ProC® Ac R

Revision bar indicates update to previous version.

CE 0197

Intended Use

ProC® Ac R is an in vitro diagnostic reagent for the quantitative, Russell Viper Venom-based determination of activated protein C resistance (APCR) as aid to diagnosis and screening for congenital factor V Leiden (FVL) mutation in patients with thromboembolic disease or at risk for APCR in human sodium citrated plasma by means of automated coagulometric methods.

For APCR testing no international reference preparation or method is available.

Summary and Explanation

Activated protein C resistance (APCR) is associated with a point mutation in the factor V gene. This factor V Leiden (FVL) mutation results in the replacement of the amino acid arginine 506 (R) with glutamine (Q) in the factor V protein[1-3]. FVL mutation slows the inactivation of factor Va by APC, causing a hypercoagulable, thrombophilic state.

The heterozygous form is present in Caucasians in 5 % of the general population but is less common or rare in other ethnic groups. Homozygosity for FVL occurs in about 1 in 5000 in Caucasians. The risk of venous thrombosis is increased from 3- to 7-fold in individuals heterozygous for FVL, and 80-fold in homozygotes. The presence of FVL is the most common cause of inherited thrombophilia and is present in about 20 % of unselected patients with a first episode of thrombosis, and 50 % of familial thrombosis[3].

APCR assays determine the "resistance" to inactivate the procoagulant function of FVa upon addition of activated protein C (APC) to a patient sample in case of FVL presence; the ratio of the clotting time with and without addition of APC is calculated. Compared to APTT-based methods for determination of APCR, the Russell-Viper venom-based method is not affected by increased FVIII concentrations or any influence of factor FIX, FXI or FXII[4].

The assay also allows the determination of acquired APC resistance in patients with newly diagnosed and symptomatic multiple myeloma, a form of blood cancer, which may be associated with an increased risk for venous thromboembolism[5].

Presence of a heparin inhibitor in the ProC APC reagent allows determination of APCR in patients under heparin therapy.

Principles of the Procedure

The ProC® Ac R test is based on the activation of endogenous protein C by incubation of plasma with *Agkistrodon contortrix contortrix* (Southern Copperhead) venom. A dilute "Russell's Viper Venom Time" (DRVVT, snake venom activator for Factor FX) is then performed on the plasma. The DRVVT is sensitive to changes in concentration of Activated Protein C. In normal individuals, activation of their protein C prolongs the result two- to three-fold, compared to plasma without activator. In individuals with Factor V (Leiden), the venom activation of Protein C induces only marginal prolongation of the result (usually less than 1.5 times the plasma without activator). To minimize the effect of other clotting variables, a ratio of clotting times obtained with and without protein C activation should be determined.

Activated Protein C resistance may also be caused by other mutations in the factor FV gene [e.g. FV (Cambridge) and FV (Hong Kong)].

Reagents

Note: ProC® Ac R 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
ProC® Ac R			
REAGENT	Lyophilized reagent containing: • diluted phospholipid rich Vipera Russelli venom (reconstituted: < 1 mg/L) • soy lecithin (reconstituted: < 10 g/L) • Calcium formate • Heparin inhibitor (reconstituted: < 0.5 g/L) • Preservative: • Sodium azide (reconstituted: < 1 g/L)	2–8 °C May be used up to the expiry date indicated on the label if stored unopened.	2–8 °C: reconstituted, 2 weeks[a]; 20–25 °C: reconstituted, 12 days[a]; 37 °C: reconstituted, 6 hours[a]; –20 °C: reconstituted, 2 weeks[a] Do not refreeze!
ACTIVATOR	Lyophilized reagent containing: • extract from snake venom, Agkistrodon contortrix (reconstituted: < 0.1 g/L) • buffers/stabilizers • Preservative: • Sodium azide (reconstituted: < 1 g/L)	2–8 °C May be used up to the expiry date indicated on the label if stored unopened.	2–8 °C: reconstituted, 2 weeks[a]; 20–25 °C: reconstituted, 12 days[a]; 37 °C: reconstituted, 6 hours[a]; –20 °C: reconstituted, 2 weeks[a] Do not refreeze!

[a] closed vial

Indication of expiration:
If there is no evidence of a vacuum when the REAGENT vial is opened and/or the REAGENT does not appear dry, it should be returned to the manufacturer. If reconstituted products show clear signs of particles or contamination or if they do not dissolve completely within 15 minutes of the addition of distilled or deionized water, please contact the manufacturer or your distributor.

Do not freeze and then thaw again more than once after reconstitution!

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.

