CE

IVD

BIOPHEN™ FVIII variant

REF 227102

R1 R2 R3 2 vials x 2.5 mL

R4 4 vials x 20 mL

HYPHEN BioMed

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INTENDED USE:

Chromogenic method for the *in vitro* quantitative determination of Factor VIII (FVIII) activity, in human citrated plasma or FVIII concentrates, using an automated method. This method is for the detection of FVIII deficiency states and monitoring of replacement therapy (except Emicizumab) on patients who are suspected of congenital or acquired deficiency and potency estimation of FVIII concentrates.
This device of *in vitro* diagnostic use is intended for professional use in the laboratory.

SUMMARY AND EXPLANATION:

Technical:[1-6]
Factor VIII is a heterodimeric glycoprotein consisting of a metal ion-linked light chain and a heavy chain and circulates in plasma at a concentration of approximately 200 ng/mL. FVIII interacts non-covalently with von Willebrand Factor which dramatically prolongs its half-life in blood circulation. Activated FVIII accelerates more than 100 000-fold the Factor X activation in the presence of Factor IXa, phospholipids and calcium ions.
Clinical:[5-11]
Hemophilia A is an X-linked recessive disorder leading to a deficiency of functional FVIII. Bleeding tendency is directly linked to the level of FVIII activity and patients are classified as mild (5% to 40% of FVIII), moderate (1% to 5% of FVIII), or severe (<1% of FVIII). Treatment for hemophilia consists of replacing the missing clotting factor (FVIII) on a regular basis (prophylaxis) or episodically (on-demand treatment). FVIII levels are reduced in von Willebrand's disease (vWD) or in case of Disseminated Intravascular Coagulation (DIC) or acquired FVIII inhibitor. Elevated concentrations of FVIII are observed in inflammatory or hepatic diseases and may be suggestive of an increased risk of venous thrombosis.

PRINCIPLE:

The BIOPHEN™ Factor VIII variant method involves the chromogenic assay of FVIII cofactor. In the presence of phospholipids (PLPs) and calcium, FVIII, activated by thrombin, forms an enzyme complex with Factor IXa, which activates Factor X. The resulting Factor Xa hydrolyzes the chromogenic substrate, leading to the release of paranitroaniline (pNa). The amount of pNA released (measured by absorbance at 405 nm) is directly proportional to the concentration of FVIII in the specimen (Factor IXa, Thrombin and Factor X are in constant excess amount).
The BIOPHEN™ FVIII variant kit is a variant method of BIOPHEN™ FVIII (221402 / 221406) where FX is from bovine origin and which is insensitive to Emicizumab.

REAGENTS:

R1 **Bovine Factor X** at approximately 7.5 µg/mL, lyophilized. Contains a fibrin polymerization inhibitor, BSA and stabilizers.
R2 **Activation Reagent,** human Factor IIa at approximately 1 NIH/mL, human Factor IXa at approximately 2 µg/mL and synthetic Phospholipids (1:40 dilution), lyophilized. Contains BSA, Calcium Chloride Dihydrate and stabilizers.
R3 **SXa-11,** chromogenic substrate specific to Factor Xa (CS-11(32)) at approximately 6 mg/mL, lyophilized. Contains stabilizers and EDTA disodium salt.
H373: May cause damage to organs through prolonged or repeated exposure.
R4 **Tris-BSA Buffer,** liquid. Contains BSA, human acid glycoprotein (AGP), stabilizers and preservatives.

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

REAGENT PREPARATION:

Gently remove the freeze-drying stopper, to avoid any product loss when opening the vial.

R1 R2 R3 Reconstitute the contents of each vial with exactly **2.5 mL of distilled water.**
Shake vigorously until **complete dissolution** (ensure that there is no deposit at the bottom of the R3 vial, if necessary, let each R3 vial stabilize for at least 15 minutes at 37°C and homogenize before use) while avoiding formation of foam and load it directly on the analyzer following Application Guide instruction.

R4 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 R4 Reagent stability after reconstitution / opening, free from any contamination or evaporation, and stored closed, is of:

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

Combination of storage are not recommended.

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 with known titration, such as:

Product Name	Reference
BIOPHEN™ Plasma Calibrator	222101
BIOPHEN™ Normal Control Plasma	223201
BIOPHEN™ Abnormal Control Plasma	223301

- For low-range calibration, dilute the calibrator in Factor VIII Deficient Plasma (DP040A/DP040K, HYPHEN BioMed).
- Automatic analyzer for chromogenic assays such as: CS-series.
- 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[12] guideline for further information concerning specimen collection, handling and storage).
For plasma storage, please refer to references[12,13,14].

PROCEDURE:

For the low range, the 6 calibration points should be prediluted in Factor VIII Deficient Plasma to obtain the calibration range from about 0.9% to 28% before loading onto the analyzer.

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

For FVIII therapeutic concentrates, pre-dilute the test specimen (**high range**) in **R4**.

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 FVIII (%) in the test specimen is directly inferred from the calibration curve, when the standard dilution is used.
- For FVIII concentrates, the measured concentration should then be multiplied by the "pre-dilution" factor.
- Lot to lot variability measured on 3 lots is: %CV ≤ 10%.
- 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.
- 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.
- FVIII assay is sensitive to direct anti-Xa.
- Depending on the variant and concentrate, discrepancy between chromogenic and clotting assays is reported[5,6,9,16].

EXPECTED VALUES:

Each laboratory has to determine its own normal range.

PERFORMANCES:

Mathematical analysis are performed using a validated statistical software built in accordance with CLSI guidelines.
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™ FVIII variant.

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 over a 5-days period, 2 series per day and 2 repetitions within each series for a sample level. Coefficient of variation (CV) for all samples is less than 10 % and 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.
This FVIII assay is insensitive to Emicizumab[15].

Clinical performances

Agreement

	CS series (n = 93)		
Analyte	**Linear regression**	**r**	**Reference / comparison method**
Factor VIII	y = 0.96x + 0.31	0.999	Factor VIII Chromogenic assay (Siemens)

Sensitivity/Specificity

	CS series (n = 93)		
Analyte	**Sensitivity**	**Specificity**	**Area under the curve**
Factor VIII	0.939	0.955	0.968

REFERENCES:

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eIFU is available on the HYPHEN BioMed website.

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
ACCEPTANCE RANGE	Acceptance range				Biological risks

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