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CE
IVD

BIOPHEN™ DiXaI

REF 221030

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

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

English, last revision: 06-2017

INTENDED USE:

The BIOPHEN™ DiXaI kit is an anti-Xa chromogenic method for the *in vitro* quantitative determination of direct Factor Xa inhibitors (**DiXaIs**), such as Rivaroxaban or Apixaban, 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:

Measurement of direct Factor Xa inhibitor concentrations may be required or it may, in some clinical situations, help in the management of patients receiving DiXaI 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™ DiXaI is a chromogenic method based on the inhibition, by the DiXaI 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 DiXaI in the sample.

[DiXaI] + [FXa (excess)] ➔ [FXa-DiXaI] + [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)). Contains mannitol.
3 x 2.5 mL vials.
R3 **Reagent 3:** Buffer: Tris-NaCl-EDTA reaction buffer, pH 7.85. Contains 1% PEG and sodium azide (0.9 g/L) preservative.
4 x 20 mL vials.

Reagent R3 contains small amounts of sodium azide (0.9 g/L), see WARNING AND CONTRA-INDICATIONS.

WARNINGS AND PRECAUTIONS:

- Biological products must be handled with all necessary precautions and considered as being potentially infectious.
- In contact with lead or copper pipes, sodium azide can generate explosive compounds.
- A yellow color indicates a contaminated substrate. Discard the vial and use a new one.
- Waste should be disposed of in accordance with applicable local regulations.
- Use only the reagents from the same batch of kits. Do not mix reagents from different kit batches when performing an assay; they are optimized for each batch of kits.
- Handle the reagents with care to avoid contamination during use. If possible, avoid reagent evaporation during use by limiting the liquid-air exchange surface. Evaporation reduces the reagent's stability in the analyzer.
- To preserve reagent stability, seal the vials after use with their respective caps.
- Aging studies, conducted over a 3-week period at 30 °C, show that the reagents can be shipped at room temperature over a short period of time, without degradation.
- The human plasma used to prepare the Factor Xa has been tested by recorded methods and is certified free of HIV antibodies, Hbs Antigen and HCV antibodies. The bovine plasma used to prepare the BSA has been tested by recorded methods and is certified free of infectious agents, in particular the causative agent of bovine spongiform encephalitis.
- If necessary, the Factor Xa concentration is adjusted for each batch in order to achieve optimum reactivity and linearity for the assay.
- Create a plasma blank if this latter 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.
- For in vitro diagnostic use.

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

REAGENT PREPARATION AND STABILITY:

The reagents are freeze-dried under a vacuum in their vials. To avoid any product loss when opening the vial, gently remove the freeze-drying stopper.

R1 **Reagent 1**: **Human Factor Xa**
Reconstitute the contents of each vial with exactly **2.5 mL distilled water**, shake vigorously until fully dissolved.
Allow to stabilize for 30 min. at room temperature (18-25°C), shaking occasionally.
Homogenize the reagent prior to use.
Reagent stability after reconstitution, excluding any contamination or evaporation, and subject to storage in the original vial, is of:

- **15 days** at 2-8°C.
- **7 days** at room temperature (18-25°C).
- **2 months** frozen at -20°C or less*

R2 **Reagent 2**: **Freeze-dried Factor Xa-specific chromogenic substrate:**
Reconstitute the contents of each vial with exactly **2.5 mL distilled water**, shake vigorously until fully dissolved.
Allow to stabilize for 30 min. at room temperature (18-25°C), shaking occasionally.
Homogenize the reagent prior to use.
Reagent stability after reconstitution, excluding any contamination or evaporation, and subject to storage in the original vial, is of:

- **2 months** at 2-8°C.
- **7 days** at room temperature (18-25°C).
- **2 months** frozen at -20°C or less*

*Thaw only once, as rapidly as possible at 37°C, adapting the incubation period to the volume of reagent. The stability of the thawed reagent should be checked under laboratory work conditions.

R3 **Reagent 3**: **Reaction buffer:**
Ready to use. Allow to stabilize for 30 min. at room temperature (18-25°C) before use.
Homogenize thoroughly before use.
Reagent stability, excluding any contamination or evaporation, and subject to storage in the original vial, is of:

- **2 months** at 2-8°C.
- **7 days** at room temperature (18-25°C).

STORAGE CONDITIONS:

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.

