HEMOCLOT™ Thrombin Inhibitors

CE

REF CK002K R1 R2 3 vials x 1 mL

REF CK002L R1 R2 3 vials x 2.5 mL

IVD

HYPHEN BioMed

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INTENDED USE:

Anti-IIa clotting method for the *in vitro* quantitative determination of Direct Thrombin Inhibitors (DTI), in human citrated plasma, using a manual or automated method. This method is for monitoring patients on Argatroban and Bivalirudin therapy and aid to diagnosis to detect anticoagulant status (Dabigatran) in patients on anticoagulants therapy.
This device of *in vitro* diagnostic use is intended for professional use in the laboratory.

SUMMARY AND EXPLANATION:

Technical:[1-3]
The HEMOCLOT™ Thrombin Inhibitors kit is an anti-IIa clotting method, based on the inhibition of constant quantity of thrombin, to determine anti-IIa activity of DTIs, using specific calibrations.
Clinical:[3-7]
Measurement of Direct Thrombin Inhibitor concentration may, in some clinical situations, help in the management of patients receiving DTI treatment (e.g.: prior to surgery, for patients presenting a risk factor associated with an hemorragic accident, for patients presenting thrombotic or hemorragic episodes, or in the event of suspected overdose).

PRINCIPLE:

HEMOCLOT™ Thrombin Inhibitors is a method (diluted thrombin time) to assay Dabigatran or other DTIs concentration on plasma. The diluted tested specimen is mixed with normal pooled human plasma, then clotting is triggered by adding a constant concentration of human thrombin, in presence of calcium. The obtained clotting time (CT) is related to the concentration of Dabigatran (or other DTI) in the tested plasma.

REAGENTS:

R1 **Normal pool plasma**, lyophilized. Contains stabilizers.
R2 **Human calcium thrombin**, highly purified, of approximately 1 NIH/mL for (h)IIa, lyophilized. Contains BSA and stabilizers.

If necessary, the R2 enzyme concentration is adjusted for each batch in order to achieve optimum reactivity and linearity for the assay.

The product is classified as non-hazardous and is not subject to labeling according to EC Regulation No. 1272/2008 [CLP].

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.
- Use only the reagents from the same batch of kits.
- Waste should be disposed of in accordance with applicable local regulations.

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 it 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.

This plasmatic reagent can be more or less turbid after reconstitution. This turbidity is mainly due to plasma lipids that, after freeze-drying, become "less" soluble and may form a slight deposit. If necessary, let each vial stabilize 10 minutes at room temperature and shake 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 Guide**.

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

Combination of storage are not recommended.

REAGENTS AND MATERIALS REQUIRED BUT NOT PROVIDED:

- Dilution buffer: Imidazole buffer (AR021B / AR021K / AR021L / AR021M / AR021N) or Physiological Saline (0.9% NaCl). Use the same buffer for all the tests performed.
- Specific Calibrators and Controls with known titration 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
BIOPHEN™ Bivalirudin Calibrator	226701
BIOPHEN™ Bivalirudin Control	225701

- Automatic analyzer for clotting assays such as: CS-series, STA-R® family, ACL-TOP® family.
- Laboratory material.

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.
Samples should be collected, prepared and stored in accordance with applicable local guidelines (for the United States, see the CLSI H21-A5[8] guideline for further information concerning specimen collection, handling and storage).
For plasma storage, please refer to references[6,8].

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**

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**
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.

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.
- The concentration of Dabigatran (or other DTI) in the test specimen is directly inferred from the calibration curve, when the standard dilution is used.
- Results are expressed in ng/mL for Dabigatran, or in µg/mL for Argatroban or Bivalirudin.
- 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 no limpid 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.
- User defined modifications are not supported by HYPHEN BioMed as they may affect performance of the system and assay results. It is the responsibility of the user to validate modifications to these instructions or use of the reagents on analyzers other than those included in HYPHEN BioMed Application Guides or these Instructions for Use.

EXPECTED VALUES:

Anti-IIa drugs are absent from normal plasmas.
For each anti-IIa drug, the 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	Bivalirudin
0-120 ng/mL	0-500 ng/mL	0-2 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 STA-R® family. 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

- 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. Amiral C. *et al.* Measurement of Bivalirudin Thrombin Inhibition Activity in Plasma with Clotting or Chromogenic Assays and Dedicated Calibrators and Controls. Thrombosis & Haemostasis: Research. 2021.
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. Amiral C. *et al.* Measurement of Bivalirudin Thrombin Inhibition Activity in Plasma with Clotting or Chromogenic Assays and Dedicated Calibrators and Controls. Thrombosis & Haemostasis: Research. 2021
8. CLSI Document H21-A5: "Collection, transport, and processing of blood specimens for testing plasma -based coagulation assays and molecular hemostasis assays; approved guideline". 2008

Changes compared to the previous version.

The following symbols may appear on the product labeling:

REF	Catalogue number	LOT	Batch code	IVD	*In-vitro* diagnostic medical device
Rx	Numerical < x> identification of reagent		See instructions for use	WHO STD	WHO standard code
	Temperature limitation		Manufacturer	YYYY-MM-DD	Use by
XXXX	CE marking of conformity with notified body ID number.	→	Reconstitution volume	CONTENTS	Contents
Cx	Numerical < x> identification of control	i- MA	See instructions in Method Application guide	CONTAINS	Contains
EXP	Expiration date	Σ	Contains sufficient for <n> tests	UNIT	Measurement unit
TARGET VALUE	Target Value		Keep away from sunlight and heat	CALx	Numerical < x> identification of calibrator
ACCEPTANCE RANGE	Acceptance range				Biological risks

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