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CE
IVD

BIOPHEN™ DTI

REF 220202

R1 R2 2 x 2.5 mL, R3 2 x 25 mL

Chromogenic method for Direct Thrombin Inhibitors (DTI) assay.

English, last revision: 04-2021

INTENDED USE:

The BIOPHEN™ DTI kit is an anti-IIa chromogenic method for *in vitro* quantitative determination of Direct Thrombin (FIIa) Inhibitors (DTI) such as Dabigatran, Hirudin and Bivalirudin, in citrated human plasma, using an automated or manual method.

SUMMARY AND EXPLANATION:

Technical:

The BIOPHEN™ DTI kit is a chromogenic anti-IIa method specific to FIIa direct inhibitor and insensitive to heparins (UFH and LMWH).[2,3]

Clinical:

Direct thrombin Inhibitors (DTI) such as Dabigatran, Hirudin, Bivalirudin are used in various contexts for prevention or treatment of thrombotic risk (eg venous thromboembolism, stroke, embolism, HIT…). When required, the DTI can be measured in plasma in case of suspicions of an excess of anticoagulant activity.[1,4]

PRINCIPLE:

BIOPHEN™ DTI is a chromogenic method based on the inhibition, by the DTI being assayed, of a constant and excess quantity of thrombin (FIIa). The residual thrombin hydrolysis the thrombin specific chromogenic substrate (CS-01(81)), releasing the paranitroaniline (pNA). The amount of pNa released (measured by absorbance at 405 nm) is inversely proportional to the concentration of DTI in the sample.

[DTI] + [FIIa (excess)] ➔ [FIIa-DTI] + [residual FIIa]
[FIIa (residual)] + Substrate ➔ Peptide + pNA

REAGENTS:

R1 **Thrombin specific chromogenic substrate (CS-01(81)),** lyophilized in presence of stabilizers, of an heparin neutralizing substance, and of a fibrin polymerization inhibitor.

R2 **Human thrombin**, purified, lyophilized in presence of stabilizers. Contains BSA.

R3 **Tris-BSA Buffer.** Tris NaCl reaction buffer. Ready to use. Contains BSA and small amounts of sodium azide (0.9 g/L).

R1 R2 ➔ **2 vials of 2.5 mL.**
R3 ➔ **2 vials of 25 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.
- In contact with lead or copper pipes, sodium azide can generate explosive compounds.
- 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.

H373: May cause damage to organs through prolonged or repeated exposure.

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 **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 30 minutes at room temperature (18-25°C), homogenize before use.

R3 Reagent is ready to use; homogenize and load it directly on the analyzer following application guide instruction.
For manual method, allow to stabilize for 30 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:

- **4 weeks** at 2-8°C.
- **24 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.

R3 Reagent stability after opening, free from any contamination or evaporation, and stored closed, is of:

- **8 weeks** at 2-8°C.
- **7 days** at room temperature (18-25°C).
- **Stability on board of the analyzer: see the specific application.**

If the substrate become yellow, this indicate a contamination. Discard the vial and use a new one.

REAGENTS AND MATERIALS REQUIRED BUT NOT PROVIDED:

Reagents:

- Distilled water.
- 20% acetic acid or 2% citric acid (end point method).
- Reference material for the DTI to be assayed (international or internal, pharmaceutical preparation…) or specific calibrators and controls with known titration, such as:

Calibrators	BIOPHEN™ Dabigatran Plasma Calibrator / Calibrator Low	Plasma Hirudin Standard Low / High	BIOPHEN™ Bivalirudin Calibrator
Reference	222801 / 222901	SC020K / SC020L	226701
Controls	**BIOPHEN™ Dabigatran Control Plasma / Low**	**Plasma Hirudin Control**	**BIOPHEN™ Bivalirudin Control**
Reference	224701 / 225001	SC025K	225701

Also refer to the specific application guide of the analyzer used.

Materials:

- Spectrophotometer or automatic instrument for chromogenic assays.
- Stopwatch; Calibrated pipettes; 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[5] guideline for further information concerning specimen collection, handling and storage).
For plasma storage, please refer to references[5,6].

PROCEDURE:

The kit can be used for kinetics, automated or manual (endpoint) methods. Perform the test at **37°C** and read color intensity at **405nm**.

Assay method (manual method):

1. Reconstitute the calibrators as indicated in the specific instructions. Calibrators plasmas should be diluted in R3 buffer as described in the table below:

Calibrator	Reference	Dilution in R3
BIOPHEN™ Dabigatran Plasma Calibrator	222801	**1:10**
BIOPHEN™ Dabigatran Calibrator Low	222901	**1:2**
BIOPHEN™ Bivalirudin Calibrator	226701	**1 :2**

For Hirudin, alternatively, a normal plasma pool supplemented with a known amount of Hirudin can be used. Calibrator plasmas should be prepared and then diluted in R3 buffer as described in the table below.

