PROTHROMBIN TIME

(ISI 1.5) & (ISI 1.00)

INTENDED USE

Vitro Thromboplastin Reagent is intended for the performance of the one stage prothrombin time test and assays for Factors II, V, VII and X.

METHOD

Vitro reagent. Single. Liquid, ready for use reagent.

CLINICAL SIGNIFICANCE

The arrest of bleeding depends upon primary platelet plug formed along with the formation of a stable fibrin clot. Formation of this clot involves the sequential interaction of a series of plasma proteins in a highly ordered and complex manner and also the interaction of these complexes with blood platelets and materials released from the tissues.

Tissue Thromboplastin, in the presence of calcium, is an activator, which initiates the extrinsic pathway of coagulation, which includes plasma coagulation factors VII, X, V, Prothrombin and Fibrinogen. During oral anticoagulant therapy most of these factors are depressed, as also during the deficiencies of clotting factor activity which may be hereditary or acquired.

Prothrombin Time determination is the preferred method for presurgical screening, determination of congenital deficiency of factors II, V, VII and X and for monitoring of anticoagulant therapy and as a liver function test.

The first standardized one-stage prothrombin time test was developed by Dr. Armand Quick in 1935. It has become the basic coagulation screening test for the diagnosis of congenital and acquired deficiencies of the extrinsic pathway involving Factors I, II, V, VII and X.1, 2 Oral anticoagulants such as Coumarin and Dicumarol interfere with the liver's production of the vitamin K dependent clotting factors II, VII, IX and X. Therefore, the prothrombin time test is used to monitor oral anticoagulant therapy since it measures three of the four factors involved.

ASSAY PRINCIPLE

Tissue thromboplastin, in the presence of calcium ions, is an activator which initiates the extrinsic pathway of coagulation. When a mixture of tissue thromboplastin and calcium ions is added to normal anticoagulated plasma, the clotting mechanism is initiated leading to formation of a fibrin clot. If a deficiency exists within the extrinsic pathway, the time required for clot formation will be prolonged depending on the severity of the deficiency.

REAGENTS

VITROPLASTIN is a liquid ready to use Calcium Thromboplastin Reagent, which is derived from rabbit brain.

Each batch of reagents undergoes rigorous quality control at various stages of manufacture for its sensitivity and performance.

- **REAGENT STORAGE AND STABILITY**

1. Store the reagent at 2-8°C. **DO NOT FREEZE.**
2. The shelf life of the reagent is as per the expiry date mentioned on the reagent vial label. The uncontaminated reagent is stable for:

18 months at 2 – 8 °C,
1 week at 18 - 25 °C,
2 days at 37 °C

SPECIMEN

Plasma obtained from whole blood collected with 3.2% or 3.8% sodium citrate as an anticoagulant is the specimen of choice.

Blood may be collected with evacuated test tubes, a 2-syringe technique, or with a butterfly and syringe technique. Accurate coagulation studies depend on the correct whole blood to anticoagulant ratio. According to NCCLS guidelines, blood specimens with hematocrits (HCT) of < 55% should be obtained by adding 9 parts of freshly collected whole blood to one part anticoagulant.7 For blood specimens with hematocrits > 55%, adjust the amount of whole blood added to the anticoagulant according to the following formula.

$$\text{Parts whole blood to one part anticoagulant} = \frac{0.6}{(1 - \text{HCT}) \times 9}$$

Particular care should be taken when using evacuated test tubes. These tubes are designed to draw 9 parts blood to 1 part anticoagulant. If the hematocrit is determined to be abnormal, blood should be drawn into a syringe and an appropriate amount mixed with an adjusted volume of citrate anticoagulant.

Though no special preparation of the patient is required prior to sample collection by approved techniques, it is preferable that patients are not heavily exercised before blood collection.

Fasting or only light non-fatty meals prior to blood collection provide samples with a desirable opacity.

Withdraw blood without undue venous stasis and without frothing into a plastic syringe fitted with a short needle of 19 to 20 SWG. The vein puncture must be a 'clean' one and, if there is any difficulty, take a new syringe and needle and try another vein. Transfer the blood into anticoagulated tubes, after detaching the needle from the syringe. Do not delay mixing blood with anticoagulant. Avoid foam formation during mixing.

Centrifuge immediately for 15 minutes at 1500-3000 rpm (approximately 1500 g) on a laboratory centrifuge and transfer the plasma into a clean test tube. It should be ensured that the plasma is free from platelets (PFP). Cap the test tubes to prevent deterioration of the samples. Plasma must be tested preferably immediately. However if the specimen are held at 22-24°C then they may be tested within 2 hours and if the specimen is held at 2-4°C then they may tested within 3 hours.

PROCEDURE

Manual Method

1. Aspirate from the reagent vial enough reagent for immediate testing requirements in a thoroughly clean and dry test tubes (Plastic tubes are preferred).
2. Bring this reagent to room temperature before pre-warming at 37°C for testing purposes.
3. To a 12 x 75 mm tube add 0.1 ml of plasma (PPP) and place the tube in a water bath for 3 to 5 minutes at 37°C.
4. To the tube **forcibly** add 0.2 ml of VITROPLASTIN reagent (pre-warmed at 37°C for at least 3 minutes) and simultaneously start a stop watch. Shake tube gently to mix contents.
5. Gently tilt the tube back and forth and stop the stopwatch **as soon as the first fibrin strand is visible and the gel/clot formation begins.** Record the time in 'seconds'.
6. Repeat steps 4-6 for a duplicate test on the same samples.
7. Recap the reagent vial and replace immediately to 2-8°C.
8. Find the average of the duplicate test values. This is the Prothrombin Time (PT).

If a coagulation instrument is being used to perform the tests, the instrument manufacturer's instructions must be strictly adhered to.

