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IVD

BIOPHEN™ DiXal

REF 221030

R1 R2 3 vials x 2.5 mL

R3 4 vials x 20 mL

English, revision: 12-2023

INTENDED USE:

Anti-Xa chromogenic method for the *in vitro* quantitative determination of direct Factor Xa inhibitors (DiXaIs), in human citrated plasma, using an automated method. This method is to monitor anticoagulant status, in the frame of specific clinical situations, for patients on oral anticoagulant therapy (Apixaban, Rivaroxaban and Edoxaban)
This device of *in vitro* diagnostic use is intended for professional use in the laboratory.

SUMMARY AND EXPLANATION:

Technical[1-4]***:***
The BIOPHEN™ DiXal kit is a two stages chromogenic method specific to FXa direct inhibitors and insensitive to heparins (UFH and LMWH).
Clinical[1-8]***:***
Measurement of direct Factor Xa inhibitor concentration may be required or it may, in some clinical situations, help in the management of patients receiving DiXal treatment (e.g.: prior to emergency surgery, for patients presenting a risk factor associated with an hemorrhagic accident, for patients presenting thrombotic or hemorrhagic episodes, or in the event of suspected overdose).

PRINCIPLE:

BIOPHEN™ DiXal is a chromogenic method based on the inhibition, by the DiXal being assayed, of a constant and excess quantity of Factor Xa (FXa). The residual Factor Xa hydrolyses the FXa-specific chromogenic substrate, releasing paranitroaniline (pNa). The amount of pNa released (measured by absorbance at 405 nm) is inversely proportional to the concentration of DiXal in the sample.

REAGENTS:

R1 **FXa (h):** Purified human Factor Xa at approximately 10 U/mL, lyophilized. Contains BSA and stabilizers.
R2 **Factor Xa-specific chromogenic substrate (CS-11(65))** at approximately 2 mg/mL, lyophilized. Contains stabilizers.
R3 **Tris-NaCl-EDTA reaction buffer,** pH 7.85, liquid form. Contains a heparin neutralizing substance. Contains preservatives and stabilizers.

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.
- Any serious incident that has occurred in relation to the device shall be reported to the manufacturer and the competent authority of the Member State in which the user and/or the patient is established.
- Summary of Safety and Performance (SSP) is available in the European database on medical devices (see Eudamed public website: https://ec.europa.eu/tools/eudamed or on request to HYPHEN BioMed).

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.

R3 Reagent is ready to use; homogenize while avoiding formation of foam and load it directly on the analyzer following Application Guide instruction.

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

- **14 days** at 2-8°C.
- **Stability on board of the analyzer: see the specific Application Guide**.

If the substrate becomes yellow, this indicates a contamination. Discard the vial and use a new one.

REAGENTS AND MATERIALS REQUIRED BUT NOT PROVIDED:

- Specific Calibrators and controls:

Calibrators	BIOPHEN™ Apixaban Calibrator / Calibrator Low	BIOPHEN™ Rivaroxaban Plasma Calibrator / Calibrator Low	BIOPHEN™ Edoxaban Calibrator / Calibrator Low
References	226201 / 226101	222701 / 226001	226501 / 226401
Controls	**BIOPHEN™ Apixaban Control / Control Low**	**BIOPHEN™ Rivaroxaban Control Plasma / Control Low**	**BIOPHEN™ Edoxaban Control / Control Low**
References	225301 / 225201	224501 / 225101	225501 / 225401

- Automatic analyzer for chromogenic assays such as: CS-series, STA-R® family, ACL-TOP® family, CN-series.
- Laboratory material.
- When required, Tris-NaCl-EDTA buffer (AR032A / AR032K).

Please note that the applications on other analyzers can be validated by the instrument manufacturer in accordance with the requirements of the REGULATION (EU) 2017/746 under their responsibility as long as the intended purpose is not modified.

TRACEABILITY:

Certificates of traceability and Instructions for Use of above calibrators and controls are available on the HYPHEN BioMed website. For more information refer to Instructions for Use of above calibrators and controls.

SPECIMEN COLLECTION AND PREPARATION:

Collection, preparation and storage of Platelet Poor Plasma (PPP) should be made according to laboratory or other validated methods[1,9-11].
The blood (9 volumes) should be carefully collected onto the trisodium citrate anticoagulant (1 volume) (0.109 M, 3.2%) by clean venipuncture.
CLSI H21-A5[9] and studies[11]:

- Plasma should remain at room temperature for no longer than 4 hours.
- If assays will not be completed within 4 hours, plasma should be frozen at −20 °C or below.
- Plasma samples should be thawed at 37°C, only once.

