BioClot-PT
Prothrombin Time (ISI~1.1)

PRODUCT CODE
CR001

INTENDED USE
BioClot-PT reagent intended for the determination of Prothrombin Time (PT) in liquid format

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 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.

PRINCIPLE
Tissue Thromboplastin in the presence of calcium activates the extrinsic pathway of human blood coagulation mechanism. When **BioClot-PT** 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.

REAGENT
BioClot-PT reagent is ready to use liquid Calcified Thromboplastin Reagent, which is derived from rabbit brain in addition with stabilizers and preservatives.

ADDITIONAL REQUIREMENTS
Test tubes (plastic tubes are preferred), 0.1 mL and 0.2 mL pipettes, water bath or heating block at 37ºC. Fresh normal plasmas for establishing MNPT.

STORAGE AND STABILITY
Store tightly closed at 2-8°C, ***Do Not Freeze***. The expiry of the reagent is as per the date mentioned on the label. The uncontaminated reagent is stable as per the labeled expiry date at 2-8ºC, 1 week at 18-25ºC, 2 days at 37ºC.

PRECAUTIONS
The reagent is for "in vitro" diagnostic use. Avoid exposure of the reagent to elevated temperature. Homogenize each time it is used. All patient samples should be handled as if they were capable of transmitting infection. All reagents and samples must be discarded according to the local regulations in force.

SPECIMEN AND SAMPLE PREPARATION
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 sodium citrate anticoagulant. Avoid foam formation during mixing.

Centrifuge immediately for 15 minutes at 1500 RPM on a 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 preferably immediately. However if the specimens are held at 2-4ºC then they may be tested within 3 hours.

PROCEDURE
Manual Method
1. Bring the reagent vial to room temperature (20-30°C). Gently mix the contents of the vial to homogenize the suspension completely.
2. Aspirate from the reagent vial enough PT Reagent for immediate use in a thoroughly clean and dry test tube. (Plastic test tubes are preferred).
3. Recap the reagent vial and replace immediately to 2-8°C.
4. Prewarm the dispensed PT Reagent 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).
5. To Plastic tube add 100µL of plasma (PPP) and place the tube in a water bath for 3 to 5 minutes at 37°C.
6. To the tube forcibly add 200µL of **PT (ISI$^{-1.1}$)** reagent (prewarmed at 37°C for at least 3 minutes) and simultaneously start a stopwatch. Shake the tube gently to mix contents.
7. 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'.
8. Repeat steps 5-7 for a duplicate test on the same sample.
9. 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 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 Prothrombin Ratio 'R'

$$R = \frac{\textbf{Mean PT of the patient plasma in seconds}}{\textbf{MNPT (Mean Normal PT) for the reagent}}$$

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 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.

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EXPECTED VALUES

Normal values using **BioClot PT (ISI~1.1)** 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 their own normal range. It is mandatory that each laboratory must establish its own MNPT for each lot of PT ($ISI^{-1.1}$).

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 $INR = (R)^{ISI}$, Lot specific ISI for the reagent.

Prothrombin Ratio 'R'

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

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.

Alternatively the INR value can be read off directly from **BioClot** PT 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 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 hemolyzed 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/ 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 agents such as antihistamines, butabarbital, phenobarbital, caffeine, oral contraceptives and vitamin K. The PT may be prolonged by corticosteroids, EDTA, asparaginase, clofibrate, erythromycin, ethanol, tetracycline, aspirin and anticoagulants such as heparin and warfarin.
10. It is important that each laboratory express 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. Homogenization of **BioClot PT (ISI$^{-1.1}$)** reagent suspension before use is important to achieve accurate and consistent results.

PERFORMANCE CHARACTERISTICS

Precision

The Precision of Prothrombin time determination is highly dependent on the method used. Precision studies were performed on coagulometer by assaying normal and abnormal control plasmas with **PT (ISI$^{-1.1}$)**. One normal control plasma and one abnormal control plasma in replicates of 10 were used to determine inter assay and intra-assay precision of the clotting times (seconds).

Inter-assay n=10	Mean	SD	% CV
Normal control plasma	13.2	0.01	0.07
Abnormal control plasma	32.0	0.38	1.27

Intra-assay n=10	Mean	SD	% CV
Normal control plasma	13.3	0.1	0.08
Abnormal control plasma	32.1	0.06	0.19

SENSITIVITY

Activity Factor %	Clotting time with **PT (ISI$^{-1.1}$)** with factor deficient plasmas (seconds)			
	Factor II	Factor V	Factor VII	Factor X
100	20.3	24.7	25.9	21.9
50	23.2	32.0	33.9	25.9
25	27.5	38.2	47.1	32.1
12.5	31.6	42.0	68.6	38.3
6.25	35.6	48.0	113.0	45.3
3.12	37.0	51.8	220.0	52.1

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

SYMBOL ON LABELS

Symbol	Signify	Symbol	Signify
REF	Catalogue Number	SIZE	Pack Size
	Expiry Date	VOL	Volume
	Storage Condition	LOT	Lot Number
	Instruction for Use	IVD	In Vitro Diagnostics
	Manufacturing Date		Manufacturer
	Number of Tests	2	For Single Use Only
EC REP	EC Representative		

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