INNOVANCE® Free PS Ag

Revision bar indicates update to previous version.

CE 0197

Intended Use

INNOVANCE® Free PS Ag is an in vitro diagnostic reagent for the quantitative, WHO-standardized determination of free protein S antigen 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 turbidimetric 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)

Free protein S antigen is considered the functional anticoagulant fraction of total protein S. In view of the better robustness of free PS Ag assays compared to PS activity assays, the free PS Ag assay is the recommended method for initial PS testing[4].

Principles of the Procedure

Polystyrene particles were coated with two different monoclonal antibodies both specific for free protein S. The resulting latex reagent aggregates when mixed with samples containing free protein S. The degree of aggregation is directly proportional to the concentration of free protein S in the test sample and is detected turbidimetrically via the increase in turbidity.

Reagents

Note: INNOVANCE® Free PS Ag 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
INNOVANCE® Free PS Ag			
REAGENT	Ready to use liquid containing: • polystyrene particles (<2 g/L) coated with monoclonal mouse antibodies to free protein S (0.076 g/L) in buffered solution • Gentamicin sulfate (6.25 mg/L) • Amphotericin B (0.625 mg/L) • Preservative: • Sodium azide (< 1 g/L)	2–8 °C May be used up to the expiry date indicated on the label if stored unopened.	2–8 °C: once opened, 8 weeks[a]

BUFFER	Ready to use liquid containing:
	• saline buffer
	• heterophilic blocking reagent, of murine origin (0.1 g/L)
	• Preservatives:
	  • Sodium azide (< 1 g/L)
	  • reaction mass of 5-chloro-2-methyl-2H-isothiazol-3-one / 2-methyl-2H-isothiazol-3-one (3:1) (0.0182 g/L)
		2–8 °C
		May be used up to the expiry date indicated on the label if stored unopened.
			2–8 °C:
			once opened, 8 weeks[a]

[a] closed vial

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! INNOVANCE Free PS Ag BUFFER
Hazardous ingredient: reaction mass of 5-chloro-2-methyl-2H-isothiazol-3-one / 2-methyl-2H-isothiazol-3-one (3:1).
H317: May cause an allergic skin reaction.
P280: Wear protective gloves/protective clothing/eye protection/face protection. **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.

CAUTION! POTENTIAL BIOHAZARD
INNOVANCE Free PS Ag REAGENT**, INNOVANCE Free PS Ag** BUFFER
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
INNOVANCE Free PS Ag BUFFER
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.

Preparing Reagents

INNOVANCE® Free PS Ag components are liquid and ready to use. Before use twist off caps, place the components on the analyzer and start measurement.

Avoid foam formation. Otherwise, remove bubbles from reagent surface prior to placing on the analyzer.

All kit components are lot-specific. The combination of lots other than those specified for the particular kit lot may lead to incorrect results.

Specimen Collection and Handling

Specimen type: Platelet poor citrated human plasma.

Collecting the Specimen

To obtain plasma, carefully mix 1 part sodium citrate solution (0.11 mol/L, 3.2 %) with 9 parts venous blood, avoiding the formation of foam. An evacuated tube system or syringe may be used. Centrifuge the blood tube as soon as possible for at least 15 minutes at 1500 to 2500 × g. Plasma can be stored on the cells or can be removed from the cellular components and stored in a closed tube at room temperature. Please refer to CLSI Guideline H21-A5[5] for further details. The manufacturer's instructions for the sampling equipment must also be observed.

Storing the Specimen

15 to 25 °C	24 hours
≤ −18 °C	3 months

Preparing frozen Specimen

Freeze plasma within 4 hours of blood collection at ≤ −18 °C. Frozen plasma should be thawed within 10 minutes at 37 °C and homogenized by carefully mixing without foam formation. Then carry out the determination of free protein S within 4 hours.

Procedure

Materials Provided

REF	Contents		
OPGL03	INNOVANCE® Free PS Ag		
	INNOVANCE® Free PS Ag reagent INNOVANCE Free PS Ag REAGENT	4 ×	3.4 mL
	INNOVANCE® Free PS Ag Buffer INNOVANCE Free PS Ag BUFFER	4 ×	3.0 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
REF B4234-25 REF B4265-37	OV BUFFER, Dade® Owren's Veronal Buffer or CA SYSTEM BUFFER, Dade® CA System Buffer for use on CS* Systems
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.

