio, PTR).

The PT is critically dependent on the characteristics of the thromboplastin used in the assay. Thromboplastin is comprised of tissue factor and phospholipid, both of which are necessary for the activation of factor X by factor VII. Different thromboplastin preparations vary considerably in their ability to initiate coagulation in the presence of decreased clotting factors. For example, "responsive" thromboplastins produce less rapid activation of factor X by factor VII than "unresponsive" thromboplastins, resulting in a relatively greater prolongation of the PT for a given decrease in vitamin K dependent coagulation factors. The PT result is also influenced by the instrument used in the assay, the plasma standard, drug-drug interactions, drug-food interactions, and other factors.

## What the PT Measures

The PT is functional determination of the extrinsic (tissue factor) pathway of coagulation and is extremely sensitive to the vitamin-K dependent clotting factors (factors II, VII, and X). Tissue factor (factor III) is a transmembrane protein that is widely expressed on cells of non-vascular origin, which activates factor VII during the initiation of the extrinsic coagulation pathway. A cascade mechanism results in fibrin production and clot formation.

The PT is a widely used laboratory assay for the detection of inherited or acquired coagulation defects related to the extrinsic pathway of coagulation.



## Performing the PT (Cont'd)

**Normal Values and Critical Limits:** 8.8 - 11.6 seconds (Jordan CD, Flood JG, Laposata M, and Lewandrowski KB: Normal reference laboratory values. N. Engl. J. Med. 327: 718-724, 1992).

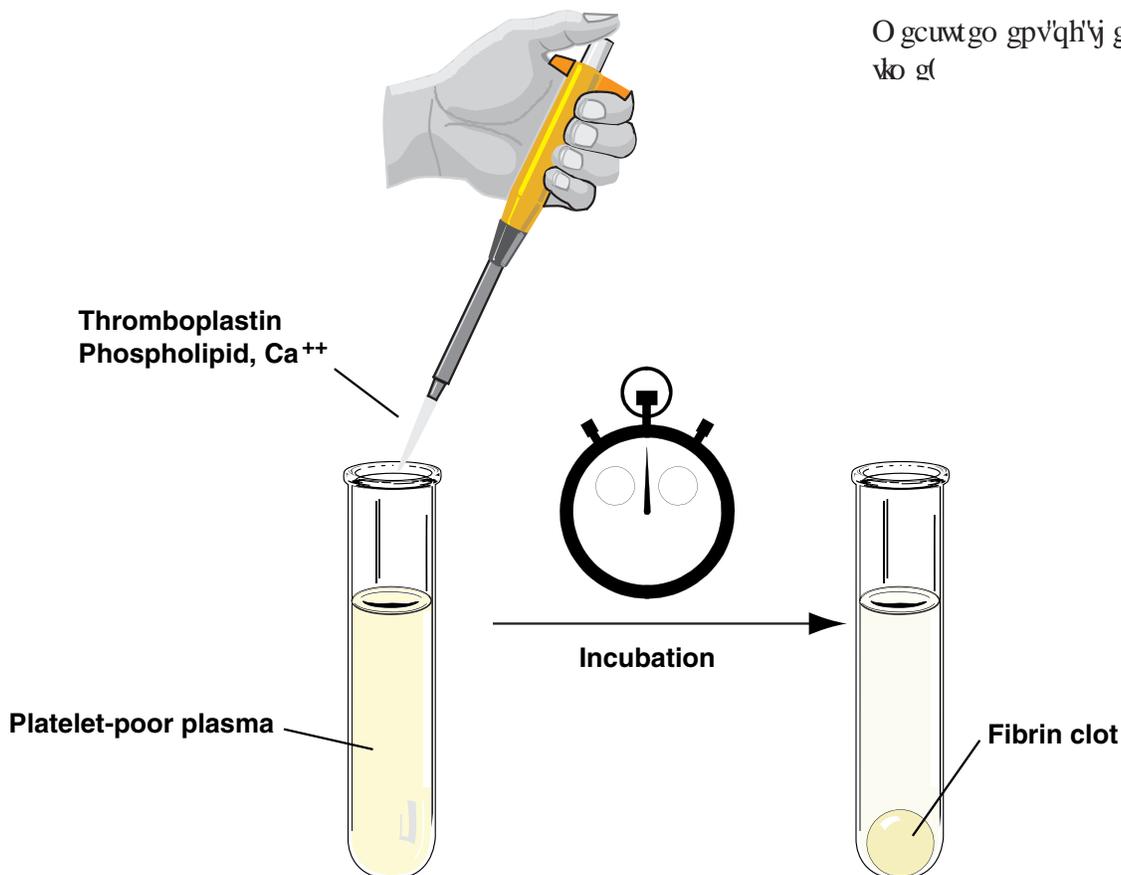
**Interferences:** Lipemia and hyperbilirubinemia interfere with the detection of clot formation by photo-optical methods. The results of the PT may be affected by a wide variety of factors, including the manner of blood coagulation, the type of container, the type of anticoagulant, specimen transport and storage conditions, incubation time and temperature, assay reagents, and the method of end point detection.

