Intended Use
Spectrum Diagnostics SP-NORMOPLASTIN reagent is 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 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, VII, X, and 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 coagulation mechanism. When SP-NORMOPLASTIN reagent is added to normal anticoagulated 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-NORMOPLASTIN 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
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: 1 year 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 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 trisodium citrate (0.11 mol/L, 3.2%).

Procedure
Manual Method
1. Aspiration 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-NORMOPLASTIN reagent (pawarmed 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.
7. Repeat steps 4-6 for a duplicate test on the same samples.
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 manufacturers 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)\text{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 average of such PT results in seconds=MNPT.

Expected Values
Normal values using SP-NORMOPLASTIN 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 its own normal range. It is mandatory that each laboratory must establish its own MNPT for each lot of SP-NORMOPLASTIN.

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 plasma regardless of interlab variations or reagent in question.

\[ \text{ISI} = \frac{\text{Lot specific ISI for the reagent}}{\text{Mean normal PT}} \]

And, \[ R = \frac{\text{Patient PT}}{\text{ISI}} \]
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 = 1.5

\[
\frac{21.0}{13.5} = 1.5
\]

INR = \((1.5) \times 1.5 = 1.8\)

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

Remarks

1. It is recommended that controls with known factor activity should be run simultaneously 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, temperature of all equipments 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. Glassware and cuvettes used in the test must be scrupulously clean and free from even traces of acids / alkalis 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 corticosteroids, 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-NORMOPLASTIN reagent suspension before use is important to achieve accurate and consistent results.

References


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