PrecisionBioLogic

CRYO*check*™ IVD

QUANTITATIVE PROTEIN C CLOTTING ASSAY

CLOT C ™

Intended Use

CRYO*check* Clot C is a clot-based assay intended for the quantitative determination of protein C activity in citrated human plasma.

Summary and Principle

Protein C is a vitamin K-dependent zymogen synthesized in the liver as a single chain polypeptide with a molecular weight of 62,000 Da. In the presence of thrombin, calcium and phospholipids, protein C is converted to an active serine protease which acts as a potent inhibitor of procoagulant factors Va and VIIIa[1,2]. This inhibition is further enhanced by protein S, a cofactor to protein C.

Protein C deficiency has both congenital and acquired etiologies of clinical interest. Acquired deficiencies are found in oral anticoagulant therapy (OAT)[3], liver disease[4], and disseminated intravascular coagulation (DIC)[5], while congenital deficiencies are commonly associated with an increased risk of venous thrombosis[6] and characterized as follows:

Deficiency	Protein C Antigen Levels	Protein C Activity Levels
Type I	diminished	diminished
Type II	normal	diminished

CRYO*check* Clot C functions by direct activation of protein C in the patient sample using Protein C Activator. The common pathway of coagulation is initiated with a Russell's viper venom (RVV-X) reagent to convert factor X to Xa and bypassing all factors above the common pathway[7]. Patients with a protein C deficiency or dysfunction will have shortened CRYO*check* Clot C clotting times relative to patients with normal levels of functional protein C. The clotting time is proportional to the amount of functional protein C in the patient's plasma and this can be quantified using a calibration curve.

Reagents

Protein C Deficient Plasma (PC Deficient)

Contains citrated pooled normal human plasma that has been depleted of protein C by immunoadsorption.

Clot C Activator (Activator)

Contains Protac®* isolated from the venom of Agkistrodon contortrix capable of activating protein C in human plasma, Russell's viper venom, phospholipids, heparin neutralizing agents, buffers and stabilizers.

C & S Diluent

Available separately from Precision BioLogic (catalog # CSD).

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[8].

Storage, Preparation and Handling

When stored at -70 °C or below, CRYO*check* Clot C is stable to the end of the month indicated on the product packaging.

Thaw 1 vial each of **PC Deficient** and **Activator** 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 format. Immediately after thawing, vortex or vigorously mix **Activator** only, for 5–10 seconds. Allow thawed reagents to acclimate to room temperature (18 to 25 °C) for **30 minutes** and invert each reagent gently prior to use. A stir bar is required for the **Activator** when placing on-board an automated instrument. Alternatively for manual methods, swirl Activator prior to performing tests.

Thawing Table	
Aliquot Size	**37 °C (± 1 °C) Waterbath**
3.0 mL	6 minutes
1.5 mL	5 minutes

CRYO*check* Clot C may be used for up to eight hours after preparation. When not in use, CRYO*check* Clot C reagents should be capped in the original vials and maintained at 2 to 8 °C. Allow refrigerated reagents to acclimate to room temperature (18 to 25 °C) and invert gently prior to use. Reagents may be capped in the original container and refrozen at -70 °C within eight hours and stored for up to 30 days. Refrozen reagents are stable for up to six hours when prepared according to Storage, Preparation and Handling instructions above. Recalibration is recommended.

NB: CRYO*check* Clot C components are lot-specific and should not be interchanged with other lot numbers.

Availability

Product	Catalog #	Format	Number of Tests
Clot C	CCC-30	PC Deficient 5 x 3.0 mL Activator 5 x 3.0 mL	300
	CCC-15	PC Deficient 5 x 1.5 mL Activator 5 x 1.5 mL	150

Instruments

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

Procedure

Materials Provided

- Protein C Deficient Plasma **(PC Deficient)**
- Clot C Activator **(Activator)**

Materials Required but not Provided

- C & S Diluent
- 0.025 M $CaCl_2$
- Waterbath capable of maintaining 37 °C (± 1 °C)
- Floatie for thawing vials in waterbath
- Coagulation instrument or assay system
- Calibration 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)
- Linear-linear graph paper
- Plastic test tubes (e.g. 12 x 75 mm)
- Coagulation reaction cuvettes
- Plastic disposable pipettes
- Volumetric pipette
- Timer

Specimen collection and Preparation

Patient samples should be collected into 105 - 109 mmol/L sodium citrate dihydrate anticoagulant (3.2%) 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 2 to 4 °C. If samples are not to be tested within four hours then plasma should be removed from the cells and frozen at -20 °C for up to two weeks or -70 °C for up to six months in accordance with the Clinical Laboratory Standards Institute (CLSI) guidelines[9].

