



**Hemoclot
Thrombin Inhibitors
(ex. «Hemoclot Hirudin »)**

**Technical File
(Ref. #ACK002K)**

**Clotting assay for the quantitative
measurement of hirudin and other direct
thrombin inhibitors in plasma**

(offering an excellent sensitivity to Argatroban®)

Nov 2007

HEMOCLOT THROMBIN INHIBITORS Technical File (Ref. ACK002K)

Assay principle

For measuring hirudin, or any other direct thrombin inhibitor, in plasma, first, the diluted tested plasma is mixed with a normal pooled human plasma (R1). Clotting is then initiated by adding a constant amount of highly purified human thrombin, in the α form (R2). The clotting time measured is directly related to the concentration of hirudin in the tested plasma.

Associated Plasma Calibrators and Controls are available for Hirudin.

Intended use:

Quantitative measurement of hirudin and other direct thrombin inhibitors in plasma, with a clotting method based on the inhibition of a constant and defined concentration of thrombin.

Kit presentation:

ACK002K: 3 x 10 tests (using KC10 or manual method).

Reagent (R1): 3* 1ml normal plasma pool, lyophilised and stabilized.

Reagent 2 (R2): 3*1ml purified human calcium thrombin, lyophilised and stabilized.

Protocol:

- Specimen: Citrated human plasma, EDTA anticoagulated human plasma, other biological sample.
- Working dilution: **1:8 for the low concentration range (0 to 2 μ g/ml hirudin in plasma)**
1:20 for the high concentration range (0 to 5 μ g/ml hirudin in plasma)

* Assay :

100 μ L of normal pooled plasma (R1)

50 μ L of calibration solution or of tested plasma (dil 1:8 [low range] or 1:20 [high range]).

1 min. at 37°C

100 μ L of thrombin (R2), preincubated at 37°C, starting the stop watch.

Record CT (sec)

Assay Characteristics:

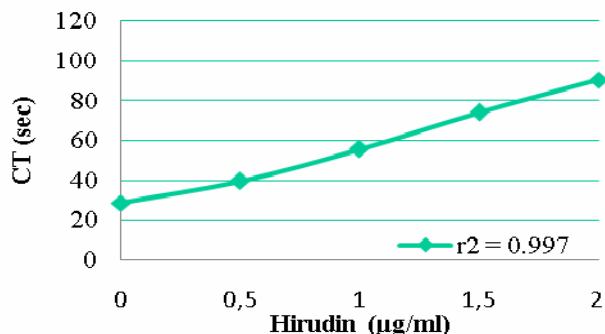
- The reagents do not contain heparin inhibitors. Presence of heparin or of other anti-thrombin substances, different from the one to be tested, may interfere in the assay and prolong the clotting time. Therefore, any anti-thrombin activity present in the tested plasma is not masked and this allows avoiding any underestimation of an existing hypocoagulability, as the result from the presence of an anti-thrombin substance.
- The assay is optimised for hirudin concentration, expressed in μ g/mL. The specific activity for hirudin drugs can vary from product to product or from lot to lot (from < 10,000 ATU*/mg to > 15,000 ATU*/mg). The curves are constructed respectively to the hirudin concentration. If a calibration by hirudin activity, expressed in ATU* (*Anti-Thrombin Unit)/mL, is needed, or when a different thrombin inhibitor is used, the user must take into account the specific anti-thrombin activity of the preparation used.
- Total assay time : **3 minutes** or below
- Can be used with: manual, semi-automated or automated methods.

