Protein S Ac

Revision bar indicates update to previous version.

CE 0197

Intended Use

Protein S Ac is an in vitro diagnostic reagent for the quantitative, WHO-standardized determination of protein S activity as aid to diagnosis of congenital or acquired protein S deficiencies in patients at risk for or suspected to have protein S deficiency in human sodium citrated plasma by means of automated coagulometric methods.

Summary and Explanation

Protein S is a vitamin K-dependent protein with essential roles in the regulation of thrombosis and hemostasis. Protein S acts as a non-enzymatic cofactor of two major natural anticoagulants[1]:

- Cofactor of activated protein C (APC): In the presence of calcium, protein S forms a complex with APC that binds to phospholipid surfaces and accelerates the APC-catalyzed proteolytic inactivation of the factors FVa and FVIIIa.
- Cofactor of TFPI (tissue factor pathway inhibitor): Protein S enhances the formation of the FXa-TFPI complex and the subsequent inhibition of the TF-FVIIa complex.

Of the circulating protein S, approximately 60 % of the protein is non-covalently bound to C4b-binding protein (C4BP), a regulator of the classical complement pathway. The remaining approx. 40 % of the total protein S fraction, i.e., free protein S, represent the physiologically active form[2].

Hereditary deficiencies of protein S are associated with an increased risk for venous thrombosis. They account for 2 % of unselected patients with venous thrombosis and up to 7.6 % of patients younger than 70 years with thrombosis. The rate of venous thrombosis among protein S–deficient members of symptomatic families is increased 5 to 11.5-fold compared with unaffected relatives. Among the general population, the risk is increased approximately 2-fold[3].

Inherited protein S deficiencies are classified into three types[2]:

- type I is defined by a reduction in both total and free protein S levels (quantitative defect)
- type II is rare and characterized by a decreased protein S activity but normal levels of total and free protein S (qualitative defect),
- type III corresponds to normal levels of total protein S but low levels of free protein S (distribution defect).

Acquired protein S deficiencies, indicated by decreased free protein S levels, are caused by several mechanisms[4]:

- increased consumption (thrombosis, surgery, DIC)
- decreased synthesis (liver disease, vitamin K deficiency, anti-vitamin K therapy, newborn)
- redistribution of complexed protein S (inflammation, acute phase reaction, oral contraceptives, estrogen replacement, pregnancy)

Testing for protein S activity is sensitive for all quantitative and qualitative protein S defects[4].

Principles of the Procedure

Protein Ca proteolytically cleaves FVa which is generated during the activation of the coagulation cascade by RVV (venom of Vipera russelli Russell's viper venom). In this reaction protein S acts as a cofactor which accelerates the reaction. As a result, the coagulation time increases proportionally

to the activity of protein S in the sample. The addition of deficient plasma ensures that the test mixture has a sufficient supply of fibrinogen, FV and the other necessary coagulation factors.

Coagulation is triggered at the level of factor FX by the FX activator of RVV. FXa forms thrombin from prothrombin under the action of the remaining FVa. The resulting thrombin finally converts fibrinogen to fibrin.

The coagulation time can be detected mechanically, optically or by other measurement principles.

Reagents

Note: Protein S Ac can be used on automated coagulation analyzers. Sysmex provides Reference Guides (Application Sheets) for several coagulation analyzers. The Reference Guides (Application Sheets) contain analyzer/assay specific handling and performance information which may differ from that provided in these Instructions for Use. In this case, the information contained in the Reference Guides (Application Sheets) supersedes the information in these Instructions for Use. Please also consult the instruction manual of the instrument manufacturer!

Reagent	Description	Storage	Stability
Protein S Ac			
DEFICIENT	Lyophilized reagent containing: • human plasma, citrated, with an unmeasurable residual activity of protein S	2–8 °C May be used up to the expiry date indicated on the label if stored unopened.	15–25 °C: reconstituted, 8 hours[a]; ≤ –18 °C: reconstituted, 2 months[a]
REAGENT	Lyophilized reagent containing: • human protein C, activated • Calcium chloride • Preservative: • reaction mass of 5-chloro-2-methyl-2H-isothiazol-3-one / 2-methyl-2H-isothiazol-3-one (3:1) (reconstituted: 5.1 mg/L)	2–8 °C May be used up to the expiry date indicated on the label if stored unopened.	15–25 °C: reconstituted, 8 hours[a]; 2–8 °C: reconstituted, 2 days[a]; ≤ –18 °C: reconstituted, 2 months[a]
ACTIVATOR	Lyophilized reagent containing: • soy lecithin (reconstituted: ~0.05 g/L) • Russell's viper venom (reconstituted: <1 mg/L) • Preservative: • Sodium azide (reconstituted: 0.5 g/L)	2–8 °C May be used up to the expiry date indicated on the label if stored unopened.	37 °C: reconstituted, 4 hours[a]; 15–25 °C: reconstituted, 8 hours[a]; 2–8 °C: reconstituted, 2 days[a]; ≤ –18 °C: reconstituted, 2 months[a]

[a] closed vial

Protein S Ac DEFICIENT: The plasma is likewise free of C4bBP.

