HEMOCLOT™ Thrombin Inhibitors

CE

IVD

REF CK002K R1 R2 3 x 1 mL

REF CK002L R1 R2 3 x 2.5 mL

Anti-IIa clotting method for the assay of Dabigatran and other direct thrombin inhibitors.

HYPHEN BioMed
www.hyphen-biomed.com
155, rue d'Eragny
95000 NEUVILLE SUR OISE
FRANCE
Tel. : +33 (0)1 34 40 65 10
Fax : +33 (0)1 34 48 72 36
info@hyphen-biomed.com

English, last revision: 07-2019

INTENDED USE:

HEMOCLOT™ Thrombin Inhibitors kit is an anti-IIa clotting method for the *in vitro* quantitative determination of Dabigatran and other Direct Thrombin Inhibitors (DTIs), on human citrated plasma, using manual or automated method.
This method is also appropriate for Argatroban, Hirudin or Bivalirudin assay.

SUMMARY AND EXPLANATION:

Technical:
Anti-IIa assays are the methods of choice for measuring Direct Thrombin Inhibitors (DTIs). HEMOCLOT™ Thrombin Inhibitors assay is an anti-IIa clotting method, based on the inhibition of constant and defined quantity of thrombin, developed to determine anti-IIa activity of DTIs (Dabigatran, Argatroban, Hirudin, Bivalirudin), using specific calibrations.
Clinical:[1,2]
Direct thrombin Inhibitors (DTI) such as Dabigatran, Hirudin, Bivalirudin, Argatroban are used in various contexts for prevention or treatment of thrombotic risk (eg venous thromboembolism, stroke, embolism, HIT…)[3]. When required, the DTI can be measured in plasma in case of suspicion of an excess of anticoagulant activity.[4,5,6]

PRINCIPLE:

The HEMOCLOT™ Thrombin Inhibitors method is a clotting assay (diluted thrombin time) to assay Dabigatran or other DTIs concentration on plasma. The diluted tested specimen is mixed with normal pooled human plasma. Clotting is then initiated by adding highly purified human thrombin, essentially in the α-thrombin form. The obtained clotting time is related to the concentration of Dabigatran (or other DTI) in the tested plasma.

REAGENTS:

R1 Normal pool plasma, lyophilized, in presence of stabilizers.
R2 Human calcium thrombin, highly purified, lyophilized in presence of stabilizers. Contains BSA.

REF CK002K ➔ R1 R2 3 vials of 1 mL.
REF CK002L ➔ R1 R2 3 vials of 2.5 mL.

WARNINGS AND PRECAUTIONS:

- Some reagents provided in these kits contain materials of human and animal origin. Whenever human plasma is required for the preparation of these reagents, approved methods are used to test the plasma for the antibodies to HIV 1, HIV 2 and HCV, and for hepatitis B surface antigen, and results are found to be negative. However, no test method can offer complete assurance that infectious agents are absent. Therefore, 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:

Gently remove the freeze-drying stopper, to avoid any product loss when opening the vial.

R1 R2 Reconstitute the contents of each vial with exactly:

REF CK002K ➔ 1 mL of distilled water.
REF CK002L ➔ 2.5 mL of distilled water.

Shake vigorously until complete dissolution while avoiding formation of foam and load directly on the analyzer following application guide instruction.
For manual method, allow to stabilize for 15 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.

R1 R2 Reagent stability after reconstitution, free from any contamination or evaporation, and stored closed, is of:

- **24 hours** at 2-8°C.
- **8 hours** at room temperature (18-25°C).
- **2 months** frozen at -20°C or less*
- **Stability on board of the analyzer: see the specific application.**

*Thaw only once, as rapidly as possible at 37°C and use immediately.

REAGENTS AND MATERIALS REQUIRED BUT NOT PROVIDED:

Reagents:

- Distilled water.
- Dilution buffer: Imidazole buffer (AR021A/AR021K/AR021L) or Physiological Saline (0.9% NaCl). Use the same buffer for all the tests performed.
- Plasma (or normal plasma pool) and reference material for the DTI to assay, or specific calibrators and controls with known titration for the DTI to assay, such as:

Product Name	Reference
Argatroban Plasma Calibrator	SC030K
Argatroban Control Plasma	SC035K
BIOPHEN™ Dabigatran Plasma Calibrator	222801
BIOPHEN™ Dabigatran Control Plasma	224701
BIOPHEN™ Dabigatran Calibrator Low	222901
BIOPHEN™ Dabigatran Control Low	225001
Plasma Hirudin Standard Low / High	SC020K* / SC020L*
Hirudin Control Plasma	SC025K
BIOPHEN™ Bivalirudin Calibrator	226701
BIOPHEN™ Bivalirudin Control	225701

*Two levels of calibrators to prepare 5 calibration points, per kit.
Also refer to the specific application guide of the analyzer used.