Test Procedure

Pipetting of samples and reagents as well as mixing and processing is performed automatically by the coagulation analyzer. For details of this processing, refer to the respective instrument specific Instruction Manual.

Performing Calibration

Calibration Material:	STANDARD PLASMA. For preparation, please follow exactly the procedure described in the chapter "Preparation of the Calibrator" in the Instructions for Use of STANDARD PLASMA.
Calibration Scheme:	6 levels, n = 2 per level
Units:	% of Norm
Typical Calibration Levels:	STANDARD PLASMA is diluted automatically by the instrument used. The respective levels are defined by the actual concentration of free protein S in the STANDARD PLASMA lot as provided in the enclosed Table of Analytical Values, and by the system-specific dilution settings for calibration.
A new calibration is required:	• for each new reagent lot of INNOVANCE® Free PS Ag. • after major maintenance or service, if indicated by quality control results. • as indicated in laboratory quality control procedures. • when required by government regulations.

Internal Quality Control

Normal range: CONTROL N
Pathological range: CONTROL P

Two levels of quality control material (normal and pathological 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. 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 control values determined in the laboratory. If the measured control value lies outside of the pre-determined range, the reagents, the standard curve and the coagulation analyzer should be checked. Do not report patient results until the problem has been identified, corrected and documented.

Assay Range

10 to 150 % of Norm with BCS® XP Systems. Assay ranges are analyzer-dependent and given in the Reference Guides (Application Sheets) for the different coagulation analyzers.

Samples initially above the assay range may be manually diluted according to the procedure specified in the Reference Guides (Application Sheets) for the different coagulation analyzers.

Results

Assay calibration is performed with STANDARD PLASMA which is calibrated against the *2nd WHO International Standard for Protein S*. INNOVANCE® Free PS Ag results are provided in % of Norm.

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

Limitations

Turbidity and particles in the samples may interfere with the assay. Therefore, samples containing particles must be centrifuged prior to testing. Lipemic or turbid samples which cannot be clarified by centrifugation (10 minutes at approximately 15000 × g) must be excluded from the assay.

Patient samples may contain heterophile antibodies (e. g. human anti-mouse antibodies (HAMA) or rheumatoid factors) that could react in immunoassays to give a falsely elevated or depressed result[6]. This assay has been designed to minimize interference from heterophile antibodies by addition of a blocking reagent. Nevertheless, complete elimination of this interference from all patient specimens cannot be guaranteed.

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.

Expected Values

Frozen plasma specimens obtained from apparently healthy donors were tested in a study using the INNOVANCE® Free PS Ag assay on the BCS® XP System with the following results:

	n	[% of Norm]
males	149	73.2–129.5
females, without oral contraceptives, without hormone replacement therapy, without pregnancy	151	65.4–138.7

Further system-specific results are given in the respective Reference Guides (Application Sheets).

In a study on BCS® XP, results of fresh and frozen samples were shown to be comparable (coefficient of correlation: 0.981, slope: 1.00, y axis intercept: 0.73 % of Norm, evaluation by Passing-Bablok regression analysis, n = 95).

As with all *in vitro* diagnostic assays, each laboratory should determine its own reference ranges for the diagnostic evaluation of patient results. Consider these limits as guidelines only.

Performance Characteristics

Note: The values cited for specific performance characteristics of the assay represent typical results and are not to be regarded as specifications for INNOVANCE® Free PS Ag.

Measuring Range

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

No cross-reactivity with protein S bound to C4b-binding protein (C4BP) is observed. Addition of C4BP to a plasma sample, leading to an increase of protein S bound to C4BP and a decrease of free protein S, reduces the test results of INNOVANCE® Free PS Ag in a concentration-dependent manner.