Protein S Ac ACTIVATOR: derived from the venom of Russell's Viper (Vipera russelli) with added phospholipids

On-board stability
Information regarding on-board stability is specified in the Reference Guides (Application Sheets) for the different coagulation analyzers.

Warnings and Precautions

For *in-vitro* diagnostic use only.

For laboratory professional use.

According to EU regulation 2017/746, any serious incident that has occurred in relation to the device shall be reported to the manufacturer and the competent authority of the EU Member State through your local distribution representative in which the user and/or patient is established.

Safety data sheets (MSDS/SDS) available upon request.

Warning! Protein S Ac REAGENT
Hazardous ingredient: reaction mass of 5-chloro-2-methyl-2H-isothiazol-3-one / 2-methyl-2H-isothiazol-3-one (3:1) (0.0118 % [w/w]).
H317: May cause an allergic skin reaction. **H412**: Harmful to aquatic life with long lasting effects. **P261**: Avoid breathing dust. **P280**: Wear protective gloves/protective clothing/eye protection/face protection. **P273**: Avoid release to the environment. **P302 + P352**: IF ON SKIN: Wash with plenty of soap and water. **P333 + P313**: If skin irritation or rash occurs: Get medical advice/attention. **P362 + P364**: Take off contaminated clothing and wash it before reuse. **P501**: Dispose of contents and container in accordance with all local, regional, and national regulations.

Warning! Protein S Ac ACTIVATOR
Hazardous ingredient: Sodium azide (1.12 % [w/w]).
H312: Harmful in contact with skin. **H412**: Harmful to aquatic life with long lasting effects. **P280**: Wear protective gloves/protective clothing/eye protection/face protection. **P273**: Avoid release to the environment. **P312**: Call a POISON CENTER or doctor/physician if you feel unwell. **P501**: Dispose of contents and container in accordance with all local, regional, and national regulations.

CAUTION! POTENTIAL BIOHAZARD
Protein S Ac DEFICIENT**, Protein S Ac** REAGENT
Each donor or donor unit was tested and found to be negative for human immunodeficiency virus (HIV) 1 and 2, hepatitis B virus (HBV) and hepatitis C virus (HCV) using either tests that are CE marked or FDA approved for this purpose. Because no known test can offer complete assurance of the absence of infectious agents, all human derived products should be handled with appropriate caution.

Caution
Protein S Ac REAGENT**, Protein S Ac** ACTIVATOR
This device contains material of animal origin and should be handled as a potential carrier and transmitter of disease.

Contains sodium azide as a preservative. Sodium azide can react with copper or lead plumbing to form explosive metal azides. On disposal, flush reagents with a large volume of water to prevent the buildup of azides. Disposal into drain systems must be in compliance with prevailing regulatory requirements.

Dispose of hazardous or biologically contaminated materials according to the practices of your institution. Discard all materials in a safe and acceptable manner and in compliance with all government requirements.

Summary of Safety and Performance (SSP) is available in the European database on medical devices (see Eudamed public website: https://ec.europa.eu/tools/eudamed). In case Eudamed is not available, SSP can be delivered by the manufacturer on request.

Preparing Reagents

Protein S Ac DEFICIENT: Dissolve in the labeled quantity of distilled water and incubate at 15 to 25 °C for 60 minutes

Protein S Ac REAGENT: Dissolve in the labeled quantity of distilled water and incubate at 15 to 25 °C for 60 minutes.

Protein S Ac ACTIVATOR: Dissolve in the labeled quantity of distilled water, incubate at 37 °C for 60 minutes in the water bath and mix gently before use.

Specimen Collection and Handling

Collecting the Specimen

To obtain the plasma, carefully mix 1 part sodium citrate solution 0.11 mol/L (3.2 %) with 9 parts venous blood, avoiding the formation of foam. Centrifuge immediately at no less than 1 500 × g for at least 10 minutes. **When removing the plasma, take care to ensure that no platelets are withdrawn.** If the sample is to be frozen repeat centrifugation, **rapidly** freeze the collected plasma in a well-closed plastic container.