PROCEDURE:

HYPHEN BioMed provides Application Guides for defined coagulation analyzer families. The Application Guides contain analyzer/assay specific handling and performance information and complement the information in these Instructions for Use.

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:

- The concentration of DiXaI (ng/mL) in the test specimen is directly inferred from the calibration curve, when the standard dilution is used.
- Lot to lot variability measured on 3 lots is: %CV = 1.6%
- 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. 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.
- In case of administration of the antidot andexanet alfa, commercial anti-FXa activity assays are reported to be unsuitable for measuring anti-FXa activity[12,13]. In these assays, the FXa inhibitor dissociates from andexanet alfa. This results in the detection of erroneously elevated anti-FXa activity levels and consequently, a substantial underestimation of the reversal activity of andexanet alfa. Data using the BIOPHEN™ DiXaI assay were not established.

EXPECTED VALUES:

Apixaban, Rivaroxaban and Edoxaban are absent from normal plasma.
For each anti-Xa drug, the normal range, therapeutic range and bleeding risk range should be defined according to current local recommendations.

PERFORMANCES:

Performances studies were conducted as described in CLSI guidelines.
The following performance data represent typical results and are not to be regarded as specifications for BIOPHEN™ DiXaI.
Mathematical analyses are performed using a validated statistical software built in accordance with CLSI guidelines.
All performances are documented in the respective Application Guides of the analyzers.

Analytical performances
Measuring Range
The measuring range is defined by the analyzer system used and is documented in the respective Application Guides of the analyzers.
Precision
Precision studies were assessed using laboratory controls and spiked pooled plasmas. Coefficient of variation (CV) for all samples is less than 8.0% for repeatability, less than 10.0% for reproducibility and less than 10.0% for within laboratory. Precision is documented in the respective Application Guides of the instruments.
Interfering substances
Interferences are defined by the analyzer system used and are documented in the respective Application Guides of the analyzers.
By the assay principle, no coagulation factor interference, such as Factor II and X, is expected. The assay is completely insensitive to heparins (UFH and LMWH) up to 2 IU/mL

Clinical performances

Agreement

	ACL-TOP® family		
Analyte	**Linear regression**	**r**	**Reference / comparison method**
Apixaban	y = 0.90x + 3.95	0.996	HemosIL® Liquid Anti-Xa
Rivaroxaban	y = 1.18x + 0.54	0.987	HemosIL® Liquid Anti-Xa

	STA-R® family		
Analyte	**Linear regression**	**r**	**Reference / comparison method**
Edoxaban	y = 0.90x + 9.15	0.972	STA® - Liquid Anti-Xa

Sensitivity/Specificity

	ACL-TOP® family				
Analyte	**Sensitivity**	**Specificity**	**LR+**	**LR-**	**Area under the curve ROC**
Apixaban	1.000	0.966	23.6	0.0	1.000
Rivaroxaban	1.000	0.942	17.3	0.0	1.000

	STA-R® family				
Analyte	**Sensitivity**	**Specificity**	**LR+**	**LR-**	**Area under the curve ROC**
Edoxaban	1.000	0.984	62.5	0.0	1.000

LR+: Likelihood ratio +
LR- : Likelihood ratio -

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3. Douxfils, J. *et al.* Laboratory testing in patients treated with direct oral anticoagulants: a practical guide for clinicians. J Thromb Haemost (2018).
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8. Ruff CT. *et al.* Association between edoxaban dose, concentration, anti-Factor Xa activity, and outcomes: an analysis of data from the randomised, double-blind ENGAGE AF-TIMI 48 trial. Lancet. (2015).
9. CLSI Document H21-A5: "Collection, transport, and processing of blood specimens for testing plasma -based coagulation assays and molecular hemostasis assays; approved guideline" (2008).
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12. Commercial anti-FXa activity assays are unsuitable for measuring anti-FXa activity following administration of andexanet alfa. European Medicine Agency [Internet]. 2020 Jun [cited 2020 Jun 17]. Available from https://www.ema.europa.eu/en/medicines/dhpc/ondexxya-andexanet-alfacommercial-anti-fxa-activity-assays-are-unsuitable-measuring-anti-fxa
13. Tomoda H. *et al*. Andexanet Alfa for Bleeding with Factor Xa Inhibitors. NEngl J Med. 2019.

e-IFU (other languages) are available on www.hyphen-biomed.com.
For customer support or Application Guides, please contact your local provider or distributor (see www.hyphen-biomed.com).

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
CE 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
UDI	Unique Device Identifier	BIO	Contains biological material of animal origin		Contains human blood or plasma derivatives
DANGER	Danger	WARNING	Warning	UK CA	UKCA marking of conformity
	Biological risks	ACCEPTANCE RANGE	Acceptance range		

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