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CE
IVD

BIOPHEN™ DiXal

REF 221030

R1 R2 3 x 2.5 mL; R3 4 x 20 mL

Chromogenic method for the assay of direct Factor Xa inhibitors (DiXals)

English, last revision: 03-2019

INTENDED USE:

The BIOPHEN™ DiXal kit is an anti-Xa chromogenic method for the *in vitro* quantitative determination of direct Factor Xa inhibitors (**DiXals**), such as Rivaroxaban, Apixaban or Edoxaban, in citrated human plasma (or purified medium), using an automated or manual method. This method is not suitable for indirect inhibitors such as Fondaparinux or heparins.

SUMMARY AND EXPLANATION:

Technical:
The BIOPHEN™ DiXal kit is a two stages chromogenic method specific to FXa direct inhibitors and insensitive to heparins (UFH and LMWH).
Clinical:
Measurement of direct Factor Xa inhibitor concentrations 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)[1-4].

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.

[DiXal] + [FXa (excess)] ➔ [FXa-DiXal] + [FXa residual]
[FXa (residual)] + Substrate ➔ Peptide + pNA

REAGENTS:

R1 **Reagent 1:** FXa (h): Purified, freeze-dried human Factor Xa. Contains BSA, Tris and stabilisers.
3 x 2.5 mL vials.
R2 **Reagent 2:** Substrate: Freeze-dried Factor Xa-specific chromogenic substrate (CS-11(65)).
3 x 2.5 mL vials.
R3 **Reagent 3:** Buffer: Tris-NaCl-EDTA reaction buffer, pH 7.85. Contains 1% PEG and small amounts of sodium azide (0.9 g/L) as preservative. Contains a heparin neutralizing substance.
4 x 20 mL vials.

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

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.

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 Reagent stability after reconstitution, free from any contamination or evaporation, and stored closed, is of:
- **15 days** at 2-8°C.
- **7 days** at room temperature (18-25°C).
- **2 months** frozen at -20°C or less*
- **Stability on board of the analyzer: see the specific application.**

R2 Reagent stability after reconstitution, free from any contamination or evaporation, and stored closed, is of:
- **2 months** at 2-8°C.
- **7 days** at room temperature (18-25°C).
- **2 months** frozen at -20°C or less*
- **Stability on board of the analyzer: see the specific application.**

R3 Reagent stability after opening, free from any contamination or evaporation, and stored closed, is of:
- **2 months** at 2-8°C.
- **7 days** at room temperature (18-25°C).
- **Stability on board of the analyzer: see the specific application.**

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

A yellow color indicates a contaminated substrate. 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 (endpoint method)
- Calibrators and specific controls with known titration, such as:

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

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

Materials:
- Spectrophotometer and chromogenic assay analyzer.
- Water-bath.
- Stopwatch, calibrated pipettes.

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[6] guideline for further information concerning specimen collection, handling and storage).
For plasma storage, please refer to references[7].

PROCEDURE:

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

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

Rivaroxaban assay:
1.Resuspend the calibrators and controls as described in the specific instructions. For the calibration curve, dilute the calibrators in R3 buffer, as described in the table below.
2. Dilute the samples in R3 buffer, as described in the table below:

Dosage	Calibrators Reference	Controls reference	Dilution in reagent R3
Rivaroxaban	222701	224501	**1/15**
Rivaroxaban low range	226001	225101	**1/3**
Samples	NA	NA	**1/15 (standard range) 1/3 (low range)**

Perform the calibration curve and test with the quality controls. If stored at room temperature (18-25°C), the diluted samples should be tested quickly. For each batch, the calibrator and control concentrations are indicated on the flyer provided with the kit.
3. Add the following to a plastic tube incubated at **37°C**:

Reagents	Volume
Calibrators, or test plasmas, or controls diluted **in** R3	200 µL
R1 **FXa (h)** pre-incubated at **37°C**	200 µL
Mix and incubate **at 37°C for exactly 1 minute**, then add the following:	
R2 **Substrate** pre-incubated **at 37°C**	200 µL
Mix and incubate at **37°C,** for **45 seconds exactly**	
Stop the reaction by adding:	
Citric acid (2%)*	400 µL
Mix and measure the optical density at **405 nm** against the corresponding blank.	

*Or acetic acid (20%). The resulting yellow colour is stable for 2 hours.
The sample blank is obtained by mixing the reagents in the reverse order of that of the test: Acetic acid (20%) or citric acid (2%), substrate, Factor Xa(h), diluted test sample.
Measure the optical density at 405 nm. Subtract the measured blank value from the test absorbance.