Storing the Specimen

Stability of the samples:

≤ −18 °C	1 month
15 to 25 °C	4 hours

Plasma stored at ≤ −18 °C is to be thawed within 10 minutes at 37 °C after which the assay is to be performed within 4 hours.

Procedure

Materials Provided

REF	Contents		
OPAP03	Protein S Ac		
	Protein S Ac Deficient Plasma Protein S Ac DEFICIENT	6 × →	1 mL
	Protein S Ac APC Reagent Protein S Ac REAGENT	2 × →	2 mL
	Protein S Ac Starting Reagent Protein S Ac ACTIVATOR	2 × →	5 mL

Materials Required but not Provided

Item	Description
REF ORKL17	STANDARD PLASMA, Standard Human Plasma
REF ORKE41	CONTROL N, Control Plasma N
REF OUPZ17	CONTROL P, Control Plasma P
Coagulation analyzers[b], such as:	• AUTOMATED BLOOD COAGULATION ANALYZER CS-2500 (CS-2500 System) • AUTOMATED BLOOD COAGULATION ANALYZER CS-5100 (CS-5100 System)

[b] Availability of analyzers may vary by country.

Please note that the applications on other analyzers can be validated by the instrument manufacturer in accordance with the requirements of the REGULATION (EU) 2017/746 under their responsibility as long as the intended purpose and performance are not modified.

Internal Quality Control

Normal range: CONTROL N

Pathological range: CONTROL P

Two levels of quality control material (normal and pathologic range) have to be measured at start of the test run, with each calibration, upon reagent vial changes and at least every eight hours on each day of testing. The controls should be processed just like the samples. Each laboratory should determine its own quality control range, either by means of the target values and ranges provided by the manufacturer of the controls or by means of its own range established in the laboratory. If the control values lie outside the range determined beforehand, then the reagent, calibration curve and coagulation analyzer should be checked. Do not release patient results until the cause of deviation has been identified and corrected.

Results

The results are obtained using a reference curve prepared beforehand by serially diluting STANDARD PLASMA with Protein S Ac DEFICIENT. Sample testing must follow the determination of the reference curve immediately. Care is to be taken to ensure that conditions for establishing the reference curves and for performing the subsequent measurements are the same (e.g. temperature of the reagents).

Limitations

The precision of the test is highly dependent on the precision of pipetting, especially in the case of the small sample volumes!

With deep-frozen samples, the recovery of protein S may be diminished if platelets and leukocytes were not carefully separated off during withdrawal of the plasma. Before samples are frozen they must be centrifuged a second time.

Direct thrombin inhibitors and direct Xa inhibitors may or may not falsely elevate Protein S activity[2]. Vitamin K antagonists (VKA) will result in reduced Protein S activity[2].

Heparin activities up to 3 U/mL (UFH and LMWH) do not disturb the test.

The existence of a mutation in FV at its protein Ca cleavage site may lead to diminished recovery of protein S[5].

The antiphospholipid antibodies (e.g. Lupus anticoagulant) may be accompanied by either increased[6] or decreased[7] Protein S activity test results.

FVIII activities up to 400 % do not interfere.

The manufacturer has validated use of these reagents on various analyzers to optimize product performance and meet product specifications. Please note that the applications on other analyzers can be validated by the instrument manufacturer in accordance with the requirements of the REGULATION (EU) 2017/746 under their responsibility as long as the intended purpose and performance are not modified. User defined modifications are not supported by the manufacturer as they may affect performance of the system and assay results. It is the responsibility of the user to validate modifications to these instructions or use of the reagents on analyzers other than those included in Application Sheets or these Instructions for Use.

Results of this test should always be interpreted in conjunction with the patient's medical history, clinical presentation and other findings.

Expected Values

In a study with ostensibly healthy subjects (n = 218) using the CA-1500 System the following data were obtained (5^{th} - 95^{th} Percentile):

60 - >130 % , Median: 95 % (Men: 75 - >130 %, Median: 104 %; women without oral contraceptives: 59 - 118 %, Median: 90 %; women with oral contraceptives: 52 - 118 %, Median: 81 %).

Reference intervals vary from laboratory to laboratory depending on the population served and the technique, method, equipment and reagent lot used. Therefore, each laboratory must establish its own reference intervals or verify them whenever one or more of the aforementioned variables are changed.

Performance Characteristics

Measuring Range

The measuring range for the CA-1500 System extends from 10 to 140 % of Norm.