Stability of reconstituted reagents

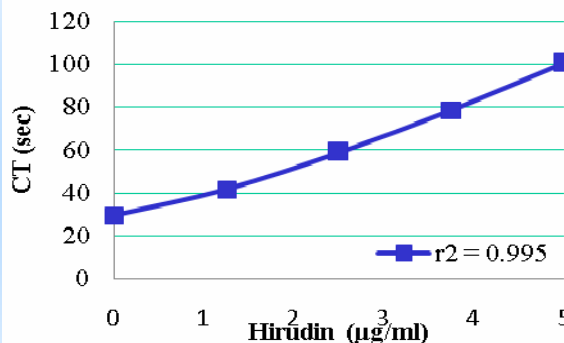
Excellent preservation of performances of reconstituted reagents, stored at 2-8°C for 48 hours or at RT for 24 hours, or frozen at -20°C or below, compared with those of freshly reconstituted vials.

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Hirudin assay: low range on STA-R



Hirudin assay: high range on STA-R



Conclusion : a good linearity is obtained for a dynamic range of 0 to 2µg/ml (“low range”) or to 5 µg/ml (“high range”) hirudin in plasma, using the standards « low or high range protocols » (ie dilution 1:8 or 1:20) in the Hemoclot thrombin Inhibitors kits (ref ACK002K).

Assay of Argatroban reactivity

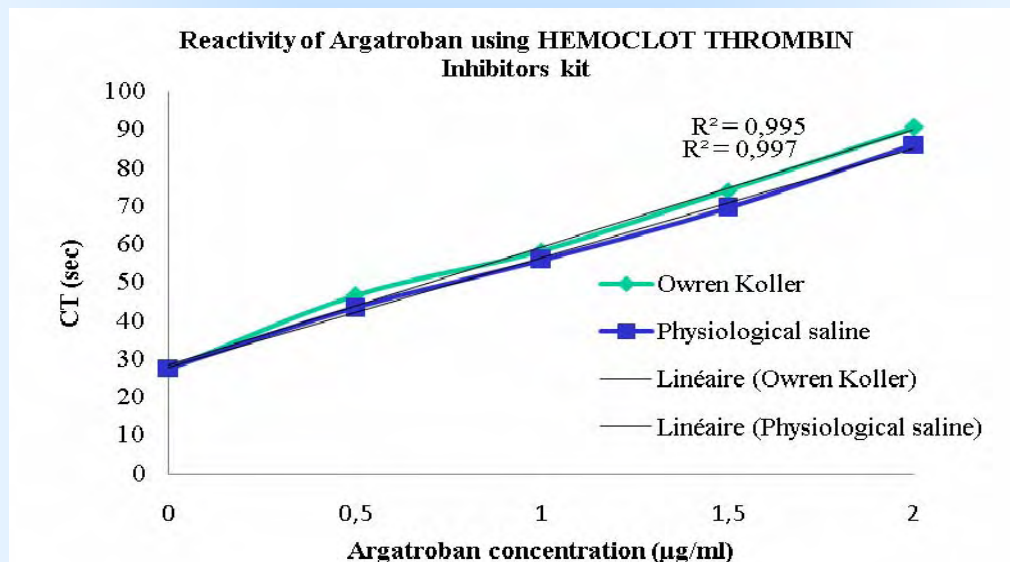
Reagents:

Argatroban (Mitsubishi Tanabe Pharma Corporation): 100mg/ml pharmaceutical solution, diluted in “Tris 0.05M, NaCl 0.15M, pH 7.50, BSA1%” buffer to 1mg/ml, then in a normal frozen plasma pool to 100µg/ml, then to 10µg/ml.

The calibration curve (0 to 2 µg/ml argatroban) is then prepared from the 10µg/ml solution, in the same normal plasma pool.

Method : Hemoclot Thrombin inhibitors (ACK002K); according to the insert, using the water bath protocol : “low range”: the preliminary prepared calibration curve is assayed at the 1:8 dilution in Owren Koller buffer or Physiological saline.

Results:



Conclusion : a good linearity is obtained for a dynamic range of 0 to 2µg/ml argatroban in plasma, using the standard « low range protocol » (ie dilution 1:8) in the Hemoclot thrombin Inhibitors kits ref ACK002K).