PrecisionBioLogic

CRYO*check*™ IVD

FACTOR DEFICIENT PLASMAS

FACTOR VIII DEFICIENT PLASMA WITH VWF

Intended Use

CRYO*check* Factor VIII Deficient Plasma with VWF is for clinical laboratory use as a deficient substrate in the quantitative determination of Factor VIII activity in 3.2% citrated human plasma based on the activated partial thromboplastin time (APTT) assay on an automated instrument. It is intended to be used in identifying factor VIII deficiency and as an aid in the management of hemophilia A in individuals aged 2 years and older. For in vitro diagnostic use.

Summary and Principle

Deficiencies in coagulation factors may have congenital or acquired etiologies and can compromise in vivo hemostasis[1]. Factor VIII (antihemophilic A factor) is a glycoprotein with a molecular weight of at least 250,000 Da[2]. It is present in vivo as a complex with von Willebrand Factor (VWF) and is necessary for intrinsic coagulation. Plasma samples deficient in coagulation factor VIII exhibit a prolonged APTT. Factor VIII deficiency (hemophilia A) is commonly diagnosed through the use of a modified APTT assay. When a patient sample is mixed with factor VIII deficient plasma, the degree of correction of the APTT is proportional to the level of factor VIII in the patient plasma[3,4].

Reagents

CRYO*check* Factor VIII Deficient Plasma with VWF is plasma which has been immunodepleted of factor VIII and which contains normal levels of von Willebrand Factor (VWF). Factor VIII has been assayed at less than 1% of normal antigen and activity levels while VWF antigen and activity levels are >50%.

For PRESCRIPTION USE ONLY

Storage, Preparation and Handling

When stored at -70 °C or below CRYO*check* Factor VIII Deficient Plasma with VWF is stable to the end of the month indicated on the product packaging.

Thaw each vial at 37 °C (± 1 °C) in a waterbath. **The use of a dry bath or heating block for thawing is not recommended.** Thawing times are important and should be strictly adhered to. The use of a timer is

recommended. Refer to the Thawing Table for recommended thawing times based on aliquot size. Invert thawed plasma gently prior to use.

Thawing Table	
Aliquot Size	**37 °C (± 1 °C) Waterbath**
1.0 mL	4 minutes
1.5 mL	5 minutes

CRYO*check* Factor VIII Deficient Plasma with VWF may be used for up to 24 hours after thawing when stored on-board the analyzer (maintained at 15 ± 1 °C), or when capped in the original vial and maintained at 2 to 8 °C. Invert the refrigerated plasma gently prior to use. Thawed material should be discarded after 24 hours and should not be refrozen.

Availability

Product	Catalog #	Format
CRYO*check* Factor VIII Deficient Plasma with VWF	FDP08VWF-10	25 vials x 1.0 mL
	FDP08VWF-15	25 vials x 1.5 mL

Instruments

Each lab should prepare the local instrument in accordance with the manufacturer's instructions for use.

Procedure

After thawing and preparing CRYO*check* Factor VIII Deficient Plasma with VWF, use in accordance with established laboratory procedures for the quantitative assessment of factor VIII.

Materials Provided

- CRYO*check* Factor VIII Deficient Plasma with VWF

Materials Required but not Provided

- Waterbath capable of maintaining temperature at 37 °C (± 1 °C)
- Floatie for thawing vials in waterbath
- Assay reagents (e.g. APTT reagent, Calcium Chloride, Assay Diluent (Buffer))
- Coagulation instrument
- Calibrator plasma (e.g. CRYO*check* Normal Reference Plasma)
- Quality Control Material (e.g. CRYO*check* Reference Control Normal, CRYO*check* Abnormal 1 Reference Control, CRYO*check* Abnormal 2 Reference Control)
- Siliconized 4 mL glass vials (optional)
- Timer
- Transfer pipette

Standard Curve Preparation

Methods may vary according to instrumentation used. Consult the instrument manufacturer's instruction manual for recommended factor assay (intrinsic protocols).

1. Prepare calibrator plasma, APTT reagent, $CaCl_2$ reagent, and assay diluent (buffer) according to their corresponding instructions for use.
2. The calibrator plasma is diluted using the assay diluent. Below is an example of a dilution profile when using a calibrator plasma with a FVIII activity level of 100%. Results will vary based on the FVIII activity in the calibrator plasma and instrument used.

Tube No.	Proportion of Buffer	Proportion of Calibrator Plasma	% Factor VIII
1	85	15	150
2	90	10	100
3	95	5	50
4	97.5	2.5	25
5	99.9	0.1	10
6	99.95	0.05	5
7	99.98	0.02	2
8	100	0	0

3. The analyzer measures the APTT clot time of each diluted calibrator sample and creates a standard curve based on the target Factor VIII value.
4. The curve is used to report Factor VIII activity of test samples assayed using this method.