Apixaban assay:
1. Resuspend the calibrators and controls as described in the specific instructions. For the calibration curve, dilute the calibrators in R3 buffer, as described in the table below.
2. Dilute the samples in R3 buffer, as described in the table below:

Dosage	Calibrators reference	Controls reference	Dilution in reagent R3
Apixaban	226201	225301	**1/40**
Apixaban low range	226101	225201	**1/6**
Samples	NA	NA	**1/40 (standard range) 1/6 (low range)**

Perform the calibration curve and test with the quality controls. If stored at room temperature (18-25°C), the diluted samples should be tested quickly. For each batch, the calibrators and control concentrations are indicated on the flyer provided with the kit.
3. Add the following to a plastic tube incubated at **37°C**:

Reagents	Volume
Calibrators, or test plasmas, or controls diluted **in** R3	200 µL
R1 **FXa (h)** pre-incubated at **37°C**	200 µL
Mix and incubate **at 37°C for exactly 1 minute**, then add the following:	
R2 **Substrate** pre-incubated **at 37°C**	200 µL
Mix and incubate at **37°C,** for **45 seconds exactly**	
Stop the reaction by adding:	
Citric acid (2%)*	400 µL
Mix and measure the optical density at **405 nm** against the corresponding blank.	

*Or acetic acid (20%). The resulting yellow colour is stable for 2 hours.
The sample blank is obtained by mixing the reagents in the reverse order of that of the test: Acetic acid (20%) or citric acid (2%), substrate, Factor Xa(h), diluted test sample.
Measure the optical density at 405 nm. Subtract the measured blank value from the test absorbance.

Edoxaban assay:
1. Resuspend the calibrators and controls as described in the specific instructions. For the calibration curve, dilute the calibrators in R3 buffer, as described in the table below.
2. Dilute the samples in R3 buffer, as described in the table below:

Dosage	Calibrators reference	Controls reference	Dilution in reagent R3
Edoxaban	226501	225501	**1/15**
Edoxaban low range	226401	225401	**1/4**
Samples	NA	NA	**1/15 (standard range)** **1/4 (low range)**

Perform the calibration curve and test with the quality controls. If stored at room temperature (18-25°C), the diluted samples should be tested quickly. For each batch, the calibrators and control concentrations are indicated on the flyer provided with the kit.
3. Add the following to a plastic tube incubated at **37°C**:

Reagents	Volume
Calibrators, or test plasmas, or controls diluted **in** R3	200 µL
R1 **FXa (h)** pre-incubated at **37°C**	200 µL
Mix and incubate **at 37°C for exactly 1 minute**, then add the following:	
R2 **Substrate** pre-incubated **at 37°C**	200 µL
Mix and incubate at **37°C,** for **45 seconds exactly**	
Stop the reaction by adding:	
Citric acid (2%)*	400 µL
Mix and measure the optical density at **405 nm** against the corresponding blank.	

*Or acetic acid (20%). The resulting yellow colour is stable for 2 hours.
The sample blank is obtained by mixing the reagents in the reverse order of that of the test: Acetic acid (20%) or citric acid (2%), substrate, Factor Xa(h), diluted test sample.
Measure the optical density at 405 nm. Subtract the measured blank value from the test absorbance.

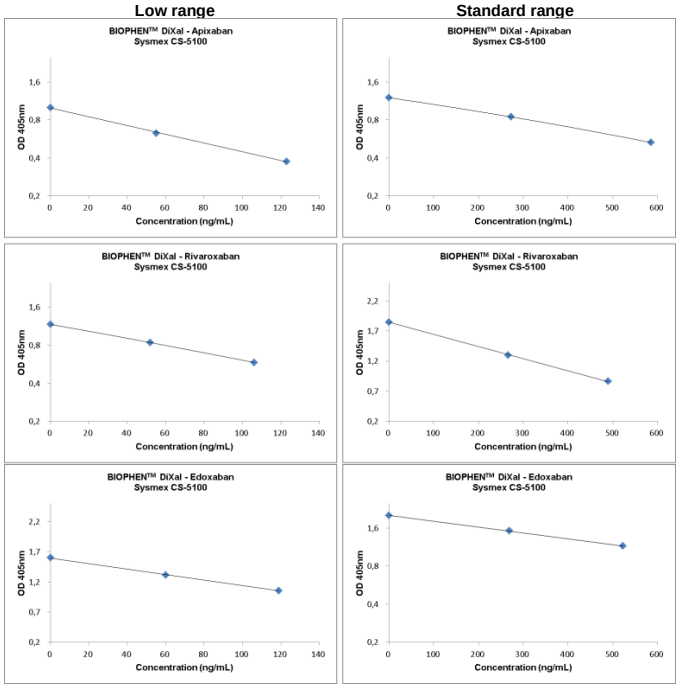
Create a plasma blank if sample is icteric, lipaemic, haemolysed, or if its color differs from the standard plasmas.
When employing the kinetic method, use ΔOD 405 instead of OD 405.

