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HEMOCLOT Thrombin time (T.T.)

CE **# CK011K**

Kit for the Thrombin Time determination on plasma

6 x 20 tests

For in vitro diagnostic use only

Last revision: 14/08/2014

METHOD:

Reagent for the determination of Thrombin Time (TT) on human citrated plasma, using a clotting method, which can be manual, semi-automatic or automatic.

ASSAY PRINCIPLE:

Measurement of the clotting time induced by bovine thrombin, in presence of calcium, on plasma, and exploration of the anti-thrombin activities.
Excellent sensitivity to low concentrations of heparin in plasma (from 0.05 to 0.10 IU/ml of Unfractionated Heparin (UFH), or from > 0.20 IU/ml Low Molecular Weight Heparin (LMWH) in plasma).

SPECIMEN:

Human plasma collected on citrate anticoagulant.

REAGENTS:

6 vials of highly purified **bovine Thrombin**, stabilised and lyophilised in presence of calcium.

MATERIAL REQUIRED BUT NOT PROVIDED:

- **100 µL Pipettes**.
- Clotting instrument for semi-automatic or automatic coagulation assays, fibrometer or electromagnetic water bath.
- Distilled water
- Quality control plasmas.

PREPARATION, STORAGE AND STABILITY OF REAGENTS:

Unopened reagents, must be stored at 2–8 °C, in their original packaging box. They are then stable until the expiration date printed on the label.

Preparation: **Calcium-Thrombin**: each vial must be restored with 2 ml of distilled water, in order to obtain a Calcium-Thrombin solution, containing a concentration of about 1.0 NIH/ml of Calcium-Thrombin*, ready to use. Shake thoroughly until complete dissolution of the content (vortex). Incubate at room temperature (18-25°C) for 15 min, while shaking the vial from time to time. Homogenise the content before each use.

Stability of restored reagent:
This solution is stable, at least:

- 48 hours at room temperature
- 7 days at 2-8°C.

***The exact Thrombin concentration can vary from lot to lot and is adjusted for each lot in order to offer a high sensitivity Thrombin Time assay.**

Note: The stability studies at 30°C show that the reagents can be shipped at room temperature without damage.
Note: Source bovine plasma used for the extraction of proteins included in the preparation of **HEMOCLOT TT** were tested with registered methods and found negative for bovine infectious diseases, notably for the bovine spongiform encephalopathy. However, no assay may warrant the total absence of infectious agents. Any product of bovine origin must then be handled with all the required cautions, as being potentially infectious.

ASSAY PROCEDURE:

Specimen collection:

Blood (9 vol.) must be collected on 0.109M citrate anticoagulant (1 vol.); plasma supernatant is decanted following a 20 min. centrifugation at 2,500 g; citrated plasma should be tested within 4 hours (or within 2 hours for plasmas from patients under heparin therapy) or stored frozen at –20°C or below for up to 1 month, and thawed for 15 min. at 37°C just before use.

Refer to GEHT or NCCLS guidelines for further instructions on specimen collection, handling and storage.

Tested plasma:

Plasma must be tested undiluted.

Assay protocol:

Manual Method:
Thrombin must be incubated at 37°C in its original vial or in a plastic tube.
In a test tube or in the reaction cuvette of the clotting instrument, introduce:

- 100 µl of citrated plasma
  Incubate for 1 minute at 37°C, then introduce
- 100 µl of Calcium Thrombin, starting the stopwatch
  Note exactly the clotting time.

Automatic Method:
Adaptations on the main coagulation analyzers are available upon request.

RESULTS AND USUAL VALUES:

As indicative values obtained on KC10 or STA, for normal plasmas, Thrombin Time is usually in the range:

15 sec. to 25 sec.

Thrombin time (TT) is abnormal if: **TT > 25 seconds.**

Each laboratory should establish its own usual range, which can vary according to the lot and instrument used.

QUALITY CONTROL:

Use of quality control plasmas allows validating an homogeneous reactivity from run to run, for a same lot of reagent.
The obtained clotting time for a same sample and a same reagent lot can vary according to the instrument used and the clot detection sensitivity adjustment.
Each laboratory should establish and validate its own usual range, mean and standard deviation, in its specific test conditions.

PROLONGED THROMBIN TIME AND CLINICAL INTEREST:

- A prolonged Thrombin Time (≥ 25 seconds) can result from:
  - Presence of antithrombin activity induced by therapy (Heparin, hirudin).
  - 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.

PERFORMANCES:

- As an example, the « usual TT range » has been determined for citrated normal human plasmas using:

Instrument	KC10	STA	Amax Destiny (mechanical)	Amax Destiny (optical)	ACL (research) (optical)
N	56	31	31	31	31
Mean TT (sec)	20.8	21.6	23.2	18.0	17.3
SD	1.7	1.8	1.7	1.6	1.2
Min-Max	16.9-24.5	17.0-25.8	20.9-26.9	15.8-21.6	15.4-20.3

(Using KC10, obtained TT was ≤ 22.5 seconds for ≈ 86% of the plasmas).

- An excellent sensitivity is obtained to low concentrations of heparin present in the tested plasma (from 0.05 to 0.10 IU/ml of Unfractionated Heparin (UFH), and from 0.20 IU/ml Low Molecular Weight Heparin (LMWH)). As indicative values, the following TT (in seconds) were obtained on normal citrated human plasma, with addition of UFH or LMWH, using:

Instrument	KC10	STA	Amax Destiny (mechanical)	Amax Destiny (optical)	ACL (research) (optical)
Initial plasma	19.3	21.5	23.2	18.2	17.2
UFH 0.05 IU/ml	30.6	41.7	36.7	25.2	25.6
UFH 0.10 IU/ml	>120	>120	>120	>120	>120
LMWH 0.20 IU/ml	35.0	53.3	>120	32.1	29.1
LMWH 0.30 IU/ml	84.0	>120	>120	82	41.7

- Good sensitivity to hirudin, using KC10, from > 0.25 ATU/ml in plasma.
- Accuracy: as an example, the following results were obtained using KC10 instrument, on normal human plasma:

TT (sec)	N	Intra assay CV	N	Inter assay CV
21.6	10	1.8%	8	4.0 %

LIMITS:

- Various common substances or treatments can affect TT results. An additional investigation should be realized to determine the origin of each unexpected abnormal result.
- The obtained clotting time for a same sample and a same reagent lot can vary according to the instrument used and the clot detection sensitivity adjustment.
Each laboratory should establish and validate its own usual range, mean and standard deviation, in its specific test conditions.
In the same way, many variables (ex: different sources of heparin) can affect the obtained results: each laboratory should consequently establish its own heparin therapeutic range.
- Using the KC10 instrument, there was no significant interference up to <1mg/ml purified fibrinogen degradation products (FDP), up to <0,25 mg/ml bilirubin, or up to <2,5 mg/ml haemoglobin added in plasma.
- Any sample presenting an abnormal aspect (ex: partial coagulation…) should be rejected.

REFERENCES:

Samama MM., Elalamy I., Conard J., Achkar A., Horellou MH., « Hémorragies et thromboses : du diagnostic au traitement », Paris : Masson, 15-16, 60, 2004.