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	BIOPHEN™ Apixaban Calibrator Low	BIOPHEN™ Rivaroxaban Calibrator	BIOPHEN™ Rivaroxaban Calibrator Low
References	226201	226101	222701	226001
Inspections	**BIOPHEN™ Apixaban Control**	**BIOPHEN™ Apixaban Control Low**	**BIOPHEN™ Rivaroxaban Control**	**BIOPHEN™ Rivaroxaban Control Low**
References	225301	225201	224501	225101

Materials:

- Spectrophotometer and chromogenic assay analyzer.
- Stopwatch, calibrated pipettes.

SPECIMEN COLLECTION AND PREPARATION:

Specimens should be prepared and stored in accordance with applicable local guidelines (for the United States, see the CLSI H21-A5 guidelines for further information on collection, handling and storage)[5].

- Specimens:

Human plasma obtained from anti-coagulated blood (trisodium citrate).

- Collection:

The blood (9 volumes) should be carefully collected onto the trisodium citrate anticoagulant (1 volume) (0.109 M) by clean venipuncture. Discard the first tube.

- Centrifugation:

Within two hours, use a laboratory-validated method to obtain platelet-poor plasma, for example at least 15 minutes at 2500 g at room temperature (18-25°C) and allow the plasma to settle in a plastic tube.

- Plasma storage[6]:
  - 4 hours at room temperature (18-25°C)
  - 1 month at -20°C.
  - 18 month at -70°C.

Frozen plasma specimens should be thawed rapidly at 37°C, then shaken thoroughly and tested immediately. Resuspend any precipitate by shaking vigorously immediately after thawing and before use.

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**.

Automated method:
Applications for the various analyzers are available on request. **See the specific application 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	Calibrator references	Control references	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 within 2 hours. 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	Calibrator references	Control references	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 within 2 hours. 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.

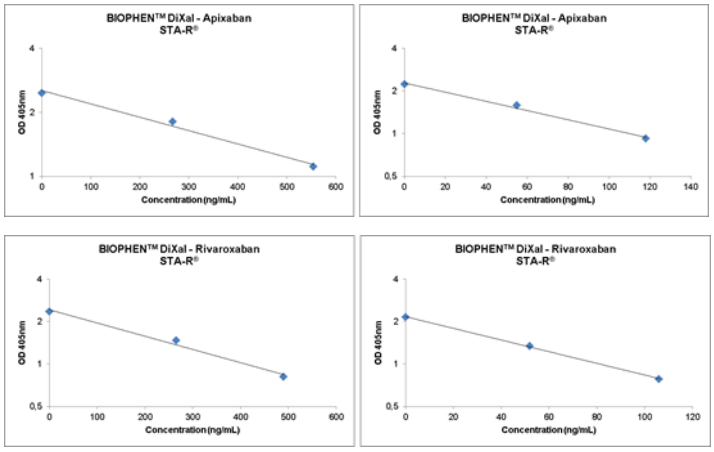
Measure the optical density at 405 nm. Subtract the measured blank value from the test absorbance.

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 and Rivaroxaban. 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, obtained by the STA-R® method, are given by way of example only. The calibration curve established for the assay series must be used.

Standard range **Low 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.
- The concentration of DiXal in the test sample is inferred directly from the calibration curve.
- The results are expressed, for example, in **ng/mL** of DiXal.
- 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. The laboratory is responsible for validating any changes made to these instructions for use.
- 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.
- Any plasma displaying a coagulum or showing signs of contamination 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 and Rivaroxaban are not found in normal plasma.
The normal internal, therapeutic range and hemorrhagic risk range should be defined according to applicable local guidelines.
The results should be interpreted according to the patient's clinical and biological status.

PERFORMANCE:

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

	Rivaroxaban assay				Apixaban assay			
Measurement range	0-500 ng/mL		0-100 ng/mL		0-600 ng/mL		0-120 ng/mL	
	N	CV%	N	CV%	N	CV%	N	CV%
Intra-test	30	2.17	30	2.50	30	1.47	30	1.78
Inter-test	10	3.35	10	6.97	10	4.10	12	5.85

- **The assay was optimised to avoid any plasma factor interference. The assay is completely insensitive to indirect Factor Xa inhibitors such as heparins 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 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.

REFERENCES:

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5. 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
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SYMBOLS:

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

Changes compared to the previous version.