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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 (FVIII) activity in 3.2% citrated human plasma based on the activated partial thromboplastin time (APTT) assay. 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 FVIII 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 FVIII deficient plasma, the degree of correction of the APTT is proportional to the level of FVIII in the patient plasma[3,4].

Reagents

CRYO*check* Factor VIII Deficient Plasma with VWF is normal human citrated plasma that has been immunodepleted of FVIII and which contains normal levels of VWF, buffered with HEPES. Factor VIII has been assayed at <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 -40 to -80 °C, 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 using the waterbath "floatie" thawing device (provided separately). **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.

Low relative humidity is associated with increased evaporation of uncapped reagents, which may decrease on-board stability. For optimum on-board stability, room temperature and humidity should be controlled.

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.

Instruments: Instrumentation Laboratories (IL) ACL TOP and ACL TOP Family 50 Series.

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 FVIII.

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 FVIII value.
4. The curve is used to report FVIII 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 two hours of collection when maintained at room temperature. If samples are not to be tested within two 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. Load the thawed CRYO*check* Factor VIII Deficient Plasma with VWF vial(s) onto the instrument. Note: 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. Factor VIII 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 FVIII activity.

The assay reference range was established using 136 citrated plasma samples collected from normal ostensibly healthy individuals using three lots of CRYO*check* Factor VIII Deficient Plasma with VWF in a modified APTT assay on two IL ACL TOP instruments according to CLSI EP28- A3c[9]. The reference interval was determined to be 62–163% FVIII activity by calculating the non-parametric 95% confidence interval (2.5th to 97.5th percentiles).

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 at four sites (one internal and three external) according to CLSI EP09c[10] to compare the accuracy of FVIII activity measurement when using CRYO*check* Factor VIII Deficient Plasma with VWF in a modified APTT assay relative to a comparator device. The internal site used an IL ACL TOP 500, and the three external sites used IL ACL TOP 700 CTS, IL ACL TOP 750 CTS and IL ACL TOP 700 instruments, respectively. Aliquots of human plasma from normal ostensibly healthy individuals, from patients with congenital or acquired hemophilia A, patients with hemophilia B,

patients with von Willebrand disease, hemophilia A patients on recombinant FVIII replacement therapies, and patients with other factor deficiencies (N=366) 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	
Site 1	115	1.15	1.12 to 1.19	0.17	-0.38 to 0.64	0.93
Site 2	125	1.19	1.16 to 1.22	-0.26	-0.48 to -0.09	0.97
Site 3	108	1.10	1.06 to 1.15	0.97	0.24 to 1.99	0.98
Site 4	18	1.22	0.95 to 1.31	1.19	-3.99 to 13.96	0.98
Overall	**366**	**1.16**	**1.15 to 1.18**	**-0.08**	**-0.25 to 0.13**	**0.96**

Absolute predicted biases at medical decision levels are reported below.

FVIII Activity (%)	Predicted Absolute Bias (%)	Lower CI (%)	Upper CI (%)
1	0.37	-1.42	2.17
5	0.91	-0.79	2.62
50	6.97	5.85	8.09
100	13.70	11.84	15.56

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[11]. 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 precision of <10% CV for all controls and normal sample, and < 0.5 SD for the two hemophilia A (low and very low) samples.

Sample	Mean FVIII Activity (%)	Within-Run Precision		Total Precision	
		SD	%CV	SD	%CV
CRYO*check* Reference Control Normal	100.4	3.5	3.4	5.7	5.7
CRYO*check* Abnormal 1 Reference Control	37.7	1.7	4.6	2.3	6.2
CRYO*check* Abnormal 2 Reference Control	11.3	0.4	3.7	0.6	5.2
High FVIII Plasma Sample	164	6.1	3.7	9.8	6.0
Low FVIII Plasma Sample (Moderate Hemophilia A)	2.0	0.2	9.3	0.2	11.1
Very Low FVIII Plasma Sample (Severe Hemophilia A)	0.1	0.0	18.2	0.0	24.1

Reproducibility:

Reproducibility studies were conducted at three sites (one internal and two external) on IL ACL TOP 500, IL ACL TOP 700 CTS, and IL ACL TOP 750 CTS analyzers using three lots of CRYO*check* Factor VIII Deficient Plasma with VWF in accordance with CLSI EP05-A3[11]. The study quantified one normal and two abnormal reference controls and three patient plasma samples representing very low, low and high levels of FVIII activity. Each sample was measured with each product lot in triplicate, twice a day for five days. The pooled data across three sites demonstrated a reproducibility <10 % CV for all controls and high FVIII plasma sample, and <0.5 SD for the low and very low FVIII plasma samples.

