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HEMOCLOT THROMBIN INHIBITORS

Reference CK002K-RUO

Clotting assays for the quantitative measurement of Dabigatran and other direct thrombin inhibitors by anti-thrombin chronometric method

FOR RESEARCH USE ONLY.
NOT FOR USE IN DIAGNOSTIC PROCEDURES.

Last revision: 17/03/2015

INTENDED USE:

HEMOCLOT THROMBIN INHIBITORS is an in-vitro assay intended to be used for the quantitative measurement of Dabigatran and other direct thrombin inhibitors (DTIs), such as Argatroban or Hirudin, in human citrated plasma, with a clotting method based on the inhibition of a constant and defined concentration of thrombin.
This kit is for research use only and should not be used for patient diagnosis or treatment.

ASSAY PRINCIPLE:

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

REAGENTS:

Each kit contains:

- **R1 (Reagent 1)**: 3 vials of **1 mL** of normal pooled citrated plasma, lyophilized.
- **R2 (Reagent 2)**: 3 vials of **1 mL** of highly purified human calcium thrombin, very pure, stabilized with additives, and lyophilized.

Warning: Thrombin (R2) is prepared by activation of purified prothrombin extracted from human plasma. Human plasmas used for the pool (R1) and thrombin preparation (R2) were tested with registered methods and found negative for HIV antibodies, HBs Ag and HVC antibodies. Bovine Serum Albumin (BSA) was prepared from bovine plasma, which was tested for the absence of infectious agents, and collected from animals free from BSE. However, no assay may warrant the total absence of infectious agents. Any product of biological origin must then be handled with all the required cautions, as being potentially infectious.

REAGENTS AND MATERIALS REQUIRED BUT NOT PROVIDED:

Reagents:

- Distilled water, preferentially sterile.
- Dilution buffer: 0.15M NaCl physiological saline solution, or Imidazole type buffer. **The same diluent must be used for all the tests performed.**
- Normal plasma (or plasma pool) and reference material for Dabigatran or other assayed DTI, or calibration and quality control plasmas titrated for the assayed DTI. The following references are available:

	Argatroban	Dabigatran	Dabigatran Low	Hirudin
Calibrators	**SC030K-RUO** (5 levels, ready to use)	**222801-RUO** (3 levels, ready to use)	**222901-RUO** (3 levels, ready to use)	**SC020K-RUO** (low range) or **SC020L-RUO** (high range) (by kit, 2 calibrators for 5 points)
Controls	**SC035K-RUO**	**224701-RUO**	**225001-RUO**	**SC025K-RUO**

Materials:

- Pipettes with dispensing volumes of 20 µL, 50 µL and 100 µL.
- Pipette with a variable dispensing volume from 50 µL to 1,000 µL.
- Semi-automatic or automatic coagulation instrument, or fibrometer or electromagnetic water bath and stop watch.

STORAGE CONDITIONS:

Reagents must be stored at 2-8°C, in their original packaging box. They are then stable, before any use, until the expiration date printed on the box.

Note: Stability studies for 3 weeks at 30°C show that the reagents can be shipped at room temperature for a short period without damage.

PREPARATION AND STABILITY OF REAGENTS:

- **R1: Normal pooled plasma:**

Reconstitute each vial with **exactly 1 mL** of distilled water. Shake until complete dissolution of the contents. Let stabilize for at least 15 min at room temperature (18-25°C) while shaking the vial from time to time. **Homogenize before each use.**

- **R2: Human calcium Thrombin:**

Reconstitute each vial with **exactly 1 mL** of distilled water. Shake until complete dissolution of the contents. Let stabilize for at least 15 min at room temperature (18-25°C) while shaking the vial from time to time. **Homogenize before each use.**

Stability of restored reagents, provided that any contamination or evaporation is avoided, kept in the original vial or in a plastic tube, is:

- **8 hours at room temperature (18-25°C).**
- **24 hours at 2-8°C.**
- **2 months frozen in the original vial at –20°C.**

Cautions:

- Freezing conditions and stability of the thawed product should be checked in the working conditions of the laboratory user.
- In order to improve stability, reagents must be closed with their original screw cap following each use.
- Reagents must be handled with care, in order to avoid any contamination or evaporation during use.
- Reagents are closed under vacuum. Remove carefully the stopper, in order to avoid any loss of powder when opening the vials.
- Incubating the reconstituted vials at RT allows stabilizing the reagents, and obtaining a homogeneous reactivity.
- To limit as much as possible any evaporation of the reagents during use, e.g., by using chimneys.
- Use only reagents from kits with the same lot number.
- **Do not mix reagents from kits with different lots when running the assay. Reagents are optimized for each lot of kits.**

SPECIMEN COLLECTION:

Preparation and storage of samples are performed as recommended by GEHT or NCCLS/CLSI.

