Protein C Reagent

PROTEIN C | COAG

Revision bar indicates update to previous version.

CE 0197

Intended Use

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

Summary and Explanation

Protein C is a vitamin K-dependent zymogen that has essential roles in the regulation of thrombosis and hemostasis. Physiologic activation of protein C occurs at the surface of endothelial cells, with acceleration by thrombomodulin. The activated protein C (APC), together with the cofactor protein S, limits thrombin generation by inactivation of the activated coagulation factors FV and FVIII via limited proteolysis. APC also exhibits potent cell-protective and anti-inflammatory properties[1].

Congenital, heterozygous protein C deficiency occurs in about 3 % of unselected patients with a first venous thrombosis and no known malignancy, and up to 9 % of patients younger than 70 years with venous thrombosis, increasing the risk for venous thrombosis 7-fold. Homozygous protein C deficiency is often incompatible with life without anticoagulation or replacement therapy, and can present with purpura fulminans and disseminated intravascular coagulation (DIC) in the newborn period[2].

Protein C activity determination is part of the thrombophilia test panel[2–4]. The diagnosis of protein C deficiency should be established only after other acquired causes of protein C deficiency are excluded.

Acquired protein C deficiency can be seen with warfarin or other vitamin K–antagonist therapy, vitamin K deficiency, liver disease, disseminated intravascular coagulation, renal insufficiency, proximal to acute thrombosis, and postoperatively, among others. Protein C is decreased during episodes of intravascular coagulation and sepsis[3].

Protein C activity can be determined by chromogenic or coagulometric methods. The carboxylation defect of protein C induced by therapy with vitamin K antagonists results in lower activity levels observed with coagulometric methods compared to chromogenic methods[2].

Principles of the Procedure

Protein C in the patient sample is activated by addition of a specific snake venom contained in the PROTEIN C | COAG | ACTIVATOR. Activated protein C inhibits Factor FV and Factor FVIII contained in the added Protein C Deficient Plasma. This inhibition reaction prolongs the subsequent APTT test. The prolongation of the APTT is thus a measure of the protein C content of the patient sample. Graduated dilutions of a standard plasma permit a standard curve to be established from which the protein C content of patient samples can be read in percent of norm.

Reagents

Note: PROTEIN C COAG can be used manually or 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 C Reagent PROTEIN C COAG			
ACTIVATOR	Lyophilized reagent containing: • extract from snake venom, Agkistrodon contortrix (reconstituted: 1 U/mL) • Stabilizer	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, 7 days[a]
REAGENT APTT	Ready to use liquid containing: • purified soy phosphatides (~1 g/L) • 1.0×10^{-4} M ellagic acid • Stabilizers • Preservative: • Phenol (1.65 g/L)	2–8 °C May be used up to the expiry date indicated on the label if stored unopened. Do not freeze!	15–25 °C: once opened, 24 hours[a]; 2–8 °C: once opened, 7 days[a]

DEFICIENT	Lyophilized reagent containing:
	• human plasma, citrated, with protein C content (reconstituted: < 1 %)
	• Stabilizer:
	  • HEPES
	• Heparin neutralizer:
	  • Hexadimethrine bromide
		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, 7 days[a]

[a] closed vial

Reconstituted PROTEIN C COAG ACTIVATOR and PROTEIN C COAG DEFICIENT can be frozen and thawed once, in the original vial. Frozen PROTEIN C COAG DEFICIENT must be thawed at 37 °C within 10 minutes and should not be exposed to 15 to 25 °C for longer than 2 hours after thawing.

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.

PROTEIN C | COAG | ACTIVATOR
Hazardous ingredient: extract from snake venom, Agkistrodon contortrix.
May produce an allergic reaction.

CAUTION! POTENTIAL BIOHAZARD

PROTEIN C | COAG | DEFICIENT
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 C | COAG | ACTIVATOR
This device contains material of animal origin and should be handled as a potential carrier and transmitter of disease.

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

Reconstitute PROTEIN C | COAG | ACTIVATOR in 3 mL of distilled water.

Reconstitute PROTEIN C | COAG | DEFICIENT in 1 mL of distilled water, invert gently to dissolve.

PROTEIN C | COAG | REAGENT | APTT must be warmed to ambient temperature (15 to 25 °C) and inverted gently to avoid foaming.

Green sediment of ellagic acid/phospholipid may form upon standing. To resuspend, mix gently by inversion immediately before use.

Calcium Chloride Solution must be warmed to 37 °C in a test tube.

All other reagents must be allowed to stand for at least 10 minutes at room temperature (15 to 25 °C) before use.

Specimen Collection and Handling

Collecting the Specimen

To obtain plasma samples, carefully mix 1 part sodium citrate solution 0.11 mol/L (3.2 %) with 9 parts venous blood, avoiding the formation of foam. Centrifuge the blood specimen immediately at 1 500 × g for no less than 15 minutes at room temperature and remove the supernatant plasma. Please refer to CLSI document H21-A5 for further details[5].

Storing the Specimen

Store fresh plasma samples at 15 to 25 °C for up to 4 hours.

Stability of the samples:

15 to 25 °C	4 hours
≤ −18 °C	1 month

Plasma samples stored at ≤ −18 °C must be thawed at 37 °C within 10 minutes. Plasma samples should not be exposed to 15 to 25 °C for longer than 2 hours after thawing. Do not refreeze.