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

CALIBRATION:

The BIOPHEN™ DiXal test can be calibrated for the analysis of various anti-Xa analytes: Apixaban, Rivaroxaban, Edoxaban. Kits containing calibrators specific to these analytes and covering the dynamic test range are available from HYPHEN BioMed (see the 'REAGENTS AND MATERIALS REQUIRED BUT NOT PROVIDED' paragraph) and can be used to generate the calibration curve specific to the assayed analyte.
The following calibration curves are given by way of example only. The calibration curve established for the assay series must be used.

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 defined, preferably for each test series, and at least for each new reagent batch, or after analyser maintenance, or when the measured quality control values fall outside the acceptable range for the method.
Each laboratory must define its acceptable 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:
  - **Rivaroxaban low range**, use a **Lin-Log** scale (ng/mL – OD).
  - **Rivaroxaban standard range,** use a **Lin-Lin** scale (ng/mL – OD).
  - **Apixaban**, use a **Lin-Lin** scale (ng/mL – OD) for both ranges.
  - **Edoxaban low range**, use a **Lin-Lin** scale (ng/mL – OD).
  - **Edoxaban standard range**, use a **Lin-Log** scale (ng/mL – OD).
- The concentration of DiXal (in **ng/mL**) in the test sample is inferred directly from the calibration curve, when the standard dilution is used.
- The results should be interpreted according to the patient's clinical and biological status.

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:

Apixaban, Rivaroxaban and Edoxaban are not found in normal plasma.
The normal interval, therapeutic range and hemorrhagic risk range should be defined according to applicable local guidelines.

PERFORMANCE:

- **The lower limit and the measurement range are defined by the analytical system used.**
- For the standard range, the calibration range is about **0 to 500 ng/mL Rivaroxaban/Edoxaban and about 0 to 600 ng/mL Apixaban.**
- For the low range, the calibration range is about **0 to 100 ng/mL Rivaroxaban and about 0 to 120 ng/mL Apixaban/Edoxaban.**
- Performance studies were conducted internally on 1 batch of reagent on Sysmex CS-series. Performance was assessed using the laboratory's controls. The following results were obtained:

	Rivaroxaban assay				Apixaban assay			
	Standard range		Low range		Standard range		Low range	
	N	CV%	N	CV%	N	CV%	N	CV%
Intra-test	30	1.5	30	1.7	30	1.8	30	1.8
Inter-test	20	2.3	20	2.2	20	3.2	20	2.9

	Edoxaban assay			
	Standard range		Low range	
	N	CV%	N	CV%
Intra-test	40	2.1	40	2.0
Inter-test	120	2.7	120	5.1

- **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) at usual concentrations.**
- Specific, sensitive assay, offering a high degree of flexibility over the measurement range according to the working dilution used.
- The test is optimized and calibrated relative to the Rivaroxaban/Apixaban/Edoxaban concentration. The calibration curves are established with a concentration expressed in ng/mL. If another direct Factor Xa inhibitor is used, the user must take into consideration the specific anti-Xa activity of the substance used.
- Correlation with reference method (LCMS :MS vs BIOPHEN™ DiXal Edoxaban) :
  Sysmex CS-5100 : n = 142 y = 1.00x + 13.10 r = 0.997
  Refer to the specific application guide for each analyzer.
- Interferences: see specific application guide for each analyzer.

REFERENCES:

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2. Pernod G, *et al.*. Management of major bleeding complications and emergency surgery in patients on long-term treatment with direct oral anticoagulants, thrombin or factor-Xa inhibitors. Proposals of the Working Group on Perioperative Haemostasis (GIHP). Ann Fr Anesth Reanim. 2013.
3. Douxfils J, *et al*. Comparison of calibrated chromogenic anti-Xa assay and PT tests with LC-MS/MS for the therapeutic monitoring of patients treated with rivaroxaban. Thromb Haemost. 2013.
4. Douxfils J, *et al.* Impact of Apixaban on routine and specific coagulation assays: a practical laboratory guide. Thromb Haemost. 2013 June.
5. 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. ;385(9984):2288-95. 2015.
6. CLSI Document H21-A5 : "Collection, transport, and processing of blood specimens for testing plasma -based coagulation assays and molecular hemostasis assays; approved guideline". Fifth Edition, 28, 5, 2008.
7. Douxfils J, *et al.* Non-VKA Oral Anticoagulants: Accurate Measurement of Plasma Drug. BioMed Research International. 2015.
8. Mauge L. and Alhenc-Gelas M. Stabilité pré-analytique des paramètres de la coagulation: revue des données disponibles. Ann Biol Clin. 2014.

SYMBOLS:

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

R1 H315: Causes skin irritation
H319: Causes serious eye irritation