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PrecisionBioLogic

CRYO*check*™ IVD

FACTOR DEFICIENT PLASMAS

FACTOR X DEFICIENT PLASMA

Intended Use

CRYO*check* Factor X Deficient Plasma is for clinical laboratory use as a deficient substrate in the quantitative determination of factor X activity in 3.2 % citrated human plasma based on the prothrombin time (PT) assay. It is intended to be used in identifying factor X deficiency and in the management of factor X deficiency. For in vitro diagnostic use.

Summary and Principle

Deficiencies in coagulation factors may have congenital or acquired etiologies and can compromise in vivo hemostais[1]. Factor X (Stuart factor) is a two-chained glycoprotein with a molecular weight of 59,000 Da and is important for both intrinsic and extrinsic coagulation[2]. Plasma samples deficient in coagulation factor X exhibit a prolonged PT and activated partial thromboplastin time (APTT). Factor X deficiency is commonly diagnosed through the use of a modified PT assay. When a patient sample is mixed with factor X deficient plasma, the degree of correction of the PT is proportional to the level of factor X in the patient plasma[3].

Reagents

CRYO*check* Factor X Deficient Plasma consists of normal citrated human plasma, which has been depleted of factor X by immunoadsorption. The plasma is then buffered with HEPES buffer, aliquoted, and rapidly frozen. Factor X has been assayed at less than 1 % of normal levels by both functional and antigenic methods. Other factors have been assayed and results are provided on the Quality Control Certificate that accompanies each lot number.

FOR PRESCRIPTION USE ONLY

Product ingredients: Citrated human plasma, buffered with 0.01 M HEPES.

Storage, Preparation and Handling

When stored at -40 to -80 °C, CRYO*check* Factor X Deficient Plasma 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). Thawing 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. Allow thawed plasma to acclimate to room temperature (18 to 25 °C) and invert each reagent gently prior to use.

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

When first thawed, CRYO*check* Factor X Deficient Plasma may be used for up to eight hours if capped in the original vial and maintained at 2 to 8 °C. Allow refrigerated plasma to acclimate to room temperature (18 to 25 °C) and invert gently prior to use. **Thawed material should be discarded after eight hours and should not be refrozen.**

Availability

Product	Catalog #	Format
CRYO*check* Factor X Deficient Plasma	FDP10-10	25 vials x 1.0 mL
	FDP10-15	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 X Deficient Plasma, use in accordance with established laboratory procedures for the quantitative assessment of factor X.

Material Provided

- CRYO*check* Factor X Deficient Plasma

Materials Required but not Provided

- Waterbath capable of maintaining 37 °C (± 1 °C)
- Floatie for thawing vials in waterbath
- Assay reagents (PT reagent)
- Owren-Koller Buffer or equivalent
- Coagulation instrument or assay system
- Calibration plasma (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)
- 2-cycle log-log graph paper
- Plastic test tubes (e.g. 12 x 75 mm)
- Sample cups
- Plastic disposable pipettes
- Volumetric pipette
- Timer

Standard Curve Preparation

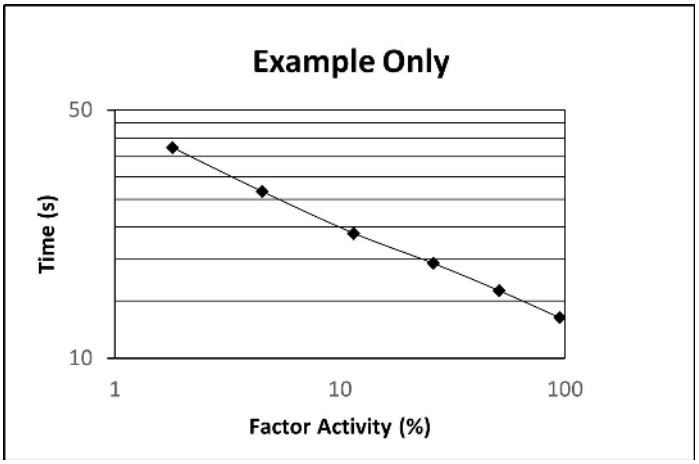
Methods may vary according to the instrumentation used. Consult the instrument manufacturer's instruction manual for recommended factor assay (extrinsic) protocols.