Sensitivity

The limit of detection and the limit of quantitation for the INNOVANCE® Free PS Ag assay were determined on the BCS® XP System consistent with CLSI guideline EP17-A[7]. Results are based on determinations with 60 samples containing no and 60 samples containing low levels of free protein S. The limit of detection is the lowest concentration that can be detected reliably. The limit of quantitation is the lowest concentration that can be quantitatively determined.

Limit of Detection[c] 1.9 % of Norm

Limit of Quantitation[d] 5.0 % of Norm

[c] with a type I and type II error level of 5 %,

[d] with a total error of ≤ 46 %.

Precision

Precision studies were conducted with the BCS® XP System, as described in the CLSI guideline EP05-A3[8], using CONTROL N and CONTROL P as well as two plasma pools covering the decision and the pathological range, respectively.

Sample	n	Mean [% of Norm]	Repeatability CV [%]	Within-Device/Lab Precision CV
CONTROL N	80	85.2	2.0	2.9
CONTROL P	80	30.7	1.3	4.9
Plasma pool decision range	80	57.7	1.7	1.9
Pathological plasma pool	80	14.8	3.3	6.6

Other system specific results are given in the respective Reference Guides (Application Sheets).

The reproducibility was assessed by the manufacturer for INNOVANCE® Free PS Ag based on proficiency testing information in 2019. The overall reproducibility median CV% was found to be <5 % including lot, instrument, laboratory and operator variability factors.

Antigen Excess

The INNOVANCE® Free PS Ag assay on the BCS® XP System shows no high-dose hook effect up to 598 % of Norm free protein S.

Method Comparison

A study was performed with frozen samples to compare the INNOVANCE® Free PS Ag assay on the BCS® XP System to the CS-5100 System for the measurement of free Protein S. The results from the Passing-Bablok regression analysis are summarized in the following table:

n	Concentration range of plasma samples investigated	Correlation Coefficient	Regression equation
346	12.4–138.4 % of Norm	0.994	y = 0.95 x + 4.13 % of Norm

Other system specific results are given in the respective Reference Guides (Application Sheets).

Interference

The INNOVANCE® Free PS Ag assay was evaluated for interference on the BCS® XP System and CA-1500 System according to CLSI guideline EP7-A2[9]. A relative difference between control samples (without the interferent) and test samples (containing the interferent) exceeding 10 % is considered interference.

The potential interference by bilirubin, hemoglobin and triglycerides is described in the Reference Guides (Application Sheets) for the individual coagulation analyzers.

	No Interference up to...	
Interferent	**BCS® XP System**	**CA-1500 System**
Cholesterol	625 mg/dL (16.2 mmol/L)	625 mg/dL (16.2 mmol/L)
Fibrinogen	11 g/L (32.4 µmol/L)	11 g/L (32.4 µmol/L)
Unfractionated heparin (UFH)	7.5 IU/mL	7.5 IU/mL
Low molecular weight heparin (LMWH)	10 IU/mL	10 IU/mL
Rheumatoid factors (RF)	2120 IU/mL	2120 IU/mL
Platelets (fresh or frozen plasma)	24.8×10^7 / mL (24.8×10^{10} / L)	24.8×10^7 / mL (24.8×10^{10} / L)

Technical Assistance

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

Current Version of Application Sheets

INNOVANCE® Free PS Ag 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

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7. CLSI. Protocols for determination of limits of detection and limits of quantitation; Approved Guideline. CLSI document **EP17-A** [ISBN 1-56238-551-8]. CLSI, 940 West Valley Road, Suite 1400, Wayne, PA 19087-1898 USA, 2004.
8. CLSI. Evaluation of Precision of Quantitative Measurement Procedures; Approved Guideline – Third Edition. CLSI document **EP05-A3** [ISBN 1-56238-968-8)] CLSI, 950 West Valley Road, Suite 2500, Wayne, PA 19087-1898 USA, 2014.
9. CLSI. Interference testing in clinical chemistry; Approved Guideline–Second Edition. CLSI document **EP7-A2** [ISBN 1-56238-584-4]. Clinical Laboratory Standards Institute, 940 West Valley Road, Suite 1400, Wayne, Pennsylvania 19087-1898 USA, 2005.

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		

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