PrecisionBioLogic

CRYO*check*™ IVD

CHROMOGENIC FACTOR IX

Intended Use

CRYO*check* Chromogenic Factor IX is for clinical laboratory use in the quantitative determination of factor IX activity in 3.2% citrated human plasma. It is intended to be used in identifying factor IX deficiency and as an aid in the management of hemophilia B in individuals aged 2 years and older. For in vitro diagnostic use.

Summary and Principle

Factor IX is a critical component in the normal blood clot-forming process; upon activation of the coagulation cascade, it enables activation of FX and subsequent downstream thrombin activation, which leads to cleavage of fibrinogen and formation of a polymerized fibrin clot. Hemophilia B is a genetic disorder caused by missing or defective clotting factor IX, which leads to prolonged bleeding. A definitive diagnosis of hemophilia B depends on determination of factor IX activity. The standard of care in the treatment of hemophilia B is the replacement of the missing or defective FIX via intravenous infusion of FIX concentrate. Individuals with hemophilia B require factor IX testing while on replacement therapy to confirm factor IX levels achieved are optimum and to ensure that the dosing regimen for a particular product for a particular patient is suitable.[1]

CRYO*check* Chromogenic Factor IX is a chromogenic factor IX assay. In this assay, FIX activity is determined in a chromogenic method, in which human FIX is activated by human FXIa and where formed FIXa activates human FX in the presence of human FVIII, calcium ions and phospholipid. Similar to in vivo conditions, FVIII is activated by thrombin which is generated during the incubation. The amount of FXa formed is related to the FIX activity and is determined from the hydrolysis of a chromogenic FXa 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 FIX present in the sample based on a standard curve.[2]

Reagents

CRYO*check* Chromogenic Factor IX consists of three reagents containing human factors (FII, FVIII, FX, FXIa), bovine factor V, FXa substrate and a tris-based buffer.

For PRESCRIPTION USE ONLY

Storage, Preparation and Handling

When stored at -70 °C or below CRYO*check* Chromogenic Factor IX 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 each reagent gently prior to use.

Thawing Table	
Aliquot Size	**37 °C (± 1 °C) Waterbath**
≤ 2.3 mL (Reagents)	3 min
10.0 mL (Diluent Buffer)	8 min

CRYO*check* Chromogenic Factor IX may be used for up to 24 hours after thawing when stored on-board the analyzer, or up to 48 hours when capped in the original vial and maintained at 2 to 8 °C. Invert the refrigerated reagents gently prior to use. Reagents that have been in use for ≤ 4 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 eight hours on board the instrument.

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

Availability

Product	Catalog #	4 Vial Sets, Each Set Containing
CRYO*check* Chromogenic Factor IX	CCCF09	Reagent 1: 1 x 0.75 mL (grey cap) Reagent 2: 1 x 2.3 mL (black cap) Reagent 3: 1 x 1.0 mL (orange cap) Diluent Buffer: 1 x 10.0 mL (white cap)

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

After thawing and preparing CRYO*check* Chromogenic Factor IX, use in accordance with established laboratory procedures for factor IX activity measurements.

Materials Provided

- **Reagent 1:** Human FVIII, human FX, bovine FV and a fibrin polymerization inhibitor
- **Reagent 2:** Human FXIa, human FII, calcium chloride and phospholipids.
- **Reagent 3:** FXa substrate containing EDTA and a thrombin inhibitor.
- **Diluent Buffer:** Tris buffer solution containing 1% BSA and a heparin antagonist.

Materials Required but not Provided

- Waterbath capable of maintaining temperature at 37 °C (± 1 °C)
- Floatie for thawing vials in waterbath
- Coagulation instrument

- Timer
- Transfer pipette
- 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)

Standard Curve Preparation

Methods may vary according to instrumentation used. Calibrate using two vial sets of CRYO*check* Chromogenic Factor IX and a calibrator with a known FIX activity value (e.g. CRYO*check* Normal Reference Plasma). Protocols for coagulation instruments are available upon request.

Specimen Collection and Preparation

Patient 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.[3] 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 three months. Samples should not undergo more than two freeze-thaw cycles prior to testing.

Assay Procedure

1. Prepare CRYO*check* Chromogenic Factor IX 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. Protocols for coagulation instruments are available upon request.
4. Load samples on the instrument.
5. Measure the FIX activity of plasma samples using the appropriate instrument protocol.

Results and Interpretation

FIX results are reported in % activity where 100% FIX activity is equivalent to 1.0 IU/ mL. FIX values recovered below the laboratory established normal range may be indicative of hemophilia B. FIX deficiency caused by Hemophilia B can be divided into three categories: mild (5% - <40%), moderate (1 – 5%) and severe (< 1%).[4]

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.[5] 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.[6]

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 FIX using the SSC/ISTH Secondary Coagulation Standard Plasma that is traceable to the WHO International Standard for Factor IX.

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 FIX.

The assay reference range was established using 128 citrated plasma samples collected from normal ostensibly healthy individuals using three lots of CRYO*check* Chromogenic Factor IX on two IL ACL TOP instruments according to CLSI EP28- A3c[7]. The reference interval was determined to be 79-155% FIX activity by calculating the non-parametric 95% confidence interval (2.5th to 97.5th percentiles).

