PrecisionBioLogic

CRYO*check*™ IVD

QUANTITATIVE PROTEIN S CLOTTING ASSAY

CLOT S ™

Intended Use

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

Summary and Principle

Protein S is a vitamin K-dependent glycoprotein with a molecular weight of ~70,000 Da. The mature protein circulates in plasma at a total concentration of 25 µg/mL, 40% free and 60% reversibly bound to C4b binding protein[1]. The free form of protein S acts as a cofactor to enhance the anticoagulant property of activated protein C, a potent inhibitor of procoagulant factors Va and VIIIa[2].

Protein S deficiency has both congenital and acquired etiologies of clinical interest. Congenital deficiencies are commonly associated with an increased risk of venous thromboembolic disease[3,4,5], while acquired abnormalities are associated with anti-vitamin K therapy[6], liver disease[6], disseminated intravascular coagulation (DIC)[6], oral contraceptives[7], oestrogen therapy[8], acute phase inflammatory responses[9], pregnancy[10], and newborns[11].

CRYO*check* Clot S initiates the common pathway of coagulation in plasma using a Russell's viper venom (RVV-X) reagent to convert factor X to Xa in the presence of activated protein C (APC), bypassing all factors above the common pathway[12]. When mixed with protein S deficient plasma, samples from patients with a protein S deficiency or dysfunction will have shortened CRYO*check* Clot S clotting times relative to samples with normal levels of functional protein S. The clotting time is proportional to the amount of functional protein S in the patient's plasma and this can be quantified using a calibration curve.

Reagents

- **Protein S Deficient Plasma (PS Deficient):** Contains citrated pooled human plasma (depleted of protein S by immunoadsorption), buffers and stabilizers.
- **Clot S Activator (Activator):** Contains activated protein C, Russell's viper venom, 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[13].

Storage, Preparation and Handling

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

Thaw one vial each of **PS Deficient** and **Activator** 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 according to format.

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

Immediately after thawing:

1. Vortex or vigorously mix **PS Deficient** only for 5 - 10 seconds
2. Gently swirl **Activator** for 5 - 10 seconds
3. Allow uncapped reagents to acclimate on-board the instrument for **30 minutes**.
4. Gently mix **PS Deficient** prior to performing tests and every two hours thereafter to prevent reagent sedimentation. For detailed mixing instructions, please consult the protocol for your automated coagulation analyzer.

For manual methods, swirl **PS Deficient** prior to performing tests and every two hours thereafter.

CRYO*check* Clot S may be used for up to five hours after preparation. When not in use, CRYO*check* Clot S reagents should be capped in the original vials and maintained at 2 to 8 °C. Before use, repeat steps 1 to 4.

NB: CRYO*check* Clot S Protein S Deficient Plasma and Clot S Activator are lot specific and should not be interchanged with other lot numbers.

Availability

Product	Catalog #	Format	Number of Tests
CRYO*check* Clot S	CCS-30	PS Deficient 5 x 3.0 mL Activator 5 x 3.0 mL	300
	CCS-15	PS 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 S Deficient Plasma **(PS Deficient)**
- Clot S 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)
- 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[14].

Assay Procedure

1. Prepare CRYO*check* Clot S 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 Diluen**t).
4. To a coagulation reaction cuvette, immediately add 50 µL of the diluted test plasma, 50 µL of PS Deficient and 50 µL of Activator.
5. Mix and incubate at 37 °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 S reagents according to Storage, Preparation and Handling instructions above.

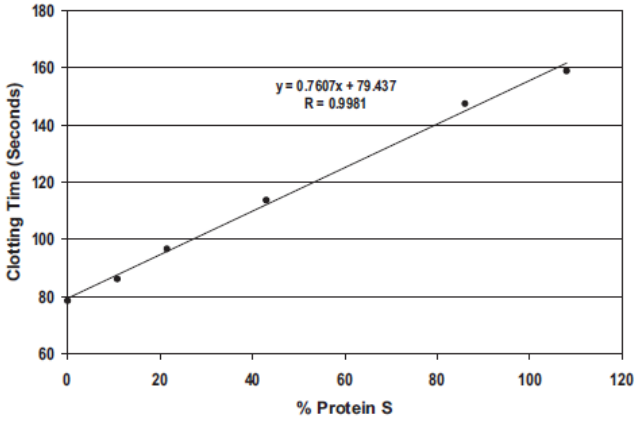
2. Prepare calibration plasma according to manufacturer's directions.
3. Prepare serial dilutions of calibration plasma from 1:7 to 1:80 in C & S Diluent according to the following table:

Tube No.	C & S Diluent (mL)	Volume of calibration Plasma	Dilution	% Factor
1	0.6	0.1	1:7	143
2	1.8	0.2	1:10	100
3	1.0	1.0 mL of Tube No. 2	1:20	50
4	1.0	1.0 mL of Tube No. 3	1:40	25
5	1.0	1.0 mL 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 S level of 100%. Always be sure to utilize the lot-specific % protein S 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 PS 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. Plot clotting times in seconds (y-axis) vs. % of protein S activity (x-axis).
8. Construct a standard curve and derive % protein S values (see Example only: CRYO*check* Clot S Calibration Curve). It is recommended that samples exceeding 140% protein S be diluted at 1:20 and re-tested. Samples below 10% protein S should be diluted at 1:5 and re-tested.

