SENSITIVE THROMBOPLASTIN REAGENT FOR PROTHROMBIN TIME (PT) DETERMINATION

**SUMMARY**

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 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 the Vitamin K dependent factors such as II, VII, IX, X, Protein C and Protein S 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, as a liver function test, determination of congenital deficiency of factors II, V, VII and X and for monitoring of patients on oral anticoagulant therapy.

**PRESENTATION**

*10621025 10620005 10620125

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UNIPLASTIN  5 ml 5 ml 12 X 5 ml

3.2% Tri-Sodium Citrate 12. 5 ml - -

INR conversion table 1 1 1

Pack insert 1 1 1

* REF 10621025 represents Precision combi pack which contains Uniplastin reagent along with 3.2% Tri-Sodium Citrate solution used for blood collection.

**REAGENT**

®

UNIPLASTIN  is a novel, highly sensitive, low opacity, ready to use liquid Calcified Thromboplastin Reagent, which is derived from rabbit brain.

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

**REAGENT STORAGE AND STABILITY**

(a) Store the reagent at 2-8°C. DO NOT FREEZE.

(b) The shelf life of the reagent is as per the expiry date mentioned on the reagent vial label. The uncontaminated reagent is stable as per the labeled shelf life at 2-8°C, 1 week at 18-25°C, 2 days at 37°C.

**PRINCIPLE**

Tissue Thromboplastin in the presence of calcium activates the extrinsic pathway of human blood coagulation mechanism.

When UNIPLASTIN® reagent is added to normal citrated plasma, the clotting mechanism is initiated, forming a solid gel clot within a specified period of time. The time required for clot formation would be prolonged if there is acquired or congenital deficiency of factors / factor activity in the extrinsic pathway of the coagulation mechanism or reduction in the activity of Vitamin K dependent clotting factors during oral anticoagulant therapy.

**NOTE**

(1) In vitro diagnostic reagent for laboratory and professional use only. Not for medicinal use. (2) UNIPLASTIN® reagent is not from human source hence contamination due to HBsAg and HIV is practically excluded. (3) Reagent contains 0.01% Thimerosal as preservative. (4) It is very important that scrupulously clean and dry micropipette tips be used to aspirate / dispense the reagent. (5) Avoid exposure of the UNIPLASTIN® reagent to elevated temperatures, contamination and undue stress due to high and low temperature exposure cycles. Immediately replace reagent cap after use and store at recommended temperatures only. (6) On prolonged storage at 2-8°C the thromboplastin suspension has a tendency to settle down. Homogenise the reagent by resuspending before use. (7) Do not use damaged or leaking reagents.

**ADDITIONAL MATERIAL REQUIRED**

12 x 75 mm test tubes (plastic tubes are preferred), 0.1 ml and 0.2 ml pipettes, Stop watch, Water bath or heating block at 37°C, Fresh normal plasmas for establishing MNPT.

**SAMPLE COLLECTION AND PREPARATION OF PPP**

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 lower opacity.
Withdraw blood without undue venous stasis or frothing into a plastic syringe fitted with a short needle of 19 to 20 SWG. The veinpuncture must be at 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. Mix exactly nine parts of freshly collected blood with one part of tri-sodium citrate (0.11 mol/l, 3.2%) or PROFACT, available from TULIP CAT. No. 10060020. For occasional patients with haematocrit less than 20% or greater than 55%, this ratio must be readjusted to ensure valid results. Centrifuge immediately for 15 minutes at 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 (PPPs). Cap the test tubes to prevent deterioration of 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 be tested within 3 hours.

**TEST PROCEDURE**

**Manual Method**

1. Bring the reagent vial to room temperature (20-30°C). Mix the contents of the vial to homogenise the suspension completely.
2. Aspirate from the reagent vial enough reagent for immediate testing requirements in a thoroughly clean and dry test tube. (Plastic test tubes are preferred).
3. Prewarm the reagent and bring to 37°C before use in test procedure (5-10 minutes may be required depending on the reagent volume to attain 37°C before testing).
4. Recap the reagent vial and replace immediately to 2-8°C.
5. To a 12 x 75 mm tube add 0.1 ml of plasma (PPP) and place the tube in a waterbath for 3 to 5 minutes at 37°C.
6. To the tube forcibly add 0.2 ml of UNIPLASTIN® reagent (preamered at 37°C for at least 3 minutes) and simultaneously start a stopwatch. Shake the tube gently to mix contents.
7. Gently lift 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'.
8. Repeat steps 4-6 for a duplicate test on the same sample.
9. Determine the mean of the duplicate test values. This is the Prothrombin Time (PT).

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

**CALCULATION OF RESULTS**

**Manual Method**

The results 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 $R^\circ = \frac{\text{Mean of the patient plasma PT in seconds}}{\text{MNPT for the reagent}}$.

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

It is recommended by the WHO that MNPT 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.

**EXPECTED VALUES**

Normal values using UNIPLASTIN® are between 11-15 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 sensitivity to individual factors using UNIPLASTIN® reagent suspension before use is important to achieve accurate and consistent results.

**WARRANTY**

The above values should only be used as guidelines. Each laboratory should establish sensitivity to individual factors using instruments, reagents and techniques used in their laboratory.

**BIBLIOGRAPHY**