Performance Characteristics

All studies were performed using an IL ACL TOP 700 CTS instrument unless otherwise noted.

Method Comparison:

A method comparison study was conducted at three sites (one internal and two external) according to CLSI EP09c[8] to compare the accuracy of CRYO*check* Chromogenic Factor IX relative to a comparator device. The internal site used an IL ACL TOP 700 CTS instrument, and the two external sites used an IL ACL TOP 750 CTS and IL ACL TOP 700 instruments, respectively. Aliquots of human plasma from normal ostensibly healthy individuals, from patients with von Willebrand disease, from patients with congenital and acquired hemophilia A and B and from patients on recombinant factor IX treatment (N=350) were distributed across three sites and tested with a single lot of CRYO*check* Chromogenic Factor IX. A second aliquot of each sample was tested at a central reference laboratory using a validated laboratory-developed chromogenic factor IX assay on an IL ACL TOP 700 instrument. Results were compared by Passing-Bablok regression analysis. Regression statistics showed that CRYO*check* Chromogenic Factor IX performed equivalently to the comparator method.

	N	Slope		Intercept		Pearson Correlation Coefficient (R)
		Value	95% CI	Value	95% CI	
Site 1	122	1.13	1.10, 1.15	-0.24	-0.36, -0.04	0.996 (r^2=0.993)
Site 2	112	1.17	1.13, 1.20	1.72	1.16, 2.38	0.995 (r^2=0.990)
Site 3	116	1.18	1.13, 1.25	-9.93	-15.82, -5.48	0.983 (r^2=0.966)
Overall	350	1.11	1.10, 1.13	0.10	-0.06, 0.48	0.992 (r^2=0.984)

Absolute predicted biases at medical decision levels are reported below.

FIX activity (%)	Predicted Absolute Bias (%)	Lower CI (%)	Upper CI (%)
1	0.38	-0.62	1.38
5	0.78	-0.18	1.74
50	5.29	4.63	5.94
100	10.30	9.45	11.13

Precision:

An internal precision study was performed using three lots of CRYO*check* Chromogenic Factor IX to quantify the FIX activity in three controls and three patient plasma samples according to CLSI EP05-A3[9]. 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 the high FIX plasma sample, <0.5% SD for the Very Low FIX plasma sample and <1% SD for the Low FIX plasma sample.

Sample	Mean FIX Activity (%)	Within-Laboratory Precision	
		SD	%CV
CRYO*check* Reference Control Normal	114.9	4.2	3.7
CRYO*check* Abnormal 1 Reference Control	39.3	1.8	4.5
CRYO*check* Abnormal 2 Reference Control	10.4	0.8	7.3
Very Low FIX Plasma Sample	1.2	0.2	NA
Low FIX Plasma Sample	6.1	0.6	NA
High FIX Plasma Sample	174.0	6.4	3.7

Reproducibility:

Reproducibility studies were conducted at three sites (one internal and two external) on IL ACL TOP 700 CTS, IL ACL TOP 700, and IL ACL TOP 750 CTS analyzers using three lots of CRYO*check* Chromogenic Factor IX in accordance with CLSI EP05-A3[9]. The study quantified one normal and two abnormal reference controls and three patient plasma samples representing very low, low and high levels of FIX activity. Each sample was measured in triplicate, twice a day for 5 days at each site. The data across three sites demonstrated a pooled reproducibility of <10% CV for all controls and the high FIX plasma sample; <0.5% SD for the Very Low FIX plasma sample and <1% SD for the Low FIX plasma sample.

Sample	Mean (%)	Within-Run		Between- Run		Between-Day		Between-Site		Across-Site	
		SD	%CV	SD	%CV	SD	%CV	SD	%CV	SD	%CV
CRYO*check* Reference Control Normal	113.1	5.0	4.5	0.9	0.8	1.1	1.0	1.9	1.7	6.3	5.6
CRYO*check* Abnormal 1 Reference Control	38.4	1.8	4.8	0.2	0.6	0.1	0.3	1.3	3.4	2.5	6.6
CRYO*check* Abnormal 2 Reference Control	10.8	0.8	7.5	0.2	1.7	0.0	0.0	0.2	2.0	0.9	8.6
Very Low FIX Plasma Sample	1.2	0.1	NA	0.0	NA	0.0	NA	0.0	NA	0.2	NA
Low FIX Plasma Sample	6.3	0.5	NA	0.1	NA	0.1	NA	0.2	NA	0.8	NA
High FIX Plasma Sample	168.5	7.6	4.5	0.0	0.0	1.3	0.8	4.4	2.6	10.0	6.0

Limit of Blank, Limit of Detection and Limit of Quantification:

The limit of blank (LoB) was determined in accordance with CLSI EP17-A2[10] by measuring four blank plasma samples obtained from individuals with severe congenital hemophilia B. Samples were measured in triplicate using three lots of CRYO*check* Chromogenic Factor IX over five days. The LoB was determined to be 0.4% FIX activity.

