ProC® Global

Revision bar indicates update to previous version.

CE 0197

Intended Use

ProC® Global is an in vitro diagnostic reagent for the quantitative determination of the anticoagulatory capacity of the Protein C system as aid to diagnosis of hereditary or acquired deficiency states of the Protein C system in patients with or at risk of thromboembolic disease in human sodium citrated plasma by means of automated coagulometric methods.

In addition, in combination with FACTOR V DEFICIENT the assay can be used to diagnose the presence of coagulation factor V Leiden mutation.

For ProC® Global testing no international reference preparation or method is available.

Summary and Explanation

The Protein C system is an important mechanism involved in regulating the coagulation activity. Here, activated Protein C (APC) interacts with its cofactor, protein S, thereby inactivating the procoagulant cofactors Factor VIIIa and Factor Va. Disturbances in this system increase the risk of thrombosis. Such disorders have so far been reported to primarily involve inhibitor deficiencies or defects as well as autoantibodies[1].

ProC® Global is a global coagulation assay that reflects the net effect of the PC pathway by measuring the activated partial thromboplastin time (APTT), before and after activation of endogenous protein C by Protac, a snake venom, and is sensitive to disturbances of the protein C pathway such as protein C or protein S deficiencies, presence of factor V Leiden or autoantibodies interfering with the protein C system[2]. Low ProC Global normalized ratios are associated with a higher risk of recurrent venous thromboembolism and idiopathic pregnancy loss[3–5].

In combination with a sample pre-dilution in FV-deficient plasma, ProC® Global can be used as a screening assay for the presence of factor V Leiden[6].

Principles of the Procedure

Incubation of the plasma with the Protein C activator (venom of *Agkistrodon contortrix*) and a contact phase activator causes activation of endogenous Protein C and of the intrinsic coagulation cascade. Coagulation is triggered by the addition of calcium ions. The activated Protein C - in conjunction with endogenous Protein S - inactivates the procoagulatory cofactors FVIIIa and FVa. This delays clot formation. The time taken for a clot to form is determined (PCAT = Protein C Activity-dependent Clotting Time). In plasmas with a diminished capacity of the Protein C system the coagulation time is less markedly prolonged.

The presence of a deficiency in procoagulatory factors or the presence of very high levels of heparin need to be excluded as these can cause a prolongation of the coagulation time and thus mask a deficiency in the Protein C system. The control used for this purpose is the PCAT/0 which must be less than or equal to 60 seconds. In the PCAT/0, instead of adding the Protein C activator to the plasma sample, only a buffer is added. This plausibility check is necessary particularly for patients undergoing heparin or oral anticoagulant therapy. In addition, before use in the test the sample can be mixed 1 + 4 with FACTOR V DEFICIENT. This minimizes the influence of other factors and a reduced normalized ratio is then almost exclusively due to the presence of Factor V Leiden.

Reagents

Note: ProC® Global 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
ProC® Global			
REAGENT APTT	Ready to use liquid containing: • Silicon dioxide (1.2 g/L) • soy lecithin (0.25 g/L) • Sodium chloride • HEPES • Preservative: • Sodium azide (< 1 g/L) pH 7.6	2–8 °C May be used up to the expiry date indicated on the label if stored unopened.	15–25 °C: once opened, 24 hours[a]; 15 °C: once opened, 2 days[a]; 2–8 °C: once opened, 2 weeks[b]

ACTIVATOR	Lyophilized reagent containing:
	• *Agkistrodon contortrix* venom (reconstituted: 200 U/L[c])
	• HEPES
	• Heparin neutralizer:
	  • Hexadimethrine bromide (reconstituted: 11 mg/L)
	• Preservative:
	  • reaction mass of 5-chloro-2-methyl-2H-isothiazol-3-one / 2-methyl-2H-isothiazol-3-one (3:1) (reconstituted: 10 mg/L)
	pH 7.4
		2–8 °C
		May be used up to the expiry date indicated on the label if stored unopened.
			15–25 °C: reconstituted, 24 hours[a];
			15 °C: reconstituted, 2 days[a];
			2–8 °C: reconstituted, 2 weeks[b];
			–20 °C: reconstituted, 4 weeks[b,d]

BUFFER	Ready to use liquid containing:
	• HEPES
	• Heparin neutralizer:
	  • Hexadimethrine bromide (11 mg/L)
	• Preservative:
	  • reaction mass of 5-chloro-2-methyl-2H-isothiazol-3-one / 2-methyl-2H-isothiazol-3-one (3:1) (10 mg/L)
	pH 7.4
		2–8 °C
		May be used up to the expiry date indicated on the label if stored unopened.
			15–25 °C: once opened, 24 hours[a];
			15 °C: once opened, 2 days[a];
			2–8 °C: once opened, 2 weeks[b];
			–20 °C: once opened, 4 weeks[b,d]

[a] opened vial
[b] closed vial
[c] depending on specific activity
[d] freeze once only

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! ProC Global ACTIVATOR
Hazardous ingredient: reaction mass of 5-chloro-2-methyl-2H-isothiazol-3-one / 2-methyl-2H-isothiazol-3-one (3:1) (0.0741 % [w/w]).
H315: Causes skin irritation. **H317**: May cause an allergic skin reaction. **H319**: Causes serious eye irritation. **H411**: Toxic to aquatic life with long lasting effects.
P261: Avoid breathing dust. **P264**: Wash hands thoroughly after handling. **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. **P337 + P313**: If eye irritation persists: Get medical advice/attention. **P391**: Collect spillage. **P501**: Dispose of contents and container in accordance with all local, regional, and national regulations.