Hirudin Low range (SC020K)	µg/mL	0	0.5	1	1.5	2
Normal plasma at 2 µg/mL of Hirudin (µL)		0	25	50	75	100
Normal Plasma (µL)		100	75	50	25	0
R3 buffer		900	900	900	900	900

Hirudin High range (SC020L)	µg/mL	0	1.25	2.50	3.75	5
Normal plasma at 5 µg/mL of hirudin (µL)		0	25	50	75	100
Normal Plasma (µL)		100	75	50	25	0
R3 buffer		2400	2400	2400	2400	2400

2. Dilute the specimens and controls in R3 buffer, as described in the table below:

Specimens	Reference	Dilution in R3
BIOPHEN™ Dabigatran Control Plasma	224701	**1:10**
BIOPHEN™ Dabigatran Control Low	225001	**1:2**
Samples	NA	**1:10 (standard range)** **1:2 (low range)**

Specimens	Reference	Dilution in R3
BIOPHEN™ Bivalirudin Control	225701	**1:2**
Samples	NA	**1:2**

Specimens	Reference	Dilution in R3	
		High range	Low range
Plasma Hirudin Control	SC025K	**1:25**	**1:10**
Samples	NA	**1:25**	**1:10**

Establish the calibration curve and test it with the quality controls. 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. Dispense the following to a microplate or plastic test tube incubated at **37°C**:

	Microplate	Volume
Specimen, calibrator or control diluted	50 µL	200 µL
R1 **Thrombin specific chromogenic substrate**	50 µL	200 µL
Mix and incubate **at 37°C** for **2 minutes**, then add the following:		
R2 **Human thrombin**. Pre-incubated at **37°C**	50 µL	200 µL
Mix and incubate at **37°C** for **2 minutes exactly**		
Stop the reaction by adding:		
Citric acid (2%)*	100 µL	400 µL
Mix and measure the optical density at **405nm** against the corresponding blank.		

*Or acetic acid (20%). The yellow color is stable for 2 hours.
The specimen blank is obtained by mixing the reagents in the reverse order to that of the test: Citric acid (2%), R2, R1, diluted specimen**.**
Measure the optical density at **405 nm**. Subtract the measured blank value from the absorbance measured for the corresponding test.
Create a plasma blank if sample is icteric, lipaemic, haemolysed, or if its color differs from the standard plasmas.

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.

Kinetics mode:
The assay can be run using a kinetics mode. In this case the change in absorbance is recorded from 10 to 100 seconds following the addition of substrate (ΔA405). There is then no need to subtract the blank sample, or to stop the reaction.

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

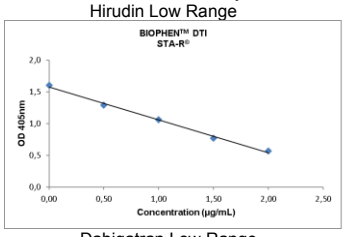
CALIBRATION:

The BIOPHEN™ DTI assay can be calibrated for the assay of Direct Thrombin Inhibitors (DTI) such as Dabigatran, Hirudin and Bivalirudin. The calibrator covering the calibration range is available from HYPHEN BioMed (see the REAGENTS AND MATERIALS REQUIRED BUT NOT PROVIDED paragraph) and can be used to establish the calibration curve.

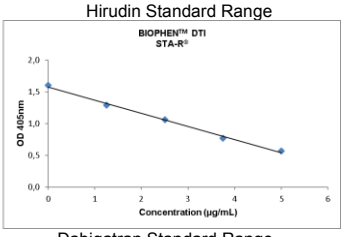
- For the standard range, the calibration range is about **0 to 500 ng/mL of Dabigatran.**
- For the low range, the calibration range is about **0 to 110 ng/mL of Dabigatran and about 0 to 2 µg/mL of Hirudin.**
- For the high range, the calibration range is about **0 to 5 µg/mL of Hirudin.**
- The calibration range is about **0 to 5 µg/mL of Bivalirudin.**