Example Only: CRYO*check* Clot S Calibration Curve



Results and Interpretation

Results are expressed as a percentage of normal protein S activity by comparison with a known standard or calibration plasma. Protein S values recovered below the laboratory established normal range may be indicative of a protein S deficiency (congenital or acquired). Each laboratory should establish its own normal reference range for protein S activity in accordance with CLSI guidelines[15].

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[16]. 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[17].

Limitations of the Procedure

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

Heparin Interference: CRYO*check* Clot S is unaffected by unfractionated heparin (UFH) or by low molecular weight heparin (LMWH) up to 1.0 IU/mL.

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

Lupus Anticoagulant: Lupus anticoagulants (LA) present in test plasma may affect CRYO*check* Clot S results.

Activated Protein C Resistance: CRYO*check* Clot S results may be affected by samples from patients with the factor V_{Leiden} mutation.

Expected Values

A normal population study was performed on 100 healthy adults. A mean protein S level of 90.3% with a two-standard deviation (SD) range of 46.7% – 133.9% was recovered. It is recommended that each laboratory establish its own normal population range.

Performance Characteristics

Reportable Range:

A reportable range for CRYO*check* Clot S of 10 to 140% protein S activity was obtained on a STA-R® instrument (Diagnostica Stago). Reportable range may vary depending on instrumentation used.

Precision:

Intra-assay reproducibility was assessed by testing one normal and one abnormal plasma (with reduced % protein S) 20 times each. To evaluate inter-assay precision, one normal sample and one abnormal sample were tested over multiple days, using multiple calibration curves. Mean, SD, and percent coefficient of variation (%CV) were as follows:

	Intra-Assay Precision			Inter-Assay Precision		
Test Sample	**Mean**	**SD**	**%CV**	**Mean**	**SD**	**%CV**
Normal	90.5	2.1	2.3	101.6	6.8	6.7
Abnormal	26.3	1.9	7.2	34.9	2.8	7.9

Correlation:

CRYO*check* Clot S was compared to another commercially available clot-based protein S test, using clinical samples from the assay target population.

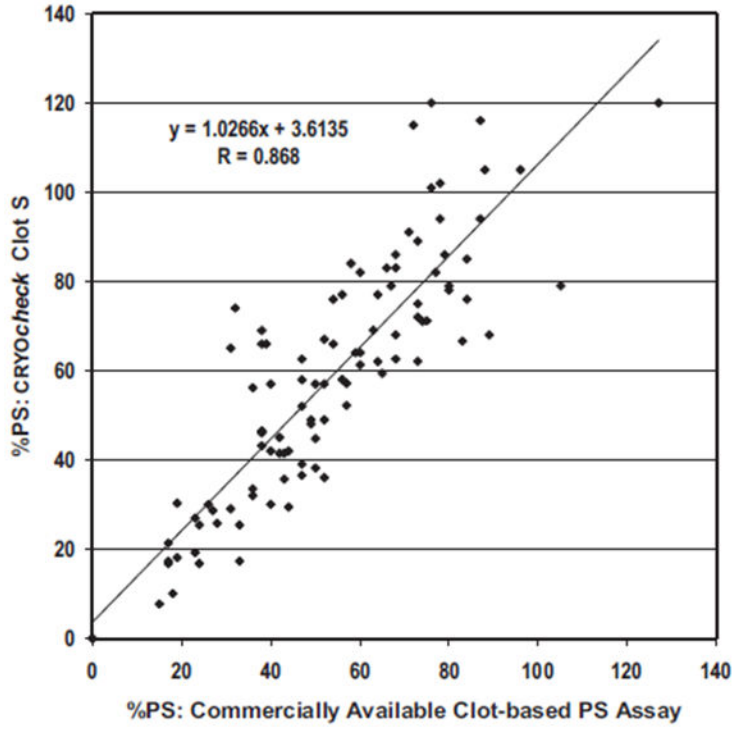


Figure 1: Correlation of protein S values determined on 100 samples from the target population.

Bibliography

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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
	Upper temperature limit		Consult electronic instructions for use

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

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