Sample	N	Mean (%)	Repeatability (Within-Run)		Between-Run		Between-Day		Between-Lot		Between-Site		Reproducibility (Total)	
			SD	%CV	SD	%CV	SD	%CV	SD	%CV	SD	%CV	SD	%CV
CRYO*check* Reference Control Normal	270	98.97	3.00	3.03	2.07	2.09	1.78	1.79	1.92	1.94	3.95	3.99	5.98	6.04
CRYO*check* Abnormal 1 Reference Control	270	37.40	1.25	3.33	0.63	1.68	0.61	1.63	0.80	2.14	0.44	1.16	1.77	4.75
CRYO*check* Abnormal 2 Reference Control	270	11.87	0.41	3.48	0.18	1.55	0.28	2.43	0.33	2.81	0.66	5.54	0.91	7.68
High FVIII Plasma Sample	269	166.37	6.54	3.93	2.08	1.25	1.45	0.87	4.34	2.61	1.78	1.07	8.44	5.07
Low FVIII Plasma Sample (Moderate Hemophilia A)	270	1.93	0.11	5.84	0.06	3.08	0.03	1.81	0.08	4.01	0.09	5.01	0.18	9.39
Very Low FVIII Plasma Sample (Severe Hemophilia A)	270	0.11	0.03	30.24	0.01	5.90	0.01	10.16	0.01	4.52	0.02	20.34	0.04	38.56

Linearity

A linearity study was conducted in accordance with CLSI EP06-Ed2[12] using three lots of CRYO*check* Factor VIII Deficient Plasma with VWF in a modified APTT assay to quantify FVIII activity of 15 samples created by combining plasma with a high FVIII concentration (~ 260%) with congenital hemophilia A patient plasma (0% FVIII). These 15 samples yielded an estimated FVIII activity in the range of 0 to 260%. The results support a linear range of 0 to 230%.

Interferences

Interference studies were conducted according to CLSI EP07[13] 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
Hemoglobin	≤1000 mg/dL
Intralipid	≤2000 mg/dL
Bilirubin (conjugated)	≤4.0 mg/dL
Bilirubin (unconjugated)	40 mg/dL
Lupus Anticoagulant	≤1.8 dRVVT ratio

Substance Tested	Sample Concentration
Warfarin	≤ INR ratio 2.72

Rivaroxaban, fondaparinux, dabigatran, emicizumab, unfractionated heparin, and low molecular weight heparin were shown to interfere with sample results.

Recovery of FVIII Replacement Products

This device accurately evaluated the potency of FVIII replacements products including Advate, Afstyla Eloctate, Jivi, Novoeight, Wilate at concentrations ranging from 0.05 to 1.0 IU/mL.

Product	Mean Percent Recovery (%)
Advate	92.84
Afstyla*	97.38
Eloctate	94.90
Jivi	104.49
Novoeight	113.33
Wilate	94.92

**Per the manufacturer's recommendation, a chromogenic assay is recommended for measurement of Afstyla. Mean percent recovery value includes 2x correction based on manufacturer's recommendation that one stage clotting assay shows an under recovery of 50%.*

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[14].

Bibliography

1. Biggs R. *Human blood coagulation, haemostasis and thrombosis 3rd ed.* Oxford: Blackwell Scientific Publications; 1984.
2. Halkier T. *Mechanisms in blood coagulation, fibrinolysis, and the complement system*. Cambridge: Cambridge University Press; 1991.
3. Triplett DA, Smith C. *Routine testing in the coagulation laboratory*. In: Triplett DA, editor. Laboratory evaluation of coagulation. Illinois: ASCP Press; 1982. p. 28-51.
4. CLSI. *Determination of Coagulation Factor Activities Using the One-Stage Clotting Assay*. 2nd ed. CLSI guideline H48. Wayne, PA (USA); Clinical and Laboratory Institute, 2016.
5. CLSI. *Collection, Transport, and Processing of Blood Specimens for Testing Plasma-Based Coagulation Assays and Molecular Hemostasis Assays*. Approved Guideline-5th ed. CLSI guideline H21-A5. Wayne, PA (USA); Clinical and Laboratory Standards Institute, 2008.
6. Srivastava A, Brewer AK, Mauser-Bunschoten EP, Key NS, Kitchen S, Llinas A et al. (2013). *Guidelines for the Management of Hemophilia*. 2nd Edition. Haemophilia. 2013; 19 (1), e1-47.
7. Cembrowski GS, Carey RN. *Laboratory Quality Management*. Chicago: ASCP Press; 1989. p. 166-171.
8. CLIA 2004 – Code of Federal regulations, 42CFR493.1269, 2004.
9. CLSI. *Defining, Establishing and Verifying Reference Intervals in the Clinical Laboratory*. Approved Guideline-3rd ed. CLSI guideline EP28-A3c. Wayne, PA (USA): Clinical and Laboratory Standards Institute, 2010.
10. CLSI. *Measurement Procedure Comparison and Bias Estimation Using Patient Samples*. 3rd ed. CLSI guideline EP09c., Wayne, PA(USA); Clinical and Laboratory Standards Institute, 2018.
11. CLSI. *Evaluation of Precision of Quantitative Measurement Procedures*. Approved Guideline – 3rd ed. CLSI guideline EP05-A3. Wayne, PA (USA): Clinical and Laboratory Standards Institute, 2014.
12. CLSI. *Evaluation of Linearity of Quantitative Measurement Procedures; A Statistical Approach*. Approved Guideline. CLSI guideline EP06-2nd Edition. Wayne, PA (USA): Clinical and Laboratory Standards Institute, 2021.
13. CLSI. *Interference Testing in Clinical Chemistry*. 3rd ed. CLSI guideline EP07. Wayne, PA (USA): Clinical and Laboratory Standards Institute, 2018.
14. Biosafety in Microbiological and Biomedical Laboratories 6th ed. Centers for Disease Control and Prevention / National Institutes of Health, 2020.

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 electronic instructions for use

EC REP European Authorized Representative (Regulatory affairs only)
Emergo Europe—Westervoortsedijk 60 6827 AT Arnhem, The Netherlands

UKRP United Kingdom Responsible Person
Emergo Consulting (UK) Limited—c/o Cr 360—UL International
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