PrecisionBioLogic

CRYO*check*™ IVD

CHROMOGENIC FACTOR VIII

Intended Use

CRYO*check* Chromogenic Factor VIII is for clinical laboratory use in the quantitative determination of factor VIII activity in 3.2 % citrated human plasma. 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

Factor VIII is a critical component in the normal blood clot-forming process; upon activation of the coagulation cascade, it binds to factors IXa and X (FIXa and FX) to enable activation of FX and subsequent downstream thrombin activation, which leads to cleavage of fibrinogen and formation of a polymerized fibrin clot. Hemophilia A is an inherited bleeding disorder caused by a decrease or functional deficiency in factor VIII (FVIII), which leads to a lifelong bleeding tendency. The standard treatment for patients with hemophilia A without inhibitors is intravenous (IV) FVIII replacement therapy with recombinant FVIII (rFVIII) or plasma-derived FVIII (pdFVIII) concentrates. CRYO*check* Chromogenic Factor VIII is used for determination of FVIII activity. Unlike clot-based FVIII activity tests, the chromogenic method offers the advantage of being less prone to interference from lipids or traces of heparin in samples[1].

CRYO*check* Chromogenic Factor VIII is a two-stage assay for use with automated coagulometers. In the first stage of the chromogenic assay, test plasma (containing an unknown amount of FVIII) is added to a reaction mixture comprised of calcium, phospholipids, purified human thrombin, and bovine FIXa and FX. This mixture swiftly activates FVIII to FVIIIa, which works in concert with FIXa to activate FX. When the reaction is stopped, FXa production is assumed to be proportional to the amount of functional FVIII present in the sample. The second stage of the assay is to measure FXa through cleavage of a FXa-specific peptide nitroanilide substrate. The product (p-nitroaniline) produces a yellow color that can be measured spectrophotometrically by absorbance at 405 nm. The color produced is directly proportional to the amount of functional FVIII present in the sample based on a standard curve.

Reagents

- **Reagent 1 (R1):** Bovine FX and a fibrin polymerization inhibitor, plus activators and stabilizer.
- **Reagent 2 (R2):** Human FIIa, bovine FIXa, calcium chloride and phospholipids.
- **Reagent 3 (R3):** FXa substrate containing EDTA and a thrombin inhibitor.
- **Diluent Buffer:** Tris buffer solution containing 1 % BSA and a heparin antagonist.

FOR PRESCRIPTION USE ONLY

Storage, Preparation and Handling

When stored at -70 °C, CRYO*check* Chromogenic Factor VIII is stable to the end of the month indicated on the product packaging.

Thaw one vial set (see Availability table for vial set description) at 37 °C (± 1 °C) in a waterbath using the waterbath "floatie" thawing device (provided separately). Thaw times are important and should be strictly adhered to. **The use of a dry bath or heating block for thawing is not recommended.** The use of a timer is recommended. Refer to the Thawing Table for recommended thawing times according to aliquot size. Ensure each reagent is thawed, and gently invert each vial prior to use.

Thawing Table	
Aliquot Size	**37 °C (± 1 °C) Waterbath**
1.25 mL	4 minutes
7.0 mL	7 minutes

When first thawed, CRYO*check* Chromogenic Factor VIII may be used for up to eight hours on board the analyzer. When not in use, the reagents should be capped in the original vials and maintained at 2 to 8 °C for up to five days. Reagents that have been in use for up to four hours may be refrozen once at -70 °C and stored for up to one month. Previously refrozen reagents can be thawed and used once for up to four hours on board the instrument.

NB: CRYO*check Chromogenic Factor VIII components are lot-specific and should not be interchanged with other lot numbers.*

Availability

Product	Catalog #	Format
CRYO*check* Chromogenic Factor VIII	CCCF08	Reagent 1 (R1): 4 vials x 1.25 mL (grey cap) Reagent 2 (R2): 4 vials x 1.25 mL (black cap) Reagent 3 (R3): 4 vials x 1.25 mL (yellow cap) Diluent Buffer: 4 vials x 7.0 mL (white cap)

Note: One vial set is comprised of one vial of each component listed above.