Assay Procedure

1. Prepare CRYO*check* Clot C reagents according to Storage, Preparation and Handling instructions above.
2. Prepare instrument according to the manufacturer's instructions for use.
3. Prepare a 1:10 dilution of test plasma (i.e. patient, calibrator or control) in C & S Diluent (**do not substitute distilled water or other buffers for C & S Diluent**).
4. To a coagulation reaction cuvette, add 50 µL of test plasma, 50 µL of PC Deficient and 50 µL of Activator.
5. Mix and incubate at 37 °C (± 1 °C) for three minutes.
6. Add 50 µL 0.025 M $CaCl_2$ and immediately initiate timer.
7. Record clotting time in seconds.

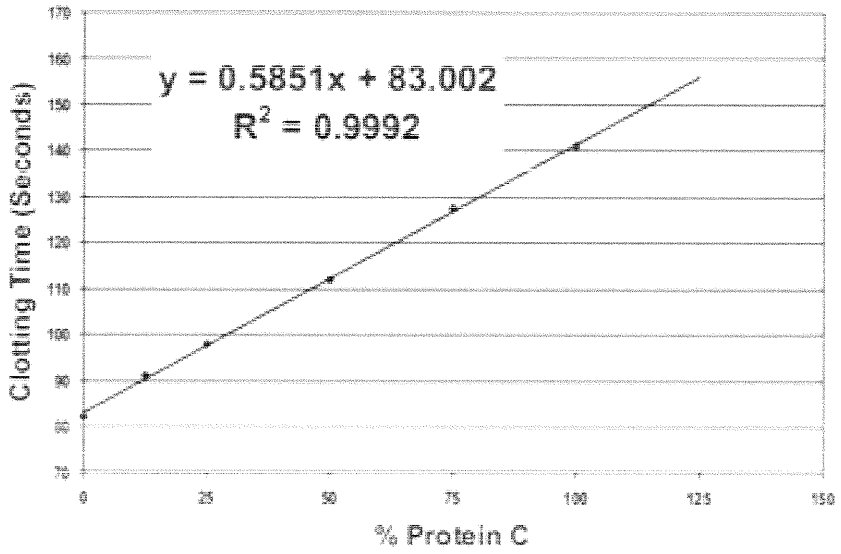
Assay Calibration

1. Prepare CRYO*check* Clot C reagents according to Storage, Preparation and Handling instructions above.
2. Prepare calibration plasma according to manufacturer's directions.
3. Prepare serial dilutions of calibration plasma from 1:10 to 1:80 in C & S Diluent according to the following table:

Tube No.	C&D Diluent (mL)	Volume of calibration Plasma (mL)	Dilution	% Factor
1	1.8	0.2	1:10	100
2	0.4	0.6 of Tube No. 1	1:15	66.7
3	1.0	1.0 of Tube No. 2	1:20	50
4	1.0	1.0 of Tube No. 3	1:40	25
5	1.0	1.0 of Tube No. 4	1:80	12.5
6	1.0	0	n/a	0

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

4. To a coagulation reaction cuvette, add 50 µL from Tube 1, 50 µL of PC Deficient, and 50 µL of Activator. Mix and incubate at 37 °C for three minutes.
5. Add 50 µL 0.025 M $CaCl_2$ and immediately initiate timer. Record clotting time in seconds.
6. Repeat steps 4 and 5 for Tubes 2 through 6.
7. On linear-linear graph paper, plot clotting times in seconds (y-axis) vs. % of 0 protein C activity (x-axis).
8. Construct a standard curve by drawing the best straight line fit through the plots (see Example only: CRYO*check* Clot C Calibration Curve).



Results

Results are expressed as a percentage of normal protein C activity by comparison with a known standard or calibration plasma. Protein C values recovered below the laboratory established normal range may be indicative of a protein C deficiency (congenital or acquired). Each laboratory should establish its own normal reference range for protein C activity in accordance with CLSI guidelines[10].

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 testing system[11]. 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[12].

Limitations of the Procedure

Factor VIII:c Interference: CRYO*check* Clot C is unaffected by factor VIII:c activity levels up to 600%.

Heparin Interference: CRYO*check* Clot C is unaffected by unfractionated heparin (UFH) or by low molecular weight heparin (LMWH) up to 1.2 IU/mL.

Direct Thrombin Inhibitors: CRYO*check* Clot C may be affected by hirudin and other direct thrombin inhibitors, resulting in falsely elevated protein C activity levels.

Lupus Anticoagulant: Interference by lupus anticoagulants (LA) has not been observed with CRYO*check* Clot C. However, since LA are heterogeneous, the possibility that some could influence CRYO*check* Clot C cannot be ruled out.

Activated Protein C Resistance: CRYO*check* Clot C is unaffected by samples from patients heterozygous for the factor V_{Leiden} mutation. CRYO*check* Clot C. may be affected by samples homozygous for this mutation.