1. Prepare assay reagents, calibration plasma, and buffer according to manufacturer's directions.
2. Make serial dilutions of calibration plasma from 1:10 to 1:320 in buffer as follows:

Tube No.	Volume of Buffer	Volume of Calibration Plasma	Dilution	% Factor
1	1.8 mL	0.2 mL calibration plasma	1:10	100
2	1.0 mL	1.0 mL of Tube No. 1	1:20	50
3	1.0 mL	1.0 mL of Tube No. 2	1:40	25
4	1.0 mL	1.0 mL of Tube No. 3	1:80	12.5
5	1.0 mL	1.0 mL of Tube No. 4	1:160	6.25
6	1.0 mL	1.0 mL of Tube No. 5	1:320	3.12

*Note: This is an **example only** of a serial dilution profile prepared using calibration plasma with a factor X level of 100 %. Always be sure to utilize the lot-specific factor X level of the calibration plasma in use. If using CRYOcheck Normal Reference Plasma, refer to the lot specific Assay Certificate.*

3. Pre-warm thromboplastin to 37 °C (± 1 °C).
4. To a coagulation reaction cuvette, add 0.1 mL of CRYO*check* Factor X Deficient Plasma and 0.1 mL of Tube No. 1 (100 % of factor). Mix and incubate according to manufacturer's directions.
5. Add 0.2 mL of pre-warmed thromboplastin and simultaneously initiate the clot timer. Record clotting in seconds.
6. Repeat steps 4 and 5 for Tube Nos. 2 to 6.
7. On log-log graph paper plot clotting times in seconds (y-axis) vs. % of factor X activity (x-axis).
8. Construct the standard curve by drawing the best straight line to fit through the plots.



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. 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 four hours of collection when maintained at room temperature in accordance with the Clinical Laboratory Standards Institute (CLSI) guidelines[4].

Assay Procedure

1. Prepare a 1:10 dilution of patient plasma with buffer.
2. Repeat steps 3 through 5 of Standard Curve Preparation, substituting diluted patient plasma for diluted calibration plasma.
3. Read the percent factor X activity from the standard curve by finding the point where the clotting time intercepts the curve, then reading the percent factor X activity off the x-axis.
4. Further dilutions of patient plasma may be prepared and tested to confirm the value.

Results and Interpretation

Factor X activity values recovered below the normal range may be indicative of a factor X deficiency (congenital or acquired). Each laboratory should establish its own normal range for factor X activity in accordance with CLSI guidelines[5].

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[6]. 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[7].

Limitations of 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.

Samples with excessive hemolysis, icterus, or lipemia, should not be used. Refer to specific PT reagent instructions for more details on interference claims. Certain anticoagulants, such as warfarin, heparin, and direct thrombin and FXa inhibitors are known interferents. The presence of nonspecific factor inhibitors and lupus anticoagulants are also known to interfere with one-stage clotting assays[5].

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 X activity.

A reference interval study was conducted in accordance with CLSI EP28-A3c[8] using three lots of CRYO*check* Factor X Deficient Plasma on the Stago Compact Max, Stago STA-R and Stago Evolution, using RecombiPlasTin 2G, or NeoPlastine CI Plus. Plasma samples from approximately 60 normal, ostensibly healthy individuals were tested. The reference interval was established by calculating the non-parametric 95 % confidence interval of the combined data and was determined to be 75 to 145 % factor X activity.

Performance Characteristics

Refer to the Quality Control Certificate for clotting factor specifications with each lot number of CRYO*check* Factor X Deficient Plasma. When used according to recommended methods, results are subject to the limitations of the assay system (i.e. reagents, instrument) in use.