Instruments

Each lab should prepare the local instrument in accordance with the manufacturer's instructions for use. Protocols for coagulation instruments are available upon request.

Procedure

Materials Required but not Provided

- Waterbath capable of maintaining 37 °C (± 1 °C)
- Floatie for thawing vials in waterbath
- Coagulation instrument or assay system (IL ACL TOP series, Stago STA-R Evolution, Stago STA-R Max, Siemens BCS XP, and Sysmex CS Series)
- Calibrator and control plasmas (e.g. CRYO*check* Normal Reference Plasma, CRYO*check* Reference Control Normal, CRYO*check* Abnormal 1 Reference Control, CRYO*check* Abnormal 2 Reference Control)
- Timer

Specimen Collection and Preparation

Patient samples should be collected into 105 to 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[2]. Patient 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 three months. Samples should not undergo more than two freeze-thaw cycles prior to testing. Note that FVIII is a labile protein. Improper handling of a specimen may give a false result.

Assay Procedure

1. Prepare CRYO*check* Chromogenic Factor VIII reagents according to Storage, Preparation and Handling instructions above.
2. Prepare instrument according to the manufacturer's instructions for use.
3. Load Reagent 1, Reagent 2, Reagent 3 and Diluent Buffer on the instrument.
4. Load samples on the instrument.
5. Measure the FVIII activity of plasma samples using the appropriate instrument protocol.

Results and Interpretation

FVIII 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 divided into three categories: mild (5 to <40 % FVIII), moderate (1 to 5 % FVIII) and severe (<1 % FVIII)[3].

Quality Control

Each laboratory should establish its own quality control (QC) ranges, either by means of the target values and ranges provided by the manufacturer of the controls or by means of its own confidence level established in the laboratory. These QC ranges may then be used to monitor and validate the integrity of the testing system[4]. 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[5].

Assay controls are available for purchase separately. These include CRYO*check* Reference Control Normal (normal control), CRYO*check* Abnormal 1 Reference Control (borderline pathological control), and CRYO*check* Abnormal 2 Reference Control (pathological control). Refer to the Assay Certificate for the expected ranges specific to each lot of control. Each lot number of these controls is assayed for FVIII using the SSC/ISTH Secondary Coagulation Standard Plasma that is traceable to the WHO International Standard for FVIII/VWF.

Expected Values

A reference interval study was conducted in accordance with CLSI EP28-A3c[6] using three lots of CRYO*check* Chromogenic Factor VIII on an IL ACL TOP instrument. Citrated plasma samples from 120 normal, ostensibly healthy individuals were tested. The reference interval was established by calculating the non-parametric 95 % confidence interval (2.5^{th} to 97.5^{th} percentiles) and was determined to be 43.2 to 159.3 % FVIII activity.

Performance Characteristics

All studies were performed using an IL ACL TOP instrument unless otherwise noted.

Method Comparison

A method comparison study was conducted at three sites (one internal and two external) according to CLSI EP09c[7] to compare the accuracy of CRYO*check* Chromogenic Factor VIII relative to a comparator device. Both external sites used IL ACL TOP 750 instruments. Aliquots of human plasma samples from normal, ostensibly healthy individuals and from patients with congenital or acquired hemophilia A and Type 1, Type 2A, Type 2B and Type 2N von Willebrand disease (N=318) were distributed across three sites and tested using a single lot of CRYO*check* Chromogenic Factor VIII. A second aliquot of each sample was tested at a central reference laboratory using Coatest SP FVIII. Results were compared by Passing-Bablok regression analysis. Regression statistics show that CRYO*check* Chromogenic Factor VIII performed equivalently to the comparator method.