Expected Values

A normal population study was performed on 126 healthy adults. A mean protein C level of 124.7% with a 2 standard deviation (SD) range of 59.6% – 189.8% was recovered. It is recommended that each laboratory establish its own normal population range.

Performance Characteristics

Reportable Range

5 to 150% protein C activity.

Precision

To evaluate inter-assay precision, 25 individual normal donor samples and 25 individual samples from patients with abnormal low protein C were tested using a single calibration curve ("Curve 1"). A second calibration curve ("Curve 2") was then established, and the same 50 samples were assayed. A correlation between Curve 1 and Curve 2 of R = 0.9899 was obtained (Figure 1).

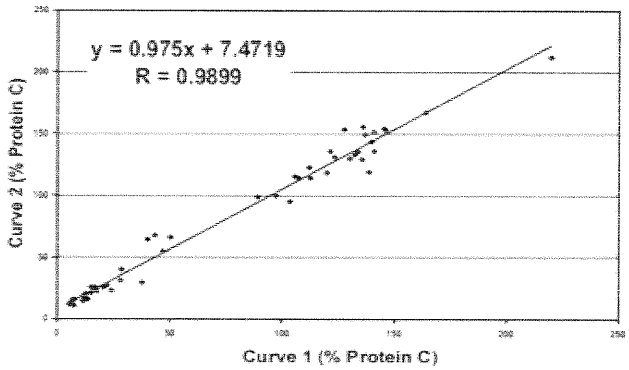


Figure 1: Inter-assay precision of CRYO*check* Clot C

Reproducibility

Intra-assay reproducibility was assessed by testing one normal and one abnormal plasma (with reduced % protein C) 20 times each. Mean, SD, and percent coefficient of variation (%CV) were as follows:

	% Protein C		
Test Sample	**Mean**	**SD**	**%CV**
Normal	121.3	7.3	6.0
Abnormal	39.7	3.4	8.5

Method Comparison

CRYO*check* Clot C was compared to another commercially available clot-based protein C test using 119 clinical samples from the target population for the assay. A correlation of R = 0.9147 was obtained (Figure 2).

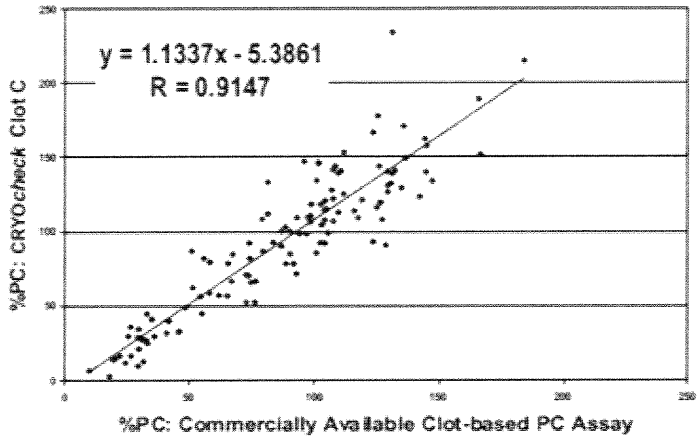


Figure 2: Correlation of protein C values determined on 119 samples from the target population.

Clinical Sample Profile:

The following clinical sample results were obtained with CRYO*check* Clot C in comparison to a commercially available chromogenic protein C assay.

Clinical Sample Category	N	% Protein C (mean ± SD)	
		CRYO*check* Clot C	Chromogenic Protein C
Normal	126	124.7 ± 32.6	107.1 ± 21.8
Oral Anticoagulation Therapy (OAT)	20	30.2 ± 23.2	49.4 ± 17.4
Heparin (UFH)	20	103.6 ± 29.4	104.4 ± 20.2
Heparin (LMWH)	20	90.2 ± 42.1	99.9 ± 31.0
Lupus Anticoagulant	20	127.9 ± 34.5	112.8 ± 20.1
Abnormal Low Protein (congenital)	5	18.8 ± 6.0	29.7 ± 6.4
Abnormal Low Protein C (acquired)	20	25.7 ± 16.9	34.8 ± 12.5
Disseminated Inta-vascular Coagulation (DIC)	20	39.4 ± 21.6	52.3 ± 18.6
Factor V_{Leiden} (homozygous)	3	55.8 ± 35.6	65.1 ± 15.9
Factor V_{Leiden} (heterozygous)	10	114.6 ± 18.4	112.4 ± 9.1
Factor Deficiency*	10	101.7 ± 18.0	N/A

* Plasmas tested included immunodepleted factors II, V, VII, VIII, IX, X, XI, XII and factor VIII and IX congenitally deficient plasma.

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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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