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

The limit of quantitation (LoQ) was determined in accordance with CLSI EP17-A2 by measuring four plasma samples with low FIX activity obtained from congenital hemophilia B donors. Samples were measured in triplicate using three lots of CRYO*check* Chromogenic Factor IX over five days. The same samples were also measured in triplicate using a single lot of a validated, laboratory-developed chromogenic factor IX assay over five days to determine the assigned values. The LoQ was determined to be 0.7% FIX activity.

Linearity:

A linearity study was conducted in accordance with CLSI EP06-A[11] using three lots of CRYO*check* Chromogenic Factor IX to quantify the FIX activity of fourteen samples created by combining plasma with a high FIX concentration (~ 230 %) with congenital hemophilia B patient plasma (0% FIX). These fourteen samples yielded an estimated FIX activity in the range of 0 to 230%. The results support a linear range of 0 to 200%.

Interferences:

Interference studies were conducted according to CLSI EP07[12] using a single lot of CRYO*check* Chromogenic Factor IX. 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	Sample Concentration
Hemoglobin	≤ 1000 mg/dL
Intralipid	≤ 2000 mg/dL
Bilirubin (unconjugated)	≤ 40 mg/dL
Bilirubin (conjugated)	≤ 23 mg/dL
Unfractionated heparin	≤ 1.2 IU/mL
Low molecular weight heparin	≤ 1.5 IU/mL
Dabigatran	≤ 0.04 mg/L
Fondaparinux	≤ 0.26 mg/L
Lupus Anticoagulant	≤ 1.8 dRVVT ratio

Rivaroxaban and warfarin interfered with the quantification of FIX activity.

Recovery of FIX Replacement Products:

This device accurately evaluated the potency of FIX replacement products including AlphaNine® SD, Alprolix®, BeneFIX®, Ixinity, Rebinyn® and Rixubis at concentrations ranging from 0.05 o 1.0 IU/mL. There was an overestimation of Idelvion*.

Product	Mean Percent Recovery (%)
AlphaNine SD	96
Alprolix	116

Product	Mean Percent Recovery (%)
BeneFIX	93
Ixinity	82
Rebinyn	117
Rixubis	102
Idelvion*	153

** Per the manufacturer's recommendations, a one stage clotting assay is recommended for measurement of Idelvion and results may vary based on the aPTT reagent in use.*

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.

Human factors II, VIII, X, and XIa were prepared from human plasma which was found to be negative when tested in accordance with current FDA required tests. Bovine serum albumin and bovine FV were prepared from bovine plasma from animals free from 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.[13]

Bibliography

1. Kitchen SK, Tiefenbacher S, Gosselin R. Factor Activity assays for monitoring extended half-life FVIII and factor IX replacement therapies. Semin Thromb Hemost 2017; 43: 331-337.
2. Marlar RA, Strandberg K, Shima M and Adcock DM. Clinical utility and impact of the use of the chromogenic vs one stage factor assays in haemophilia A and B. Eur J Haematol. 2020; 104(1): 3-14.
3. CLSI. Collection, Transport, and Processing of Blood Specimens for Testing Plasma-Based Coagulation Assays and Molecular Hemostasis Assays; Approved Guideline-Fifth Edition. H21-A5. Clinical and Laboratory Standards Institute, Wayne, PA (USA); 2008.
4. Srivastava, Brewer, Mauser-Bunschoten, Key, Kitchen, Llinas, et al. Guidelines for the Management of Hemophilia, 2nd Edition. Haemophilia. 2013; 19 (1), e1-47.
5. Cembrowski GS, Carey RN. *Laboratory Quality Management*. Chicago: ASCP Press; 1989. p. 166-171.
6. CLIA 2004 – Code of Federal regulations, 42CFR493.1269, 2004.
7. CLSI. *Defining, Establishing and Verifying Reference Intervals in the Clinical Laboratory*. Approved Guideline-3rd ed. CLSI guideline EP28-A3. Wayne, PA (USA): Clinical and Laboratory Standards Institute, 2010.
8. CLSI. *Measurement Procedure Comparison and Bias Estimation Using Patient Samples*. 3rd ed. CLSI guideline EP09c., Wayne, PA(USA); Clinical and Laboratory Standards Institute, 2018.
9. 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.
10. CLSI. *Evaluation of Detection Capability for Clinical Laboratory Measurement Procedures*. Approved Guideline-2nd ed. CLSI guideline EP17-A2. Wayne, PA (USA): Clinical and Laboratory Standards Institute, 2012.
11. CLSI. *Evaluation of Linearity of Quantitative Measurement Procedures*; *A Statistical Approach*. Approved Guideline. CLSI guideline EP06-A. Wayne, PA (USA): Clinical and Laboratory Standards Institute, 2003.
12. CLSI. *Interference Testing in Clinical Chemistry*. 3rd ed. CLSI guideline EP07. Wayne, PA (USA): Clinical and Laboratory Standards Institute, 2018.
13. Biosafety in Microbiological and Biomedical Laboratories 6th ed. Centers for Disease Control and Prevention / National Institutes of Health, 2020.

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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
	Use by	**Rx ONLY**	For prescription use only
	Temperature limitation		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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