SPECTRUM

The Creative Approach to Bioscience

SP-Ultraplastin PT Reagent ISI 1.05

REF: 622 001 (8 x 2 ml) 80 test
REF: 622 002 (8 x 6 ml) 240 test

SYMBOLS IN PRODUCT LABELLING

EC REP	Authorised Representative		Use by/Expiration Date
IVD	For in-vitro diagnostic use		CAUTION. Consult instructions for use
LOT	Batch Code/Lot number		
REF	Catalogue Number		Manufactured by
	Consult instructions for use		
	Temperature Limitation		

Intended Use

Spectrum Diagnostics **SP-ULTRAPLASTIN** is a sensitive thromboplastin reagent intended for prothrombin time (PT) determination.

Background

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 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 congential deficiency of factors II, VII, X, V and for monitoring of patients on oral anticoagulant therapy and as a liver function test.

Assay Principle

Tissue thromboplastin in the presence of calcium activates the extrinsic pathway of human blood coagulantion mechanism. When **SP-ULTRAPLASTIN** reagent is added to normal antigoagulated 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 a deficiency of factors / factor activity in the extrinsic pathway of the coagulation mechanism.

Reagent

SP-ULTRAPLASTIN is a liquid ready to use Calcium Thromboplastin reagent,which is derived from rabbit brain. Each batch of reagents undergoes vigorous quality control at various stages of manufacture for its sensitivity and performance.

Reagent Storage and Stability

Store the reagent at 2 – 8°C (Do not freeze) . the shelf life of reagent is mentioned on vial label. The uncontaminated reagent is stable for : 2 years at 2 – 8°C, 1 week at 18 – 25°C, 2 days at 37°C.

Specimen 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. The venipuncture must be a "clean" one and, if there is any difficulty, take a new syringe and needle and try anther vein. Trasfer 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%). For occasional patients with hematocrit less than 20% or greater than 55%, this ratio must be readjusted to ensure valid result. Centrifuge immediately for 15 minutes at 1500 – 3000 rpm(approximately 1500g) on laboratory centrifuge and transfer the plasma into a clean test tube. It should be ensured that the plasma is free from platelets (PPP). Cap the test tubes to prevent deterioration of samples. Plasma must be tested peferably immediately. However if the specimens are held at 2 - 4 °C then they may be 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 tube (Plastic test tubes are preferred).
2- Bring this reagent to room temperature before prewarming at 37 °C for testing purpose.
3- Recap the reagent vial and replace immediately to 2 – 8°C.
4- To a 12x75 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.
5- To the tube forcibly add 0.2 ml of **SP-ULTRAPLASTIN** reagent (prewarmed at 37 °C for at least 3 minutes) and simultaneously start a stop watch. Shake the tube gently to mix contents.
6- 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.
7- Repeat steps 4-6 for a duplicate test on the same samples.
8- Find the avarage of the duplicate test values. This is the Prothrombin Time (PT).

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

Calculation of Results

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 to 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 avarage of such PT results in seconds=MNPT.

Expected Values

•**Normal values** using **SP-ULTRAPLASTIN** 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 establesh its own normal range. It is mandatory that each laboratory must establish its own MNPT for each lot of **SP-ULTRAPLASTIN**.

•**Oral anticoagulant therapeutic range : INR = 2.0 – 3.5**

The use of INR's enables direct comparison to be made between all results on patient plasmas regardless of interlab variations or reagent in question.

The INR is calculated as INR = (R) ^{ISI}
Where ISI = Lot specific ISI for the reagent

$$\text{And, } R = \frac{\text{Patient PT}}{\text{Mean normal PT}}$$

Mean normal PT = Mean of the normal range that is specifically determined by each user laboratory for each lot of thromboplastin reagent with specific instrument and techniques routinely used for patient testing.

Example:

Patient PT result = 21 seconds
MNPT = 13.5 seconds.
ISI of reagent = 1.1

$$R = \frac{21.0}{13.5} = 1.5$$

$$INR = (1.5)^{1.1} = 1.56$$

Alternatively the INR value can be read off directly from the **SP-ULTRAPLASTIN** INR conversion table.

Remarks

(1) it is recommended that controls with known factor activity should be run simultaneosly with each test series to validate test run.
(2) incorrect mixture of blood and tri-sodium citrate, insufficient prewarming of plasma and reagent, contaminated reagents, glassware etc. are potential source of errors.
(3) Oxalated plasma may induce prolonged clotting times.
(4) Since the PT test functions correctly only at 37 ± 0.5 °C, tempreature of all equipment must be calibrated daily.
(5) 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.
(6) turbid, icteric, lipemic or grossly hemolysed samples may generate erroneous PT results.
(7) Glasswares and cuvettes used in the test must be scrupulously clean and free from even traces of acids / alkalies or detergents.
(8) plasma samples held at 4 – 8°C may undergo cold activation leading to a marked shortening of the PT.
(9) The PT may be shortened during acute inflammatory conditions which are accompanied by increase in fibrinogen levels and also by agent such as antihistamines, butabarbital, phenobarbital, caffeine, oral contraceptives and vitamin K. the PT may be prolonged by corticosteroides, EDTA, asparaginase, clofibrate, ethanol, tetracycline, aspirin and anticoagulants such as heparin and warfarin.
(10) It is important that each laboratory expresses the results in terms of INR for patients on oral anticoagulant therapy for the clinician to 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) Homogenisation of **SP-ULTRAPLASTIN** reagent suspension before use is important to achieve accurate and consistent results.

References

1.Biggs R. and R.G. McFarlane: Human Coagulation and its disorders, Blackwell Scientific Publications, Oxford, 1962.
2.Hirsh J., Dalen J.E., Deykin D., poller L.: Oral Anticoagulants : Mechanism of Action, Clinical Effectiveness and Optical Therapeutic Range, Chest : 1995 : 108 (suppl.) : 231S-246S.
3.W.H.O. Expert Committee on Biological standardization, No. 687, 1983.
4.Colman R., Hirsh J.: Haemostasis & Thrombosis, J.B. Lippincott Company, 3rd Ed., 1994.

ORDERING INFORMATION	
CATALOG NO.	**QUANTITY**
622 001	8 x 2 ml
622 002	8 x 6 ml

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