Specimen Collection and Preparation

Samples should be collected into 105 - 109 mmol/L sodium citrate dihydrate anticoagulant (3.2% w/v) in a ratio of 9 parts blood to 1 part anticoagulant in accordance with the Clinical Laboratory Standards Institute (CLSI) guidelines[5]. Plasma is derived by centrifugation at 1500 x g for 15 minutes in order to achieve platelet-poor plasma (<10,000 platelets/µL) and should be tested within four hours of collection when maintained at room temperature. If samples are not to be tested within four hours, then plasma should be removed from the cells and frozen at ≤ -70 °C for up to one month. Note that FVIII is a labile protein. Improper handling of a specimen may give a false result.

Assay Procedure

1. Prepare CRYO*check* Factor VIII Deficient Plasma with VWF according to Storage, Preparation and Handling instructions above.
2. Prepare one vial per 14 tests or pool two vials when generating a calibration curve.
3. Prepare instrument according to the manufacturer's instructions for use.
4. Prepare assay reagents (e.g. APTT reagent, $CaCl_2$, Factor Diluent) according to manufacturer's instructions for use and load on the instrument.
5. If CRYO*check* Factor VIII Deficient Plasma with VWF cannot be loaded directly onto the analyzer, it may be transferred to a 4 mL siliconized glass vial to be loaded on the instrument.
6. Load samples on the instrument.
7. Measure the FVIII activity of plasma samples using the appropriate instrument protocol.

Results and Interpretation

Factor VIII results are reported in % activity where 100% FVIII activity is equivalent to 1.0 IU/mL. FVIII values recovered below the laboratory established normal range may be indicative of hemophilia A. Hemophilia A can be classified into mild (5% to <40% FVIII), moderate (1% to 5% FVIII) and severe (<1% FVIII)[6] categories.

Quality Control

Each laboratory should establish its own quality control (QC) ranges using acceptable statistical methods. These QC ranges may then be used to monitor and validate the integrity of the test system[7]. For all coagulation tests, the laboratory must include at least two levels of control for every eight hours of operation and any time a change in reagents occurs[8].

Limitations of the Procedure

When proper control values are not obtained, assessment of each component of the test system including reagents, control plasmas, instrumentation and operator technique must be undertaken in order to ascertain that all other components are functioning properly.

Expected Values

Expected values may vary according to reagent, instrument and technique employed as well as population age and characteristics. It is recommended each laboratory establish its own normal range for factor VIII activity.

Expected factor VIII activity values are 50-150% (0.50-1.50 IU/mL).

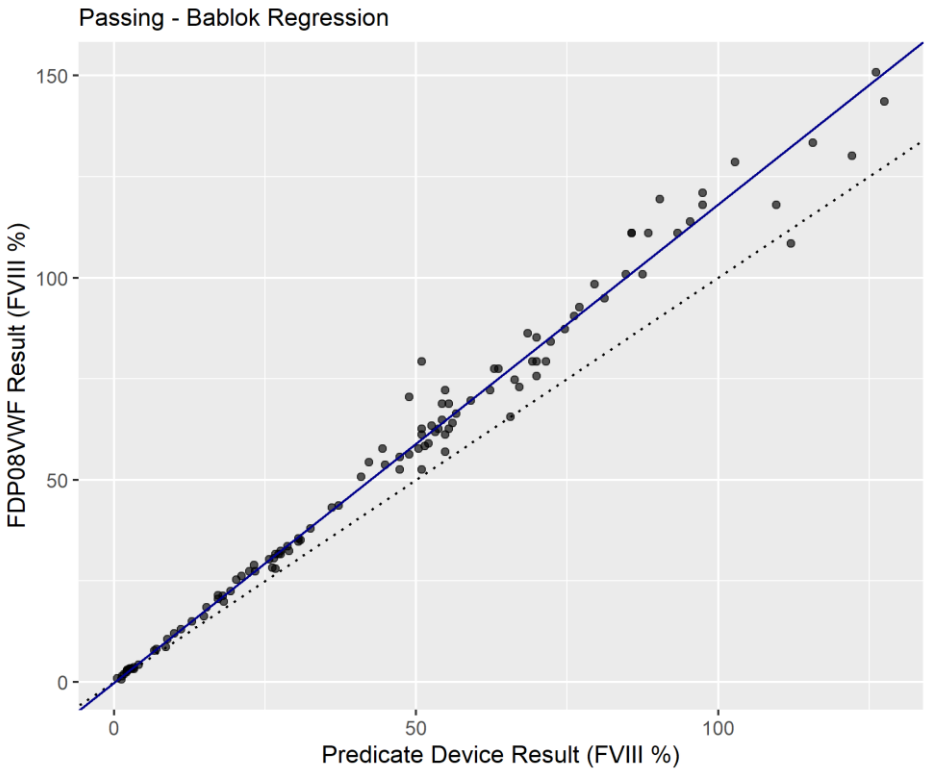
Performance Characteristics

All studies were performed using the HemosIL® SynthASil APTT reagent (with 20 mM $CaCl_2$) and IL Factor Diluent with an IL ACL TOP instrument unless otherwise noted.

