HEMOCLOT™ Thrombin Time LRT

REF CK012K

R 6 x 5 mL

CE

IVD

Clotting method for the determination of Thrombin Time with ready to use liquid reagent.

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INTENDED USE:

HEMOCLOT™ Thrombin Time LRT kit is a clotting method for the *in vitro* quantitative determination of Thrombin Time on human citrated plasma, using manual or automated method.
Reagents are in the liquid presentation, ready to use (LRT, Liquid reagent Technology).

SUMMARY AND EXPLANATION:

Technical:
The Thrombin Time is a coagulation assay measuring the time to convert fibrinogen to fibrin.
Clinical:[1]
The Thrombin Time is a screening test to assess abnormalities of fibrinogen and to detect inhibitors against thrombin or fibrin. It can be useful for the evaluation of Disseminated Intravascular Coagulation (DIC) and liver disease.
A prolonged Thrombin Time can result from:

- Presence of antithrombin activity induced by therapy (eg Heparin, hirudin, argatroban, dabigatran).
- Presence of high concentrations of Fibrin/Fibrinogen degradation products.
- Qualitative (dysfibrinogenemia) or quantitative abnormalities of Fibrinogen (deficiency, DIC, fibrinolysis, hepatic disorders including cirrhosis).

The Thrombin Time is normal in presence of a Factor XIII deficiency.

PRINCIPLE:

The HEMOCLOT™ Thrombin Time LRT kit is a reagent for Thrombin Time (TT). It measures the clotting time (CT) induced by a controlled and constant amount of bovine thrombin, in presence of calcium, on citrated plasma. The time required for the formation of a stable clot is measured in seconds.

REAGENTS:

R **Bovine Thrombin**, and Calcium, liquid form. Contains BSA, preservatives and stabilizers.
6 vials of 5 mL.

The bovine Thrombin concentration (about 1.0 NIH/mL) can vary from lot to lot and is adjusted for each lot in order to offer a high sensitivity of Thrombin Time assay to low concentrations of Unfractionated Heparin (UFH) and Low Molecular Weight Heparin (LMWH).

WARNINGS AND PRECAUTIONS:

- Some reagents provided in these kits contain materials of animal origin. Users of reagents of these types must exercise extreme care in full compliance with safety precautions in the manipulation of these biological materials as if they were infectious.
- Waste should be disposed of in accordance with applicable local regulations.
- Use only the reagents from the same batch of kits.
- Aging studies show that the reagents can be shipped at room temperature without degradation.
- This device of *in vitro* diagnostic use is intended for professional use in the laboratory.

REAGENT PREPARATION:

R Reagent is ready to use; homogenize and load it directly on the analyzer following application guide instruction.
For manual method, allow to stabilize for 30 minutes at room temperature (18-25°C), homogenize before use.

STORAGE AND STABILITY:

Unopened reagents should be stored at 2-8°C in their original packaging. Under these conditions, they can be used until the expiry date printed on the kit.

R Reagent stability after opening, free from any contamination or evaporation, and stored closed, is of:

- **4 weeks** at 2-8°C.
- **7 days** at room temperature (18-25°C).
- **Do not freeze**
- **Stability on board of the analyzer: see the specific application.**

REAGENTS AND MATERIALS REQUIRED BUT NOT PROVIDED:

Reagents:

- Distilled water.
- Specific controls such as:

Product name	Reference
BIOPHEN™ Normal Control Plasma	223201
EASYPLASMA™ Control Set	225601

Also refer to the specific application guide of the analyzer used.

Materials:

- Water-bath, semi-automatic or automatic instrument for clotting assays.
- Stopwatch; Calibrated pipettes; silicon glass or plastic test tubes.

SPECIMEN COLLECTION AND PREPARATION:

The blood (9 volumes) should be carefully collected onto the trisodium citrate anticoagulant (1 volume) (0.109 M, 3.2%) by clean venipuncture. Discard the first tube.
Specimens should be prepared and stored in accordance with applicable local guidelines (for the United States, see the CLSI H21-A5[2] guideline for further information concerning specimen collection, handling and storage).
For plasma storage, please refer to references[2,3].

PROCEDURE:

The kit can be used in manual or automated method. Perform the test at **37°C** and the clotting time, triggered by addition of thrombin, is measured.

For an automated method, application guides are available on request. See specific application guide and specific precautions for each analyzer.