Procedure

Materials Provided

REF	Contents	
OQYG11	Protein C Reagent PROTEIN C COAG	
	Protein C Activator PROTEIN C COAG ACTIVATOR	1 × → 3 mL
	APTT Reagent for Protein C PROTEIN C COAG REAGENT APTT	1 × 10 mL
	Protein C Deficient Plasma PROTEIN C COAG DEFICIENT	4 × → 1 mL

Materials Required but not Provided

Item	Description
REF ORHO37	$CaCl_2$ SOLUTION, Calcium Chloride Solution 0.025 mol/L
REF ORKE41	CONTROL N, Control Plasma N
REF OUPZ17	CONTROL P, Control Plasma P
REF OQAA33	IMIDAZOLE BUFFER, Imidazole Buffer Solution (for manual method and for some semi-automated instruments)
REF ORKL17	STANDARD PLASMA, Standard Human Plasma
–	Distilled water
–	Disposable plastic tubes
Coagulation analyzers[b], such as:	• Automated Blood Coagulation Analyzer CA-600 series (CA-600 series) • 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

Dilute 1 part patient sample + 9 parts Imidazole Buffer Solution. The dilutions should be used within 10 minutes.

Pipette the following reagents into a test tube warmed to 37 °C:

Patient plasma (diluted 1+9)	50 µL
PROTEIN C COAG DEFICIENT	50 µL
PROTEIN C COAG ACTIVATOR	50 µL
PROTEIN C COAG REAGENT APTT	50 µL
Mix and incubate for exactly 4 minutes at 37 °C.	
Calcium Chloride Solution (37 °C)	50 µL
Start the stopwatch or timer on the coagulation analyzer and determine the coagulation time.	

Note: Do not deviate from the pipetting sequence.

Performing Calibration

The calibration curve is established using a calibrator plasma with an assigned value for protein C (e.g. STANDARD PLASMA). The STANDARD PLASMA assigned value is lot specific, please refer to the Table

of Assigned Values. To establish a calibration curve, manual dilutions of the calibrator plasma, first with PROTEIN C COAG DEFICIENT and then with IMIDAZOLE BUFFER must be prepared in disposable plastic tubes. Suggested calibration points: 150 %, 100 %, 75 %, 50 %, 33 %, 10 % of Norm protein C activity. The dilutions are obtained as follows (calibrator plasma assumed to have 100 % of Norm protein C activity):

		1. dilution step		2. dilution step	
Multiplication factors[c]	Dilution	Calibrator Plasma [µL]	Protein C Deficient Plasma [µL]	Calibrator Dil. with Deficient Plasma [µL]	IMIDAZOLE BUFFER [µL]
1.50	1:6.7	60	–	–	340
1.00	1:10	50	–	–	450
0.75	1:13.3	150	50	50	450
0.50	1:20	50	50	50	450
0.33	1:30.3	50	100	50	450
0.10	1:100	20	180	50	450

[c] To obtain the protein C activity (% of Norm) for each calibration point, multiply the assigned value of calibrator by the indicated factors.

Calibrator coagulation times are measured in duplicate as described in the "Test Procedure" section using 50 µL of the respective final calibrator dilution instead of 50 µL diluted patient plasma.

A new reference curve must be generated for each equipment change and for each new lot of Protein C Reagent.

Internal Quality Control

Normal range: CONTROL N

Pathological range: CONTROL P

Two controls (one within the normal range and one within the pathological range) should be measured at least every 8 hours during each day of testing. The control material should be treated like a sample. Each laboratory should determine its own quality control range either on the basis of the target values and target ranges provided by the manufacturer of the controls or on the basis of control values determined in the laboratory. If the measured control value lies outside the range determined beforehand, then the reagents, coagulation analyzer and reference curve should be examined. Do not release patient results until the cause of deviation has been identified and corrected.

Results

PROTEIN C COAG results are reported in percent of norm protein C activity. These results should be related to the reference range for protein C testing in each laboratory.

Limitations

Therapeutic doses of hirudin or other direct thrombin inhibitors will cause erroneously increased protein C activity[6]. For some direct anti-Xa oral anticoagulants it has been shown that therapeutic levels may lead to overestimation of the protein C activity in plasma[7].

Highly elevated activities of coagulation factor FVIII yield lowered protein C values as a result of the measurement principle (modified APTT) used[8]. The presence of a mutation of Factor V at the protein C_a cleavage site (Factor V Leiden) may likewise lead to diminished recovery of protein C[9,10]. Heparin up to a concentration of 2 U/mL does not interfere. Lupus anticoagulants are reported to interfere and may lead to prolonged clotting times thus indicating apparently higher protein C concentrations[11,12]. Hemolyzed samples are not suitable for protein C determination.

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

The expected values range from 70 to 140 % of Norm[13].

Expected values for healthy individuals vary from laboratory to laboratory depending on the technique used; therefore, each laboratory must determine or verify its own expected values for its particular patient population based on the technique and instrumentation.

Performance Characteristics

Measuring Range

The PROTEIN C COAG measuring range 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.

Precision

Precision studies were performed on the BCS® by the evaluation of two levels of controls and two levels of human plasma (normal and pathological) in a manner consistent with the CLSI Guideline EP5-A. The intra-assay precision coefficient of variation ranged from 1.4 to 2.5 %, while the inter-assay precision coefficient of variation ranged from 1.1 to 4.7 %.

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

The reproducibility was assessed by the manufacturer for Protein C with PROTEIN C COAG based on proficiency testing information in 2019/2020. The overall reproducibility median CV% was found to be <9 % including lot, instrument, laboratory and operator variability factors.

Method Comparison

The PROTEIN C COAG assay was compared to a commercially available coagulation protein C assay by evaluation of 86 plasma samples ranging from 11 to 125 % of Norm protein C activity. A correlation coefficient of 0.99 was obtained, with a y-intercept value of 4.5 % and a slope of 0.91.

The values cited for the specific performance characteristics of the assay represent typical results and are not to be regarded as specifications for PROTEIN C COAG.

Technical Assistance

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

Current Version of Application Sheets

PROTEIN C COAG 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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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

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