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

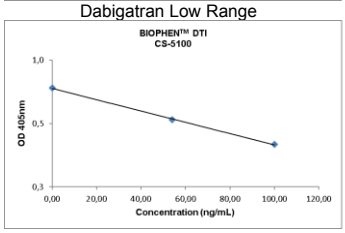
Hirudin Low Range



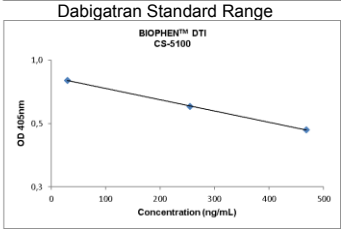
Hirudin Standard Range

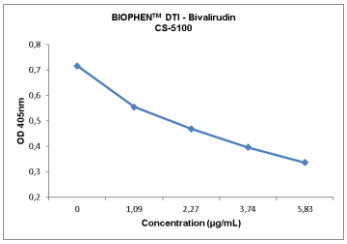


Dabigatran Low Range



Dabigatran 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, with the OD 405 nm along the Y-axis and the analyte concentration along the X-axis:
  - **Dabigatran**, use a **Log-Lin** scale (ng/mL – OD).
  - **Hirudin,** use a **Lin-Lin** scale (µg/mL – OD).
  - **Bivalirudin,** use a **Lin-Lin** scale (µg/mL – OD) in polynomial degree 3.
- When employing the kinetic method, use ΔOD 405 instead of OD 405.
- The concentration of DTI in the test specimen is directly inferred from the calibration curve, when the standard dilution is used.
- Results are expressed, for example, in ng/mL for Dabigatran or µg/mL for Hirudin and Bivalirudin.
- 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. The measured concentrations should then be multiplied by the supplementary dilution factor.

EXPECTED VALUES:

Dabigatran, Hirudin and Bivalirudin are not found in normal plasma.
The normal interval, therapeutic range and hemorrhagic risk range should be defined according to applicable local guidelines.

PERFORMANCES:

- The lower limit and the measurement range are defined by the analytical system used.
- The measuring range of Dabigatran depends on the analytical system used (about 15 to 120 ng/mL (low range) or 20 to 500 ng/mL (standard range) on STA-R®-series or CS-series.
- The measuring range of Hirudin depends on the analytical system used (about 0.15 to 2 µg/mL (low range) or 0.30 to 5 µg/mL (high range) on STA-R®-series.
- The measuring range of Bivalirudin depends on the analytical system used (about 0.5 to 15 µg/mL on Sysmex CS-series with redilution).
- Performance studies were conducted internally on 1 batch of reagent using a Sysmex CS-series and STA-R®-series. Performance was assessed using laboratory controls. The following results were obtained:

	Intra assay			Inter assays		
Echantillons	**n**	**Mean**	**CV%**	**N**	**Mean**	**CV%**
Dabigatran low range level 1	6	28.8 ng/mL	5.3	8	23.4 ng/mL	10.7
Dabigatran low range level 2	6	86.2 ng/mL	4.1	8	79.4 ng/mL	3.0
Dabigatran high range level 1	6	111.2 ng/mL	1.4	6	110.8 ng/mL	7.1
Dabigatran high range level 2	6	280.5 ng/mL	2.4	6	281.8 ng/mL	2.4
Hirudin level 1	10	1.00 µg/mL	4.8	4	1.26 µg/mL	<5%
Hirudin level 2	10	2.00 µg/mL	1.5	4	2.16 µg/mL	<5%
Bivalirudin level 1	40	1.62 µg/mL	0.7	10	1.64	2.4
Bivalirudin level 2	40	3.95 µg/mL	0.7	10	4.10	2.7

- Correlation with reference method (BIOPHEN™ DTI vs LC:MS/MS) :
  n = 101 y = 0.926x + 10.46 r = 0.987
- **The assay is completely insensitive to heparins (UFH and LMWH) at usual concentrations.**
- The influence of progressive thrombin inhibitors can be neglected because of the short incubation time.
- Interferences: refer to the specific application guide of the analyzer used.

REFERENCES:

1. Greinacher A and Warkentin T. The direct thrombin inhibitor hirudin, *Thromb Haemost*;. 2008.
2. Schramm *et al,* Development of a chromogenic susbtrate for the determination of hirudin in plasma, *Blood Coagul Fibrinolysis*. 1991.
3. Amiral J *et al.* An update on laboratory measurements of Dabigatran: Smart specific and calibrated dedicated assays formeasuring anti-IIa activity in plasma. Transfusion and Apheresis Science. 2016.
4. Poli *et al.* Diagnostic accuracy of a novel chromogenic direct thrombin inhibitor assay: clinical experiences for Dabigatran monitoring. *Thromb Haemost*. 2017.
5. CLSI Document H21-A5: "Collection, transport, and processing of blood specimens for testing plasma -based coagulation assays and molecular hemostasis assays; approved guideline". 2008.
6. Woodhams B *et al.* Stability of coagulation proteins in frozen plasma. Blood coagulation and Fibrinolysis. 2001.

SYMBOLS:

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

Changes compared to the previous version.