MediRox

CE

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MRX Thrombin time Art. No: MRX941

INTENDED USE
For the quantitative determination of thrombin time (TT) in human citrated plasma on mechanical and optical instruments.
FOR IN VITRO DIAGNOSTIC USE

SUMMARY AND PRICIPLE
Thrombin time (TT) is a common clotting test used for quantitative measurements of fibrinogen pathologies or for detecting the effects of heparin and/or thrombin inhibitors in human citrated plasma. Several factors will result in a prolongation of the time to form a clot, including low fibrinogen concentrations (hypofibrinogenemia) and malfunction of fibrinogen (hereditary dysfibrinogenemia). Thrombin time is influenced by both direct and indirect fibrinogen inhibitors; heparin, hirudin, fibrinogen degradation products, dabigatran (Pradaxa®) etc.

Plasma fibrinogen is cleaved by thrombin (factor IIa) to form fibrin, which polymerizes to form a fibrin clot[3]. Fibrinogen present in the sample is converted to fibrin by the addition of purified bovine thrombin and the time (measured in seconds) to form a clot is reported as the thrombin time.

PRODUCT DESCRIPTION
The thrombin time kit consists of: 10 x 5 mL or 10 x 2 mL vials of lyophilized bovine thrombin (2.4 or 6.0 UNIH/mL) with bovine albumin, buffer and preservative.

PRECAUTIONS:
The test reagents contain bovine thrombin. All donor animals were sourced from BSE-free herds. The cattle received ante- and post mortem health inspection by a veterinarian, and were tested free from infectious- and contagious agents. As a matter of caution, the material should, however, be treated as potentially infectious.

PREPARATION
For normal thrombin time testing:
reconstitute the content of each vial with 5 mL of distilled water.
For heparin measurement:
reconstitute the content of each vial with 2 mL of distilled water.
Swirl gently. Keep the reagent at 15-25 °C for 30 minutes and invert to mix before use. Do not shake.

STORAGE CONDITIONS AND STABILITY
Unopened reagents are stable until the expiration date shown on the vial when stored at 2-8 °C.
Stability after reconstitution, in sealed original vial: 8 days at 2-8 °C.

SPECIMEN COLLECTION AND STORAGE
It is recommended that specimen collection, handling and storage be carried out in accordance with CLSI guideline H21-A5 Vol. 28 No.5 [1]. Venous blood is collected in 3.2% sodium citrate at a ratio of 9 parts blood to 1 part anticoagulant (1:10 ratio). The ratio is critical. If using commercial vacuum tubes, a full draw must be assured. Trauma or stasis during drawing should be avoided. The presence of a clot in a specimen is cause for rejection.

INSTRUMENT/TEST PROCEDURE
Procedure (be sure final volume is within instrument specification)

PARAMETER SET UP:
Typical settings for manual analyse.
All test tubes and pipette tips should be in plastic or in siliconized glass.
To an instrument cuvette or test tube:

- Add 100 µL undiluted control or patient plasma
- Incubate for 2 minutes at 37 °C
- Add 100 µL MRX Thrombin time reagent to plasma
- Start stopwatch
- Record the clotting time in seconds

Reagents and equipment required but not provided:

1. Distilled water for reconstitution
2. Stopwatch or timing device
3. Reagent cups or plastic test tubes
4. Coagulation analyser or 37 °C water bath
5. Variable volume pipettes
6. Control Plasma

Each laboratory should optimize their own parameter set up on every individual optical/mechanical instrument.

QUALITY CONTROL
Medirox recommends the use of normal control plasma (GHI162, GHI163, GHI164, MRX171 or MRX181) for reliable quality control of the performance and at a frequency in accordance with good laboratory practise. Each laboratory is expected to establish its own quality control program to evaluate its measurement methods including the current reagent.

RESULTS
Patient results are reported in seconds.

LIMITATIONS/INTERFERING SUBSTANCES
TT results may be affected (results in prolonged clotting time) by many commonly administered drugs. For example heparin, and direct thrombin inhibitors (DTI) such as hirudin and dabigatran, and other factor II inhibitor drugs. TT is also affected by haemolysis and high bilirubin concentration.

TT results are not affected by lipids up to:

MRX941	Lipids (g/L)
2 mL	0.625
5 mL	3.75

Haemolysed or Icteric plasma should not be assayed.

EXPECTED VALUES
A reference range study was performed on ACL Top instrument using 87 plasma samples from healthy subjects, approximately equal numbers of males and females.

MRX941	n	Mean	SD	Mean±1SD
2 mL	87	7.8 s	0.52	7.3 - 8.3 s
5 mL	87	17.1 s	1.66	15.4 - 18.8 s

Each laboratory is expected to establish its own local reference range for the thrombin time measurements.

PRECISION
Within run and total (run to run and day to day) precision was assessed over multiple runs using normal plasma. Seven runs with six replicates of each sample were performed on ACL Top.

MRX941	Mean	CV% within run	CV% between run	CV% Total
2 mL	8.6 s	1.7	1.2	2.1
5 mL	15.1 s	0.9	1.0	1.3

COMPARISON
Correlation study was made between MRX941 and HemosIL TT. Samples from 52 donors were measured. Samples were run in parallel.

Assay	slope	intercept	r	reference method
MRX TT, 2 mL	0.882	0.336	0.939	HemosIL TT, 2 mL
MRX TT, 5 mL	0.927	0.239	0.963	HemosIL TT, 5 mL

HEPARIN
Heparin test was performed using normal plasma spiked with un-fractionated heparin. Measurements were performed using an ACL Top instrument and the results show that MRX941, 2 mL is sensitive for heparin in the range 0.03-0.25 IU/mL [2].

Each laboratory is expected to establish its own local heparin therapeutic range.

REFERENCES.

1. Clinical and Laboratory Standards Institute. Collection, Transport, and Processing of Blood Specimens for testing Plasma-Based Coagulation and Molecular Hemostasis. Assays; Approved Guideline - Fifith Editon, CLSI Document H21-A5; Vol. 28 No.5.
2. Ray MJ, Perrin EJ, Smith IR, et al, "A Proposed Model to Monitor Heparin Therapy Using the Concentrated Thrombin Time Which Allows Standardization of Reagents and Improved Estimation of Heparin Concentrations,"Blood Coagul Fibrinolysis, 1996, 7(5):515-21.
3. M.M. Flanders, R. Crist, G.M. Rodgers. Comparison of Five Thrombin Time Reagents. Clinical Chemistry 49, No. 1 2003; 169-172