Method Comparison

A method comparison study was conducted according to CLSI EP09c[9] to compare the accuracy of factor VIII activity measurement when using CRYO*check* Factor VIII Deficient Plasma with VWF in a modified APTT assay relative to a comparator device. Aliquots of human plasma from normal ostensibly healthy individuals, from patients with congenital or acquired hemophilia A and Type 1, Type 2A, Type 2N and Type 3 von Willebrand disease (N=112) were tested in the study. Results were compared by Passing-Bablok regression analysis. Regression statistics showed that CRYO*check* Factor VIII Deficient Plasma with VWF performed equivalently to the comparator method.

N	Slope		Intercept		Pearson Correlation Coefficient (R)
	Value	95% CI	Value	95% CI	
112	1.18	1.16 – 1.20	-0.2	-0.69 – 0.17	0.99



Precision

A precision study was performed using three lots of CRYO*check* Factor VIII Deficient Plasma with VWF as the substrate in a modified APTT assay to quantify FVIII activity in three controls and three patient plasma samples according to CLSI EP05-A3[10]. Each sample was measured with each lot of product in duplicate, twice a day for 20 days for a total of 80 replicates per sample per lot. The results demonstrated a pooled precision of <10% CV for all controls and normal sample, and ≤ 0.1% SD for the two Hemophilia A (Low and Very Low) samples.

Sample	Mean FVIII Activity (%)	Within-Laboratory Precision	
		SD	%CV
CRYO*check* Reference Control Normal	100.4	5.7	5.7
CRYO*check* Abnormal 1 Reference Control	37.7	2.3	6.2
CRYO*check* Abnormal 2 Reference Control	11.3	0.6	5.2
High FVIII Plasma Sample	164	9.8	6.0
Low FVIII Plasma Sample (Moderate Hemophilia A)	2.0	0.2	NA
Very Low FVIII Plasma Sample (Severe Hemophilia A)	0.1	0.0	NA

Linearity

A linearity study was conducted in accordance with CLSI EP06-A[11] using a single lot of CRYO*check* Factor VIII Deficient Plasma with VWF in a modified APTT assay to quantify FVIII activity of fifteen samples created by combining plasma with a high FVIII concentration (~ 260%) with congenital hemophilia A patient plasma (0% FVIII). These fifteen samples yielded an estimated FVIII activity in the range of 0 to 260%. The results support a linear range of 0 to 175%.

Interferences

Interference studies were conducted according to CLSI EP07[12] using a single lot of CRYO*check* Factor VIII Deficient Plasma with VWF in a modified APTT assay. Plasma samples were spiked with possible interferents, and 10 replicates were tested alongside 10 replicates of the corresponding blank matrix control. The following substances showed no interference at the concentration indicated.

Substance Tested	Sample Concentration
Intralipid	2000 mg/dL
Bilirubin (unconjugated)	40 mg/dL
Lupus Anticoagulant	≤1.8 dRVVT ratio

Hemoglobin at 1000 mg/dL and Bilirubin (conjugated) at 40 mg/dL were shown to interfere with sample results.

Precautions/Warnings

Do not use the product if it is thawed upon receipt, if the vial appears cracked, or if upon thawing the product appears to have clotted. Transferring the deficient substrate material into another container other than siliconized glass or polypropylene could have performance impact and is not recommended.

Any serious incident that has occurred in relation to the use of this device shall be reported to the manufacturer and the competent authority of the Member State in which the user and/or patient is established.

All blood products should be treated as potentially infectious. Source material from which this product was derived was found to be negative when tested in accordance with current required tests for transfusion-transmitted diseases. No known test methods can offer assurance that products derived from human blood will not transmit infectious agents. Accordingly, these human blood-based products should be handled and discarded as recommended for any potentially infectious human specimen[13].

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Symbols Used

IVD	In vitro diagnostic medical device		Biological risks
LOT	Batch code		Manufacturer
REF	Catalogue number	EC REP	Authorized representative in the European Community / European Union
	Use by date	**Rx ONLY**	For prescription use only
	Temperature limit		Consult instructions for use

EC REP European Authorized Representative (Regulatory affairs only)
Emergo Europe—Prinsessegracht 20, 2514 AP The Hague, The Netherlands

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