Method Comparison

Accuracy of factor X activity measurement when CRYO*check* Factor X Deficient Plasma was used in a modified PT assay (using STA Neoplastine CI Plus 10, on a Stago STA-R Evolution) was assessed through a method comparison study where the recovery of factor X activity for 44 samples was compared to a comparator product Stago FX Def. The results were analyzed according to CLSI EP09c[9] by weighted Deming regression analysis. Regression statistics show that CRYO*check* Factor X Deficient Plasma performed equivalently to the comparator method.

N	Slope		Intercept		Pearson Correlation Coefficient (R)
	Value	95 % CI	Value	95 % CI	
44	1.02	1.01, 1.04	-0.14	-1.80, 0.44	0.998 (r^2 = 0.996)

Absolute predicted biases at medical decision levels are reported below.

Medical Decision Level (FX %)	Predicted Absolute Bias (%)	Lower CI (%)	Upper CI (%)
5	-0.06	-1.60	0.49
10	0.02	-1.42	0.54
50	0.64	0.06	1.04
100	1.43	0.76	2.30
150	2.21	0.98	4.20

Precision

The precision of five lots of CRYO*check* Factor X Deficient Plasma when used with various PT reagents on various coagulation analyzers to quantify factor X activity in two levels of controls, CRYO*check* Reference Control Normal (RCN) and CRYO*check* Abnormal 1 Reference Plasma (ARP1) was assessed by analyzing these controls over 20 days in duplicate, for two runs per day using multiple operators. The resulting data were analyzed as per CLSI EP05-A3[10] to calculate within-run precision (repeatability), between-run precision, between-day precision, and within-lot precision.

The expected precision estimates using a single representative lot of CRYO*check* Factor X Deficient Plasma with different PT reagent and analyzer combinations are displayed below. The results demonstrated within-lot precision of <10 % CV for all.

HemosIL RecombiPlasTin 2G on the Compact Max									
Sample	Mean Value (%)	Within-Run		Between-Run		Between-Day		Within-Lot	
		SD	%CV	SD	%CV	SD	%CV	SD	%CV
RCN	91	3.8	4.2	0.0	0.0	2.9	3.1	4.8	5.2
ARP1	36	1.3	3.5	0.8	2.2	0.0	0.0	1.5	4.1

STA Neoplastine CI Plus 10 on the STA-R Evolution									
Sample	Mean Value (%)	Within-Run		Between-Run		Between-Day		Within-Lot	
		SD	%CV	SD	%CV	SD	%CV	SD	%CV
RCN	107	1.5	1.4	1.4	1.3	2.2	2.0	3.0	2.8
ARP1	40	0.7	1.7	0.6	1.6	0.8	2.1	1.3	3.1

HemosIL RecombiPlatisTin 2G on the STA-R Evolution									
Sample	Mean Value (%)	Within-Run		Between-Run		Between-Day		Within-Lot	
		SD	%CV	SD	%CV	SD	%CV	SD	%CV
RCN	100	2.0	2.0	1.9	1.9	0.5	0.5	2.8	2.8
ARP1	37	1.0	2.7	0.7	2.0	0.6	1.7	1.4	3.7

Linearity

A linearity study was conducted in accordance with CLSI EP06-Ed2[11] using three lots of CRYO*check* Factor X Deficient Plasma. Plasma with high factor X activity (>200 %) was combined with factor X deficient plasma (0 %) to create nine sample dilutions with estimated factor X activity in the range of 0 to 182 %. The results support a linear range of 0 to 150 %.

Precautions/Warnings

Do not use the product if it is thawed upon receipt or if the vials appear cracked, or if upon thawing the product appears to have clotted. 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.

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 method can offer complete assurance that components 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[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
	Temperature limit		Consult electronic instructions for use
	Biological risks		

CE 0123

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

CH REP Swiss Authorized Representative (Regulatory affairs only)
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