- Samples:
  Human plasma collected on citrate anticoagulant where the presence of Dabigatran or other DTI must be measured.
- Collection:
  Blood (9 vol.) must be collected on trisodium citrate anticoagulant (1 vol.) through a net venipuncture. The first tube must be discarded. The delay between the collections and the tests is ideally 1 to 2 hours and should not exceed 4 hours.
- Centrifugation:
  The centrifugation step is important and acts to separate the plasma from the platelets. Use a validated method in the laboratory to obtain a platelet-poor plasma, e.g., a minimum of 15 minutes at 2000 g at room temperature (18-25°C)
- Storage of plasma:
  - 4 hours at room temperature (18-25°C)
  - 2 months at -20°C.

OPERATING PROCEDURES:

The assay is calibrated with the DTI to be assayed. To date, this kit is validated for the determination of Dabigatran, Argatroban and Hirudin.
Measurement Ranges:

	Dabigatran Low	Dabigatran	Argatroban	Hirudin Low Range	Hirudin High Range
Measurement Range	0-120 ng/mL	50-500 ng/mL	0-2 µg/mL	0-2 µg/mL	2-4 µg/mL

The kit can also be used with other DTIs, for research use only; the protocol must be adapted as necessary to the DTI used: the calibration range can be prepared by diluting the inhibitor in normal plasma.

ANALYSIS PROCEDURES:

Assay of Dabigatran and Argatroban (manual method):

1. Prepare the calibration curve using the calibration kit specific for the DTI to be assayed. Calibrator plasmas should be diluted as described in the table below. Run the calibration curve and test it with quality controls within one hour for optimal assay performance.
2. The samples, controls and calibrators should be diluted using Dilution Buffer as described in the table below:

Assay	Calibrators Reference	Controls Reference	Working Dilution
Dabigatran	222801-RUO	224701-RUO	**1/8**
Dabigatran low	222901-RUO	225001-RUO	**1/2**
Argatroban	SC030K-RUO	SC035K-RUO	**1/8**

The dilutions must be tested within one hour. Please note that the exact concentration of the calibrators and controls are indicated for each lot on the flyer provided with the kit.

3. For the manual method, in a plastic tube incubated at 37°C, introduce:

Reagents	Amount
R1: normal plasma pool.	100µL
Diluted test plasma, calibrator or control	50µL
Shake and incubate **1 minute at 37°C**, then introduce, **and switch on the stop watch**:	
R2 : preincubated Thrombin at 37°C	100 µL
Note the clotting time (in seconds)	

Assay of Hirudin (manual method):

1. Prepare the calibration curve according to the specific instructions indicated on the calibrator insert (**Plasma Hirudin Standard Low #SC020K-RUO** or **Plasma Hirudin Standard High #SC020L-RUO**). Consider the exact concentrations ("C") indicated for each lot on the flyer provided within the kit. Calibrator plasmas should be prepared and then diluted as described in the table below. Run the calibration curve and test it with quality controls within one hour for optimal assay performance.

	Working dilution	Cal 1	Cal 2	Cal 3	Cal 4	Cal 5
Hirudin high range (SC020L-RUO) (µg/mL)	1/20	0	1.25 or C/4	2.5 or C/2	3.75 or 3C/4	5 or C
Hirudin low range (SC020K-RUO) (µg/mL)	1/8	0	0.5 or C/4	1 or C/2	1.5 or 3C/4	2 or C

2. The samples and controls should be diluted using Dilution Buffer as described in the table below:

	Control reference	Dilution with buffer	
		High range	Low range
Hirudin	SC025K-RUO	1/20 (100µL + 1900µL of buffer)	1/8 (100µL + 700µL of buffer)

The dilutions must be tested within one hour. Please note that the exact concentration of the calibrators and controls are indicated for each lot on the flyer provided with the kit.