Materials:

- Water-bath, semi-automatic or automatic analyzer 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[7] guideline for further information concerning specimen collection, handling and storage).
For plasma storage, please refer to references[6,7].

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 R2, is measured.

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

Assay method (manual method) :
1. Reconstitute the calibrators and controls as indicated in the specific instructions.
Calibrators should be diluted in the dilution buffer as described in the table below:

Calibrators	Reference	Dilution
BIOPHEN™ Dabigatran Plasma Calibrator	222801	**1:8**
BIOPHEN™ Dabigatran Calibrator Low	222901	**1:2**
Argatroban Plasma Calibrator	SC030K	**1:8**
BIOPHEN™ Bivalirudin Calibrator	226701	**1:10**

For Hirudin, calibrators should be diluted in the dilution buffer as described in the specific instructions and in the table below in order to prepare the calibration curve ("C" defines the concentration of Hirudin):

	Working dilution	CAL1	CAL2	CAL3	CAL4	CAL5
Hirudin High range (SC020L)	1:20	0 µg/mL	1.25 µg/mL or C/4	2.5 µg/mL or C/2	3.75 µg/mL or 3C/4	5 µg/mL or C
Hirudin Low range (SC020K)	1:8	0 µg/mL	0.5 µg/mL or C/4	1 µg/mL or C/2	1.5 µg/mL or 3C/4	2 µg/mL or C

2. Dilute the specimens and controls in the dilution buffer, as described in the table below:

Specimens	Reference	Dilution
BIOPHEN™ Dabigatran Control Plasma	224701	**1:8**
BIOPHEN™ Dabigatran Control Low	225001	**1:2**
Specimens	NA	**1:8 (standard range)** **1:2 (low range)**
Specimens	**Reference**	**Dilution**
Argatroban Control Plasma	SC035K	**1:8**
Specimens	NA	**1:8**
Specimens	**Reference**	**Dilution**
Hirudin Control Plasma (high range)	SC025K	**1:20**
Hirudin Control Plasma (low range)	SC025K	**1:8**
Specimens	NA	**1:20 (high range)** **1:8 (low range)**
Specimens	**Reference**	**Dilution**
BIOPHEN™ Bivalirudin Control	225701	**1:10**
Specimens	NA	**1:10**

Establish the calibration curve and test it quickly with the quality controls for optimal assay performance. If stored at room temperature (18-25°C), test the diluted specimens quickly. The exact calibrator and control concentrations for each batch are indicated on the flyer provided with the kit.

3. Introduce in the plastic tube incubated at 37°C:

	Volume
Diluted Specimen, control and calibrators.	50 µL
R1 **Normal pool plasma**	100 µL
Mix and incubate at **37°C for 1 minute (2 minutes** for Bivalirudin), then introduce, **starting the stop-watch** :	
R2 **Human calcium thrombin,** Preincubated at **37°C**	100 µL
Record the exact clotting time (CT, sec).	

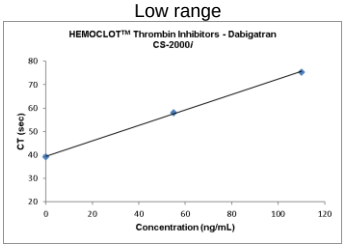
If a reaction volume other than that specified above is required for the method used, the ratio of volumes must be strictly observed to guarantee assay performance. The user is responsible for validating any changes and their impact on all results.

CALIBRATION:

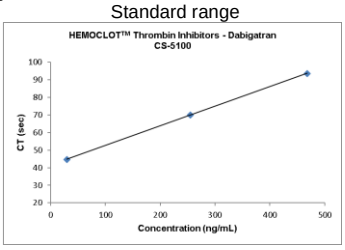
The HEMOCLOT™ Thrombin Inhibitors assay can be calibrated for the assay of different anti-IIa analytes: Dabigatran, Argatroban, Hirudin, Bivalirudin. Kits containing calibrators specific to these analytes and covering the calibration range are available from HYPHEN BioMed (see the REAGENTS AND MATERIALS REQUIRED BUT NOT PROVIDED paragraph) and can be used to establish the calibration curve specific to the assayed analyte.

The calibration curves shown below are given by way of example only. The calibration curve established for the assay series must be used.