Many physiologic factors can influence the PT as well. The PT is prolonged in cord blood and newborns due to relatively low levels of vitamin K-dependent clotting factors, which may not increase to the normal adult range until several weeks after birth. Lipemia, hyperbilirubinemia, and hemolysis interfere with the detection of clot formation by photo-optical methods and cause falsely elevated values. Heparin at therapeutic doses usually does not interfere with the PT, but PT prolongation can result in patients receiving higher doses of Heparin. In fact, due to variability in the sensitivities of different thromboplastins to heparin, a falsely prolonged PT can occur during initiation of warfarin treatment when the patient is simultaneously receiving heparin therapy.

## Clinical Significance of the PT

The PT and aPTT are the fundamental assays of the coagulation system. The principal clinical uses of the aPTT include: (1) the detection of hereditary or acquired deficiencies or defects of the intrinsic coagulation factors (Factors XII, XI, IX, VIII, prekallikrein, high molecular weight kininogen), (2) monitoring heparin anticoagulant therapy, (3) the detection of coagulation inhibitors (i.e., lupus anticoagulant), and (4) to monitor coagulation factor replacement therapy.

The aPTT is increased with hereditary or acquired intrinsic factor deficiencies < 40% (Factor VIII:C, Factor IX, Factor XI, Factor XII, vWf), lupus anticoagulants, or specific inhibitors of the intrinsic coagulation factors. Other causes of an elevated aPTT include liver disease, DIC, anticoagulant therapy, a traumatic phlebotomy, and improper specimen collection.



# Understanding the INR

The International Normalized Ratio (INR) was introduced by the World Health Organization (WHO) in the early 1980's as a means of standardizing PT results. Since there is a linear relationship between the logarithms of the PT ratios obtained with different extracts of human and rabbit brain, a calibration system was developed to relate any PT ratio to a WHO standard. For this purpose, a very responsive batch of human brain extract was designated as the first International Reference Preparation (IRP), and a correction factor (International Sensitivity Index, ISI) was developed to correlate the sensitivity of commercial thromboplastin preparations to the IRP. By definition, the ISI of the first IRP was 1.0. An additional term, the INR, was introduced to compare a given prothrombin ratio measurement to the IRP. Thus, the INR represents the prothrombin time which would have been obtained if the IRP had been used as a reagent in the test. "Responsive" thromboplastins have lower ISIs and produce longer prothrombin times than "unresponsive" thromboplastins. A low ISI is a desirable reagent property. When a reagent with a low ISI is used, the analytical precision of the prothrombin time is increased, there is better discrimination of normal and warfarin-treated patients, and a wider therapeutic range exists, enabling clinicians to make fine adjustments in anticoagulation dosage. Recent studies have shown that the ISIs of thromboplastins used in the United States vary from 0.8 to 2.24.

Commercial suppliers of thromboplastin preparations supply the ISI with each reagent lot. If the ISI is known, the INR is easily calculated by the following formula:

$$\text{INR} = \left( \frac{\text{Patient PT}}{\text{Mean Normal PT}} \right)^{\text{ISI}} = (\text{Prothrombin Ratio})^{\text{ISI}}$$

where the mean (geometric mean) normal PT is determined from at least 20 fresh plasma specimens obtained from healthy individuals.

## References

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