3. For the manual method, in a plastic tube incubated at 37°C, introduce:

Reagents	Amount
R1: normal plasma pool.	100µL
Diluted test plasma, calibrator or control	50µL
Shake and incubate **1 minute at 37°C**, then introduce, **and switch on the stop watch**:	
R2 : preincubated Thrombin at 37°C	100 µL
Note the clotting time (in seconds)	

Automated method:

Applications for the various analyzers are available upon request. **Refer to each specific application and specific cautions for each instrument.**

QUALITY CONTROL:

Using suitable commercially available quality control plasmas, titrated for the assayed DTI, allows validating the calibration curve, as well as the homogeneous reactivity from run to run, when using a same lot of reagents. The calibration curve is acceptable when linearity ($r^2 \geq 0.98$) and the concentrations measured for controls are within the acceptance range. Various control plasmas are available:

Argatroban **SC035K-RUO**
Dabigatran **224701-RUO**
Dabigatran Low **225001-RUO**
Hirudin **SC025K-RUO**
(C1 more representative for low range, and C2 for high range)

Each laboratory should verify (and adjust if required) its own target values and acceptance ranges, in the exact working conditions, for each new lot of reagents used.

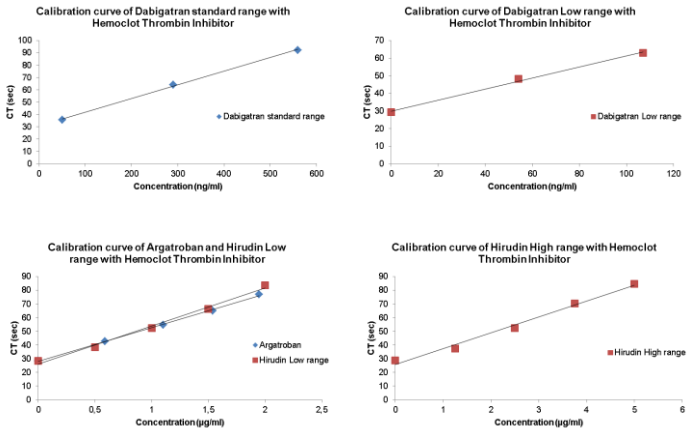
Note: Include at least one quality control at each level in each series, as per good laboratory practice. A new calibration curve must be carried out preferentially for each test series, and at least for each new lot of reagents, after each important maintenance of the analyzer, or when measured values for the quality controls are out of the acceptance range determined for the method (after checking all other parameters of the system). Each laboratory should establish and verify its own target values, acceptance ranges and expected performances, according to the combination of assayed DTI, reagents lots, instruments and protocols used, and in its exact working conditions.

EXPRESSION OF RESULTS:

On a linear graph paper, plot on abscissae the assayed DTI concentrations and on ordinates the corresponding clotting times (CT in seconds). On the calibration curve obtained, interpolate directly the corresponding DTI concentration for the tested plasma (when the standard dilution is used for the assay).
Using automated methods, the DTI concentrations are directly calculated by the analyzer, respectively to the calibration curve, and the sample dilution used.
The results obtained should be for research purposes only and not used for patient diagnosis or treatment.

EXAMPLE OF CALIBRATION CURVE:

The calibration curves below are given for example only, using the STAR instrument. Only the calibration curve generated for the series of assays performed should be used for calculating the concentrations in the assayed samples.



PERFORMANCE CHARACTERISTICS:

- The HEMOCLOT THROMBIN INHIBITORS 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.
- Example of reproducibility data using STAR instrument and lyophilized calibrators:

Lyophilized sample	Target value	Intra Assay CV%	Inter Assay CV%
Dabigatran	255 ng/mL	2.2% (N= 20)	5.3 %(N=20)
Dabigatran Low	57 ng/mL	1.1% (N=15)	3.7% (N=10)
Argatroban	0.59 µg/mL	2.3% (N=10)	2.2% (N=5)
Hirudin Low range	2.12 µg/mL	2.8% (N=10)	5.0% (N=6)

LIMITATIONS:

Blood activation, during specimen collection and plasma preparation, may interfere in the assay. Discard any sample presenting an unusual appearence (icteric, haemolysed, lipaemic…). No significant interference of excess or deficiency of other plasma factors was identified, in compliance with the test principle using diluted test plasma and a substrate plasma in excess. In order to get the optimal assay performances, the working instructions must be carefully observed. Each laboratory should establish and verify its own working range, expected values and acceptance ranges, as well as performances, in the exact laboratory working conditions (combination of reagents lots and instrument used), and for its specific application.