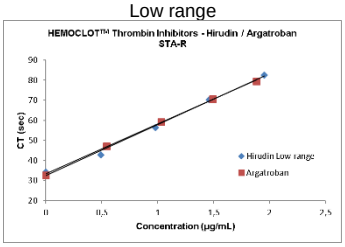
Low range



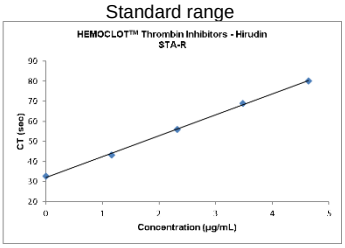
Standard range

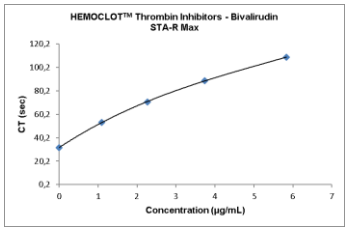


Low range



Standard range





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. A new calibration curve should be established, preferably for each test series, and at least for each new reagent batch, or after analyzer maintenance, or when the measured quality control values fall outside the acceptance range for the method.
Each laboratory must define its acceptance ranges and verify the expected performance in its analytical system.

RESULTS:

- For the manual endpoint method, plot the calibration curve lin-lin, with the clotting time (sec) along the Y-axis and the DTI concentration along the X-axis.
- Results are expressed in ng/mL for Dabigatran, or in µg/mL for Argatroban, Hirudin or Bivalirudin.
- The concentration of Dabigatran (or other DTI) in the test specimen is directly inferred from the calibration curve, when the standard dilution is used.
- If other dilutions are used, the level obtained should be multiplied by the additional dilution factor used.
- 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.
- Highly concentrated samples can be pre-diluted in a pool of normal plasmas or in Imidazole buffer. The measured concentrations should then be multiplied by the supplementary dilution factor.

EXPECTED VALUES:

Dabigatran, Argatroban, Hirudin and Bivalirudin are absent from normal plasmas.
Normal range, therapeutic range and bleeding risk range should be defined according to the current local recommendations.

PERFORMANCES:

- The lower analyzer detection limit and measuring range depend on the analytical system used.
- The calibration range are about:

Dabigatran **Low range**	**Dabigatran** **Standard range**	**Argatroban**
0-120 ng/mL	50-500 ng/mL	0-2 µg/mL
Hirudin **Low range**	**Hirudin** **High range**	**Bivalirudin**
0-2 µg/mL	2-4 µg/mL	0-5 µg/mL

- HEMOCLOT™ Thrombin Inhibitors reagent **does not contain heparin inhibitors**. Presence of heparin or thrombin inhibitors, different from the one to be tested, in the tested plasma may induce a prolonged clotting time.
- Performance studies were conducted internally on Sysmex CS-series and STAR-series. Performance was assessed using laboratory controls over a 5-day period, 2 series per day and 2 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
Dabigatran Low	30	80 ng/mL	3.7	3.0	20	77 ng/mL	2.1	1.6
Dabigatran	20	292 ng/mL	2.4	6.9	20	286 ng/mL	4.1	11.7
Bivalirudin	40	4.27 µg/mL	2.1	0.09	10	4.14 µg/mL	3.1	0.13
Argatroban	20	1.24 µg/mL	1.8	0.02	12	1.32 µg/mL	2.5	0.03
Hirudin low range	5	1.01 µg/mL	1.7	0.02	12	1.02 µg/mL	4.4	0.05

- By the assay principle, no coagulation factor deficiency interference, such as Factor II, V, X, AT, or low Fibrinogen, is expected.
- Correlation with reference method (LCMS :MS vs HEMOCLOT™ Thrombin Inhibitors, Dabigatran)[2] :
  Sysmex CS-2000i : n = 100 y = 0.926x + 10.46 r = 0.987
  For other molecules, also refer to the specific application guide of the analyzer used.
- Interferences: Refer to the specific application guide of the analyzer used.

REFERENCES:

1. Antovic J.P. *et al.* Evaluation of coagulation assays versus LC-MS/MS for determinations of dabigatran concentrations in plasma. Eur J Clin Pharmacol. 2013.
2. Amiral J. *et al.* An update on laboratory measurements of Dabigatran: Smart specific and calibrated dedicated assays for measuring anti-IIa activity in plasma. Transfusion and Apheresis Science. 2016
3. Greinacher A. and Warkentin T. The direct thrombin inhibitor hirudin. Thromb Haemost 2008.
4. Van Cott EM et *al.* Laboratory Monitoring of Parenteral Direct Thrombin Inhibitors. Semin. Thromb. Haemost. 2017.
5. Kyrle P.A. et al. Dabigatran :patient management in specific clinical settings. Wien Klin Wochenschr. 2014.
6. Gosselin R.C. *et al.* International council for standardization in haematology (ICSH) recommendations for laboratory measurement of direct oral anticoagulants. Thrombosis and Haemostasis. 2018
7. CLSI Document H21-A5: "Collection, transport, and processing of blood specimens for testing plasma -based coagulation assays and molecular hemostasis assays; approved guideline". 2008

SYMBOLS:

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

Changes compared to the previous version.