	N	Slope		Intercept		Pearson Correlation Coefficient (R)
		Value	95 % CI	Value	95 % CI	
Site 1	133	1.041	1.027, 1.058	0.720	0.252, 1.205	0.997 (r^2=0.993)
Site 2	53	1.138	1.109, 1.168	0.252	0.001, 0.409	0.998 (r^2=0.996)
Site 3	132	1.012	0.989, 1.045	-0.140	-1.768, 0.404	0.991 (r^2=0.982)
Overall	318	1.038	1.022, 1.051	0.473	0.265, 0.594	0.994 (r^2=0.987)

Absolute predicted biases at medical decision levels are reported below.

FVIII activity (%)	Predicted Absolute Bias (%)	Lower CI (%)	Upper CI (%)
1	-1.11	-1.87	-0.35
5	-0.81	-1.53	-0.08
45	2.20	1.71	2.69
50	2.57	2.09	3.06
100	6.33	5.51	7.15
150	10.09	8.71	11.47

Limit of Blank, Limit of Detection and Limit of Quantification

The limit of blank (LoB) was determined following the CLSI EP17-A2 guideline[8] by measuring four blank plasma samples obtained from individuals with severe congenital hemophilia A. Samples were measured in triplicate using three lots of CRYO*check* Chromogenic Factor VIII over five days. The LoB was determined to be 0.4 %.

The limit of detection (LoD) was determined following the CLSI EP17-A2 guideline by measuring four plasma samples with low FVIII activity obtained from congenital hemophilia A donors. Samples were measured in triplicate using three lots of CRYO*check* Chromogenic Factor VIII over five days. The LoD was determined to be 0.5 %.

The limit of quantitation (LoQ) was determined following the CLSI EP17-A2 guideline by measuring four plasma samples with low FVIII activity obtained from congenital hemophilia A donors. Samples were measured in triplicate using three lots of CRYO*check* Chromogenic Factor VIII over five days. The same samples were also measured in triplicate using a single lot of Coatest SP FVIII over five days to determine the assigned values. The LoQ was determined to be 0.5 %.

Linearity

A linearity study was conducted in accordance with CLSI EP06-A[9] using three lots of CRYO*check* Chromogenic Factor VIII. Plasma with a high FVIII concentration (~ 260 %) was combined with congenital hemophilia A patient plasma (0 % FVIII) to create fifteen sample dilutions with estimated FVIII activity in the range of 0 to 260 %. The results support a linear range of 0 to 200 %.

Precision

An internal precision study was performed using three lots of CRYO*check* Chromogenic Factor VIII in accordance with CLSI EP05-A3[10]. The study quantified one normal and two abnormal reference controls and five patient plasma samples representing very low, low, mid, normal and high levels of FVIII activity. Each sample was measured with each product lot 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 samples >1 % FVIII, and ≤0.1 SD for the very low plasma sample.

Sample	Mean FVIII Activity (%)	Within-Laboratory Precision	
		SD	%CV
CRYO*check* Reference Control Normal	80.8	4.0	5.0
CRYO*check* Abnormal 1 Reference Control	26.1	1.9	7.1
CRYO*check* Abnormal 2 Reference Control	7.8	0.8	9.9
Very Low FVIII Plasma Sample	1.0	0.1	NA
Low FVIII Plasma Sample	5.4	0.4	7.4
Mid FVIII Plasma Sample	26.0	1.7	6.7
Normal FVIII Plasma Sample	85.3	4.3	5.1
High FVIII Plasma Sample	152.1	5.7	3.8

Reproducibility

Reproducibility studies were conducted at three sites (one internal and two external) using three lots of CRYO*check* Chromogenic Factor VIII in accordance with CLSI EP05-A3. Both external sites used IL ACL TOP 750 instruments. The study quantified one normal reference control and two abnormal reference controls and three plasma samples representing very low, normal and high levels of FVIII activity. Each sample was measured with each product lot in triplicate, twice a day for five days. The data across three sites demonstrated a pooled reproducibility of <10 % CV for all controls and samples >1 % FVIII, and ≤0.1 SD for the very low plasma sample.