Assay method:
1. Reconstitute, if necessary, the controls as indicated in the specific instructions.
2. Plasma should be tested **undiluted.**

3. Introduce into a reaction cuvette, silicon glass or plastic test tube incubated at **37°C:**

Reagents	Volume
Specimens and controls non-diluted	100 µL
Incubate at **37°C** for **1 minute**, then introduce (starting the stop-watch) :	
R **Bovine Thrombin**, preincubated at **37°C**	100 µL
Record the exact clotting time (CT, sec).	

If a reaction volume other than that specified above is required for the method used, the ratio of volumes must be strictly observed to guarantee assay performance. The user is responsible for validating any changes and their impact on all results.

QUALITY CONTROL:

The use of quality controls serves to validate method compliance, along with between-test assay homogeneity for a given batch of reagents.
Include the quality controls with each series, as per good laboratory practice, in order to validate the test. Each laboratory must define its acceptable ranges and verify the expected performance in its analytical system.

RESULTS:

- The obtained CT for the sample must be compared with that of the reference normal range for the laboratory (refer to current local recommendations).
- Results can be reported as a ratio:
  TT ratio = Sample (CT, sec) / Mean of normals (CT, sec).
- The results should be interpreted according to the patient's clinical and biological condition.

LIMITATIONS:

- To ensure optimum test performance and to meet the specifications, the technical instructions validated by HYPHEN BioMed should be followed carefully.
- Any reagent presenting an unusual appearance or showing signs of contamination must be rejected.
- Any suspicious samples or those showing signs of activation must be rejected.
- Various drugs or treatments can affect TT results. An additional investigation should be realized to determine the origin of each unexpected abnormal result.
- The obtained CT for a same sample and a same reagent lot can vary according to the instrument used and the clot detection mode.
  In the same way, many variables (eg: different sources of heparin) can affect the obtained results: each laboratory should consequently establish its own heparin therapeutic range.
- The assay is sensitive to low concentrations of heparin provided the tested plasma is collected without activation and release of platelet alpha granules, which contain PF4, a heparin inhibitor.[1,2]

EXPECTED VALUES:

Thrombin Time is usually expected < 25 sec.
As an example, for one lot, the mean value from healthy adults (n=120) on Sysmex CS-5100 was 18.0 seconds with SD = 1.0 second.
Each laboratory should determine its own usual ranges (normal range, heparin sensitivity…) for each combination of lot and instrument used.

PERFORMANCES:

- The reagent is sensitive to low concentrations of plasmatic heparin (≥ 0.10 IU/mL of UFH, > 0.20 IU/mL LMWH in plasma).
- Performance studies were conducted internally on Sysmex CS-5100. Performance was assessed using laboratory controls over a 20-day period, 2 series per day and 3 repetitions within each series for a control level. The following results were obtained:

Control	Intra assay				Inter assays			
	n	Mean	CV%	SD	n	Mean	CV%	SD
Control 1	40	22.7	0.9	0.2	120	22.8	1.4	0.3
Control 2	40	37.9	1.1	0.4	120	38.5	2.0	0.8

- Correlation with reference method (HEMOCLOT™ Thrombin Time vs HEMOCLOT™ Thrombin Time LRT on Sysmex CS-5100, on T.T. seconds) :
  n = 102 y = 1.026x – 0.986 r = 0.999

- Interferences:
  No interference, on the analyzer Sysmex CS-5100 was observed with the molecules and up to following concentrations:

Intralipids	Hemoglobin	Bilirubin (F/C)	Apixaban / Rivaroxaban / Edoxaban	D-Dimer
500 mg/dL	1000 mg/dL	75 mg/dL	500 ng/mL	400 µg/mL

Also refer to the specific application guide of the analyzer used.

REFERENCES:

1. Appel I.M. *et al*. Age dependency of coagulation parameters during childhood and puberty. Journal of Thrombosis and Haemostasis. 2012.
2. CLSI Document H21-A5: "Collection, transport, and processing of blood specimens for testing plasma -based coagulation assays and molecular hemostasis assays; approved guideline". 2008
3. Woodhams B. *et al*. Stability of coagulation proteins in frozen plasma. Blood coagulation and Fibrinolysis. 2001.

SYMBOLS:

Symbols used and signs listed in the ISO 15223-1 standard, see Symbol definitions document.

Changes compared to the previous version.