ProC Global BUFFER
Hazardous ingredient: reaction mass of 5-chloro-2-methyl-2H-isothiazol-3-one / 2-methyl-2H-isothiazol-3-one (3:1) (0.00102 % [w/w]).
May produce an allergic reaction.

ProC Global ACTIVATOR
Hazardous ingredient: *Agkistrodon contortrix* venom.
May produce an allergic reaction.

Caution
ProC Global 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

Satisfactory performance of the test is assured only if the reagents used are from the specified lots; the permitted combination of reagent lots is listed on the test kit.

ProC Global ACTIVATOR: Dissolve in 2 mL of distilled water and incubate for at least 5 minutes at 15 to 25 °C with occasional swirling.

ProC Global REAGENT APTT: Shake briefly before the first use and use at room temperature (15 to 25 °C). The reagent has to be re-shaken after 24 hours of use.

Warm the ProC Global BUFFER to 15 to 25 °C and $CaCl_2$ SOLUTION 0.025 mol/L to 37 °C.

Mix carefully once more before using.

Specimen Collection and Handling

Collecting the Specimen

To obtain the plasma carefully mix 1 part sodium citrate solution (0.11 mol/L) with 9 parts venous blood, avoiding foam formation. Centrifuge immediately at no less than 1 500 × g for 10 minutes. **When withdrawing the plasma ensure that no platelets are included.** If the sample needs to be frozen, recentrifuge the withdrawn plasma and **rapidly** freeze the supernatant in a well-closed plastic container.

Storing the Specimen

Stability of the samples:

15 to 25 °C	4 hours
−20 °C	1 month

Plasma samples stored at −20 °C are to be thawed at 37 °C within 10 minutes and the determination then performed within 2 hours.

Procedure

Materials Provided

REF	Contents		
OQLS13	ProC® Global		
	APTT reagent for ProC® Global ProC Global REAGENT APTT	4 ×	5 mL
	Activator Reagent for ProC® Global ProC Global ACTIVATOR	4 × →	2 mL
	Buffer for ProC® Global ProC Global BUFFER	4 ×	2 mL

Materials Required but not Provided

Item	Description
REF ORHO37	$CaCl_2$ SOLUTION, Calcium Chloride Solution
REF ORKL17	STANDARD PLASMA, Standard Human Plasma
REF ORKE41	CONTROL N, Control Plasma N
REF OQKE17	ProC CONTROL, ProC® Control Plasma
REF ORSM19	FACTOR V DEFICIENT, Coagulation Factor V Deficient Plasma
Coagulation analyzers[e], such as:	• AUTOMATED BLOOD COAGULATION ANALYZER CS-2500 (CS-2500 System) • AUTOMATED BLOOD COAGULATION ANALYZER CS-5100 (CS-5100 System)

[e] 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.

Manual Testing

Bring the test tubes to 37 °C before the start of the test.

Pipetting scheme for the determination of the PCAT and PCAT/0:

	ProC® Global		ProC® Global / FV	
	PCAT	PCAT/0	PCAT	PCAT/0
Citrated plasma	100 µL	100 µL	20 µL	20 µL
FACTOR V DEFICIENT	-	-	80 µL	80 µL
ACTIVATOR	100 µL	-	100 µL	-
BUFFER	-	100 µL	-	100 µL
REAGENT APTT	100 µL	100 µL	100 µL	100 µL
Incubate for 3 minutes at 37 °C				
$CaCl_2$ SOLUTION (37 °C)	100 µL	100 µL	100 µL	100 µL

On adding the calcium chloride solution start the stopwatch or timer on the coagulation analyzer and determine the coagulation time.

For determination always use the same combination of reagents and analyzer as used for calibration. The PCAT and PCAT/0 of each plasma sample should be determined in the same run. On manual coagulation analyzers it is recommended to use double determinations.

Internal Quality Control

Normal range: CONTROL N

Pathological range: ProC CONTROL

Two controls (one in the normal range and one in the pathological range) have to be measured at the start of the test run, after each change of reagent vial, and at least once during an 8 hour shift. The control material should be prepared and processed in the same manner as the samples. Each laboratory should establish its own confidence intervals for the controls. This interval is generally ±2 to ±2.5 standard deviations from the mean control value. If the measured control value lies outside of the pre-determined range, then the reagents, the calibration factor and the coagulation analyzer should be checked. Before reporting the patient values, it is recommended that all steps should be documented that were taken to identify and rectify the problem. New control ranges should be defined for each new lot of reagents or controls.