Sample	Mean (%)	Within-Run		Between- Run		Between-Day		Between-Site		Reproducibility	
		SD	%CV	SD	%CV	SD	%CV	SD	%CV	SD	%CV
CRYO*check* Reference Control Normal	80.3	3.6	4.5	0.2	0.2	0.5	0.7	0.0	0.0	4.1	5.1
CRYO*check* Abnormal 1 Reference Control	25.4	1.3	5.2	0.2	0.6	0.3	1.2	1.5	5.7	2.1	8.2
CRYO*check* Abnormal 2 Reference Control	7.7	0.5	7.0	0.0	0.4	0.1	1.6	0.4	4.9	0.7	9.6
Very Low FVIII Plasma Sample	1.1	0.1	NA	0.0	NA	0.0	NA	0.0	NA	0.2	NA
Normal FVIII Plasma Sample	85.4	4.3	5.1	1.1	1.3	0.0	0.0	0.0	0.0	5.0	5.8
High FVIII Plasma Sample	156.9	7.8	5.0	0.9	0.5	0.0	0.0	6.1	3.9	11.4	7.3

Interferences

Interference studies were conducted according to CLSI EP07[11] using a single lot of CRYO*check* Chromogenic Factor VIII on an IL ACL TOP instrument. 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 up to the concentrations indicated:

Substance Tested	Test Concentration
Hemoglobin	≤1000 mg/dL
Intralipid	≤830 mg/dL
Bilirubin (unconjugated)	≤40 mg/dL
Bilirubin (conjugated)	≤11 mg/dL
von Willebrand factor	≤20 µg/mL
Unfractionated heparin	≤3.3 IU/mL
Low molecular weight heparin	≤5 IU/mL
Fondaparinux	≤0.2 mg/L
Lupus Anticoagulant	≤1.8 dRVVT ratio
Emicizumab	≤150 µg/mL
Mim8	≤8 µg/mL
Warfarin	INR ≤7

Rivaroxaban and dabigatran interfered with the quantification of FVIII activity.

The performance of this device has not been established in individuals with von Willebrand disease Type 2M.

Recovery of FVIII Replacement Products

CRYO*check* Chromogenic Factor VIII accurately evaluated the potency of FVIII replacement products including ADVATE, ADYNOVATE, AFSTYLA, ALTUVIIO, ESPEROCT, HUMATE-P, JIVI, KOVALTRY, Novoeight, Nuwiq, and wilate at concentrations ranging from 0.05 to 1.0 IU/mL. It also accurately evaluated the potency of ELOCTATE, and XYNTHA from 0.05 to 0.6 IU/mL, with an over recovery observed at 0.8 and 1.0 IU/mL. There was an underestimation of OBIZUR.

Product	Mean Percent Recovery (%)
ADVATE®	90.3
ADYNOVATE®	97.7
AFSTYLA®	89.8
ALTUVIIIO®	96.2*
ELOCTATE®	116.3
ESPEROCT®	93.2
HUMATE-P®	95.0
JIVI®	98.5
KOVALTRY®	92.0
Novoeight®	115.6
Nuwiq®	90.2
OBIZUR®	49.5
wilate®	95.2

Product	Mean Percent Recovery (%)
XYNTHA®	114.7

*Per the manufacturer's recommendations, chromogenic assays overestimate Altuviiio approximately 2.5-fold. Mean percent recovery value includes dividing by a correction factor of 2.5.

Precautions/Warnings

Do not use the product if it is thawed upon receipt or if the vials appear cracked. Transferring the material into another container other than siliconized glass or polypropylene could have a 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.

A Summary of the Safety and Performance of this device can be found on the EUDAMED database.

Human thrombin (FIIa) was prepared from human plasma which was found to be negative when tested in accordance with current FDA required tests. Bovine serum albumin and bovine FX, and FIXa were prepared from bovine plasma from animals free from Bovine Spongiform Encephalopathy (BSE). However, no known test method can offer complete assurance that components derived from human or bovine blood will not transmit infectious agents, therefore, the handling and disposal of the reagents should be made with the required caution, as being potentially infectious[12].

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Symbols Used

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

CE 0123

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