The measuring range depends on the individual application of the assay due to instrument related conditions. Application specific performance data is listed in the respective Reference Guides of the instruments.

Specificity

The test detects functionally active protein S.

Precision

For normal plasma samples measured using the CA-1500 System, the resulting variation coefficients ranged between 3.6 % and 5.1 % within the series of measurements, and between 2.0 % and 4.1 % from day to day. Total precision ranged from 4.6 to 5.5 %.

For pathologically deficient plasma samples which were measured using the CA-1500 System, the resulting variation coefficients ranged between 4.7 % and 9.2 % within the series of measurements, and between 1.9 % and 7.7 % from day to day. Total precision ranged from 4.8 to 11.6 %.

Other system specific results are given in the respective Reference Guides (Application Sheets). The reproducibility was assessed by the manufacturer for Protein S assay with Protein S Ac based on proficiency testing information in 2020. The overall reproducibility median CV% was found to be < 15 % including lot, instrument, laboratory, and operator variability factors.

Method Comparison

Protein S Ac assay was compared to a commercially available protein S assay by evaluation of 239 plasma samples.

A correlation coefficient of r = 0.92 was obtained, with a y-intercept value of 1.53 % of Norm and a slope of 0.96.

The values cited for the specific performance characteristics of the assay represent typical results and are not to be regarded as specifications for Protein S Ac test.

Technical Assistance

For customer support, contact your local technical support provider or distributor.

Current Version of Application Sheets

Protein S Ac can be used in combination with various automated coagulation analyzers. Sysmex provides Reference Guides/Application Sheets for the coagulation analyzers listed in section "Materials Required but not Provided", page 4 under the dedicated link below:

sysmex-ifu.com/ag

As the manufacturer continuously monitors the product performance and safety, the users are required to ensure that they work with the correct revision of the instructions for the product lots in use. Please periodically review the availability of new electronic labeling revisions to ensure safe use of the product.

The IFU version number is visible on each product box label. Sysmex ensures that all products lots bearing the same IFU version number are compatible with the electronic labeling provided via sysmex-ifu.com.

References

1. Dahlbäck B. Novel insights into the regulation of coagulation by factor V isoforms, tissue factor pathway inhibitorα, and protein S. J Thromb Haemost. 2017;15:1241-1250.
2. Marlar RA, Gausman JN, Tsuda H, et al. Recommendations for clinical laboratory testing for protein S deficiency: communication from the SSC committee plasma coagulation inhibitors of the ISTH. J Thromb Haemost. 2021;19(01):68–74.
3. Van Cott EM, Ledford-Kraemer M, Meijer P, et al. Protein S assays: an analysis of North American Specialized Coagulation Laboratory Association proficiency testing. Am J Clin Pathol 2005;123(5):778-85.
4. Marlar RA, Gausman JN. Protein S abnormalities: a diagnostic nightmare. Am J Hematol. 2011;86:418-21.
5. Faioni EM, Boyer-Neumann C, Franchi F, et al. Another protein S functional assay is sensitive to resistance to activated protein C. Thromb Haemost. 1994; 72: 648.
6. Lawrie AS, Lloyd ME, Mohamed F, et al. Assay of protein S in systemic lupus erythematosus. Blood Coagul Fibrinolysis. 1995; 6: 322-4.
7. Rossi E, Gatti L, Guarneri D, et al. Functional protein S in women with lupus anticoagulant inhibitor. Thromb Res. 1992; 65: 253-62.

Definition of Symbols

The following symbols may appear on the product labeling:

	Do not reuse		Use By
LOT	Batch Code	REF	Catalogue Number
	Caution		Manufacturer
EC REP	Authorized representative in the European Community	CH REP	Authorized representative in Switzerland
	Contains sufficient for <n> tests		Biological Risks
IVD	*In Vitro* Diagnostic Medical Device		Temperature Limitation
	Consult instruction for Use		Non-sterile
CE	CE marking of conformity	CE 0197	CE marking of conformity with notified body ID number. Notified body ID number can vary.
CONTENTS	Contents		Reconstitution volume
LEVEL	Level		Keep away from sunlight and heat
WARNING	Warning	DANGER	Danger
RxOnly	Prescription device (US only)	UDI	Device Identification (UDI) barcode
REACH xx/xx/xx	REACH Authorization Number		

Legal Information

Siemens Healthcare Diagnostics Products GmbH
Emil-von-Behring-Str. 76
35041 Marburg
Germany
siemens-healthineers.com