Results

To assure inter-laboratory comparability of the results, which may vary due to different analyzers, the normalized ratio (NR) is calculated. This is done by calculating the quotient of PCAT and PCAT/0, and multiplying by a calibration factor (CF) which is calculated from the measured ratio of STANDARD PLASMA (SHP) and the sensitivity value (SV) for SHP:

$NR = (PCAT:PCAT/0)_{sample} \times CF$

$CF = SV / (PCAT:PCAT/0)_{SHP}$

The calibration factor must be redetermined every month for each coagulation analyzer and each lot of reagent.

Limitations

Pre-activation of the samples due to incorrect sample withdrawal (noticeable due to systematic deviations from the reference range for the PCAT/0) can lead to false results. Deep-frozen samples may exhibit poor reproducibility if cellular constituents are not carefully separated out during plasma collection.

For determining the normalized ratio only samples should be used which have a PCAT/0 value below 60 seconds because otherwise it is not possible to clearly evaluate the effect of the Protein C system. A prolonged PCAT/0 can also be due to lupus anticoagulant.

As treatment with coumarin derivatives diminishes the activities of factors including Protein C and Protein S, samples from patients receiving oral anticoagulants are generally found to have results below the decision limit. The ProC® Global reagents contain a heparin neutralizer which enables the assay to be performed in the presence of heparin levels of up to 0.8 U/mL.

Plasmin destroys Protein C[7]. Samples from patients undergoing lysis therapy may therefore yield false positive results (shortened coagulation times).

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

PCAT and PCAT/0 were determined on a BCT using samples from healthy adults who are not carriers of the Factor V Leiden mutation. The table lists the median and the 5^{th} - 95^{th} percentile range of the results in seconds, as well as the normalized ratios.

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.

	ProC® Global			ProC® Global / FV		
	n	Median	5th–95th Percentile	n	Median	5th–95th Percentile
PCAT	234	132 s	85–>200 s	243	151 s	128–173 s
PCAT/0	234	44 s	35–55 s	243	78 s	68–91 s
normalized ratio	234	0.94	0.69–>1.56	243	0.99	0.86–1.10

Performance Characteristics

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 Guide of the instrument.

Specificity and Sensitivity

ProC® Global / FV

ProC® Global used in combination with FACTOR V DEFICIENT, and with a decision limit of 0.7 for the normalized ratio, was found to have a sensitivity of 98 % (95 % confidence range 91.8 to 99.7 %) for Factor V Leiden and a specificity of 99 % (96.4 to 99.7 %).

ProC® Global

For ProC® Global without the use of FACTOR V DEFICIENT, a normalized ratio of 0.8 was established empirically as a decision limit for detecting the presence of Factor V Leiden or a deficiency in Protein C or Protein S. This decision limit permits highly sensitive detection of the stated impairments of the Protein C system. In a clinical study the test was found to have the following sensitivity (in parentheses 95 % confidence range): 100 % (94.4 to 100.0 %) for Factor V Leiden, 92 % (80.4 to 97.7 %) for Protein C deficiency (activity < 70 % of the norm) and 89 % (79.7 to 94.7 %) for Protein S deficiency (activity < 60 % of the norm). At the same time, in a group of healthy blood donors this decision limit was found to have a specificity of 79 % (95 % confidence range 73.7 to 84.5 %). For symptomatic Protein S-deficient cases, the ProC® Global assay exhibited in a study a sensitivity of 92.3 %, after excluding cases without thrombotic manifestations from the sensitivity calculation[8]. Normalized ratios below those of normal samples may also be caused by high activities of Factor V and Factor VIII or by the presence of lupus anticoagulant[9].

Precision

On a BCT the intra-assay coefficients of variation determined for the normalized ratio were between 2.2 and 6.6 % whereas the inter-assay coefficients of variation ranged between 1.5 and 8.2 %.

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

The reproducibility was assessed by the manufacturer for ProC Global based on publicly available proficiency testing information in 2020. The overall reproducibility median CV% was found to be < 7 % including lot, instrument, laboratory and operator variability factors.

Technical Assistance

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

Current Version of Application Sheets

ProC® Global 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. Esmon CT. The protein C pathway. Chest. 2003;124(3 Suppl):26S-32S.
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3. Eichinger S, Hron G, Hirschl M, et al. Prediction of recurrent venous thromboembolism by measuring ProC Global. Thromb Haemost. 2007;98(6):1232-6.
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5. Sarig G, Lanir N, Hoffman R, et al. Protein C global assay in the evaluation of women with idiopathic pregnancy loss. Thromb Haemost. 2002 Jul;88(1):32-6.
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7. Váradi K, Philapitsch A, Santa T, Schwarz HP. Activation and inactivation of human protein C by plasmin. Thromb Haemost. 1994; 71: 615-21.
8. Gemmati D, Serino ML, Tognazzo S, et al. The reduced sensitivity of the ProC Global test in protein S deficient subjects reflects a reduction in the associated thrombotic risk. Blood Coag Fibrinolysis 2001, 12:691-7.
9. Kraus M, Noah M, Fickenscher K. The PCAT - a simple screening assay for assessing the functionality of the protein C anticoagulant pathway. Thromb Res 1995; 79: 217-22.

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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