CALCULATION

Manual Method

The result may be reported directly in terms of the mean of the double determination of PT of the test plasma in 'seconds'.

Or as a ratio 'R':

$$R = \frac{\text{Mean of the patient plasma PT in seconds}}{\text{MNPT for the reagent*}}$$

Or as International Normalized Ratio (INR), $INR = (R)^{ISI}$, where ISI = International Sensitivity Index of the reagent (Refer reagent vial label).

*It is recommended by WHO that MNPT (mean normal PT) should be established for each lot of PT reagents by each laboratory, since PT results are dependent on the combination of reagent lot, instrument and technique followed at each laboratory. Usually plasma from at least 20 normal healthy individuals should be used to establish the MNPT. The average of such PT results in seconds = MNPT.

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EXPECTED VALUES

Normal values using VITROPLASTIN are between 10-14 seconds. Between manual and Turbo densitometric instrument results a variation of 1-2 seconds may be expected. For photo optical instruments, it is recommended that each laboratory must establish their own normal range. It is mandatory that each laboratory must establish its own MNPT for each lot of VITROPLASTIN.

Oral Anticoagulant Therapeutic Range: INR = 2.0 - 3.5.

The International Normalized Ratio, or INR, was developed to standardize PT values, so that test results from different thromboplastins and coagulation analyzers become equivalent. Under the INR system, a thromboplastin is assigned an International Sensitivity Index (ISI) value. The ISI indicates the relative sensitivity of the thromboplastin compared to an international reference thromboplastin. If a thromboplastin has the same sensitivity as the reference thromboplastin, then its ISI is 1.0. A higher ISI value indicates that a thromboplastin is less sensitive than the reference thromboplastin. The ISI is used in the following formula to calculate an INR value from a PT value.

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

The ISI is usually determined by the thromboplastin manufacturer. Different ISI values are assigned for different models or classes of coagulation analyzers. The mean normal PT is determined in each laboratory by averaging the PT values from at least 20 healthy individuals.

- Alternatively, the INR value can be read off directly from Vitroplastin INR conversion table.

PERFORMANCE

Precision:

	Within Run		Between Day	
Control	Level I	Level II	Level I	Level II
Number of samples	20	20	20	20
Mean %	89	36	89	35
SD %	1.3	0.57	1.66	1.47
CV (%)	1.46	1.57	1.86	4.17

Comparison with commercially available reagent (same method): 170 plasmas located between 14% and 110% were tested

y = 1,0287 x + 0,1601 r= 0,9863

INTERFERING SUBSTANCES

Turbidity No interference up to 0,390 abs
Low Molecular weight heparin Positive interference from 0.11 IU anti Xa
Bilirubin Positive interference from 171 µmol/L
Hemoglobin No interference up to 258 µmol/L

WARNINGS & PRECAUTIONS

It is recommended that controls with known factor activity should be run simultaneously with each test series to validate test run.

1. Incorrect mixture of blood and tri-sodium citrate, insufficient pre-warming of plasma and reagent, contaminated reagents, glassware etc. are potential source of errors.
2. Oxalated plasma may induce prolonged clotting times.
3. Since the PT test functions correctly only at 37+ 0.5°C, temperature of all equipment must be calibrated daily.
4. Clotting time of patients on anticoagulant therapy depends upon the type and dosage of anticoagulant and also the time lag between the specimen collected and the last dose.
5. Turbid, icteric, lipemic or grossly hemolysed samples may generate erroneous PT results.
6. Glass wares and cuvettes used in the test must be scrupulously clean and free from even traces of if acids / alkalies or detergents.
7. Plasma samples held at 4-8°C may undergo 'cold activation' leading to a marked shortening of the PT.
8. The PT may be shortened during acute inflammatory conditions which are accompanied by increase in Fibrinogen levels and also by agents such as *antihistamines*, butabarbital, phenobarbital, caffeine, oral contraceptives and vitamin K. The PT may be prolonged by corticosteroids, EDTA, asparaginase, clofibrate, ethanol, tetracycline, aspirin, and anticoagulants such as heparin and warfarin.
9. It is important that each laboratory express the results in terms of INR for patients on oral anticoagulant therapy for the clinician to
10. adjust the dosage based on INR.
11. Since the test uses platelet poor plasma, each laboratory must calibrate the necessary force and time required during centrifugation to yield the PPP. Contamination of plasma with excess platelets could falsely elevate levels of some of the factors.
12. Homogenization of VITROPLASTIN reagent suspension before use is important to achieve accurate and consistent results.

BIBLIOGRAPHY

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3. CRC Handbook Series in Clinical Laboratory, Science, Section 1: Haematology, Volume III, 1980.
4. E.A. Loeliger, A.M.H.P Van den besselaar and S.M. Lewis, Reliability and Clinical Impact of Normalization of Prothrombin Times in Oral Anticoagulant Control - F.K. Schattauer verlag Gmbh (I985).
5. Hull R., Hirsh H., Jay R., et al., Different intensities of oral anticoagulant therapy in the treatment of proximal-vein thrombosis. N. Engl. J. Med. 1982; 307: 1676-81.
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SYMBOL DECLARATION

	Manufacturer
	Consult instructions for use
LOT	Batch code (Lot #)
REF	Catalog number
	Temperature limitation
IVD	In vitro diagnostic medical device
	Use by
	Caution. Consult instructions
	Keep away from light

ORDERING INFORMATION

PT ISI 1.50 REF	SIZE	PT ISI 1.00 REF
30101	8 x 2 ml	30201
30102	1 X 8 ml	30202
30103	2 x 8 ml	30203
30104	6 X 8 ml	30204
30105	10 X 8 ml	30205
30106	4 x 5 ml	30206

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