Danger! ProC Ac R REAGENT
Hazardous ingredient: Calcium formate (8.50 % [w/w]), Sodium azide (0.527 % [w/w]), Cetrimonium bromide (0.130 % [w/w]).
H318: Causes serious eye damage. **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. **P305 + P351 + P338**: IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing. **P310**: Immediately call a POISON CENTER or doctor/physician. **P501**: Dispose of contents and container in accordance with all local, regional, and national regulations.

ProC Ac R ACTIVATOR
Hazardous ingredient: extract from snake venom, Agkistrodon contortrix.
May produce an allergic reaction.

Caution
ProC Ac R REAGENT**, ProC Ac R** 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

- Reconstitute ProC Ac R ACTIVATOR in 2.0 mL distilled or deionized water without preservatives. The reconstituted product should be a clear, pale yellow solution with no apparent particles.
- Reconstitute ProC Ac R REAGENT in 4.0 mL distilled or deionized water without preservatives. The reconstituted product should be a translucent, pale solution with no apparent particles.
- Gently invert to mix. **Do not shake**!
- Incubate for 10 minutes at room temperature (15 to 25 °C) before use.
- Mix reagents again carefully before use.

Specimen Collection and Handling

Collecting the Specimen

Important: Treat all plasmas as potentially infectious!

To obtain the plasma, carefully mix 1 part sodium citrate solution 0.11 mol/L (3.2 %) with 9 parts freshly collected venous blood, avoiding the formation of foam. Centrifuge the blood specimen as soon as possible at 1 500 × g for no less than 15 minutes at room temperature. When separating the plasma, take care that no platelets are pipetted off as well. If the sample is to be frozen, centrifuge the separated plasma again and freeze immediately. Please refer to CLSI document H21-A5[3] for general guidelines on sample preparation and storage.

Storing the Specimen

Stability of the samples:

at 2 to 8 °C	4 hours (fresh plasma)
at −30 °C	6 months (if platelets are removed by double centrifugation prior to freezing).

Procedure

Materials Provided

REF	Contents	
OPBC03	ProC® Ac R	
	ProC® Ac R PR3V Reagent ProC Ac R REAGENT	5 × → 4 mL
	ProC® Ac R Activator Reagent ProC Ac R ACTIVATOR	5 × → 2 mL

Sufficient to perform 100 manual clotting assays (100 tests with activator and 100 tests without activator).

Note: The number of tests obtained with specific coagulation analyzers may differ.

Materials Required but not Provided

Item	Description
REF OQKE17	ProC CONTROL, ProC® Control Plasma
REF ORKE41	CONTROL N, Control Plasma N
REF B4234-25 REF B4265-37 –	OV BUFFER, Dade® Owren's Veronal Buffer or CA SYSTEM BUFFER, Dade® CA System Buffer
–	For blood collection, use sodium citrate (0.11 M or 3.2 %)
–	Distilled or deionized water without preservatives
–	Plastic test tubes
–	Pipettes for precise measurement of 100 µL, 2.0 mL and 4.0 mL
–	Water bath, maintained at 37 °C
–	Timer or stop watch
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

Manual:

Test with activator:

1. Pre-warm a sufficient amount of ProC Ac R REAGENT, allowing 0.1 mL per test, to 37 ±1 °C in a reagent reservoir.
2. Dispense 0.1 mL test plasma into a test tube.
3. Add 0.1 mL ProC Ac R ACTIVATOR to the test plasma and incubate for exactly 5 minutes at 37 ±1 °C.

4. Add 0.1 mL of the ProC Ac R REAGENT prewarmed to 37 ±1 °C, start the stop watch immediately and determine the clotting time.
5. Repeat the measurement for double determination and report the average of both determinations as the result.

Test without activator:

1. Pre-warm a sufficient amount of ProC Ac R REAGENT, allowing 0.1 mL per test, to 37 ±1 °C in a reagent reservoir.
2. Dispense 0.1 mL test plasma into a test tube.
3. Add 0.1 mL **saline solution** to the test plasma and incubate for exactly 5 minutes at 37 ±1 °C.
4. Add 0.1 mL of the ProC Ac R REAGENT prewarmed to 37 ±1 °C, start the stop watch immediately and determine the clotting time.
5. Repeat the measurement for double determination and report the average of both determinations as the result.

Internal Quality Control

Normal range: CONTROL N

Pathological range: ProC CONTROL

Two controls [one FV (Leiden) negative and one that is FV (Leiden) positive] have to be measured with each test run. A normal control plasma may be established using a pool of freshly collected normal plasmas, all tested and found to be negative for the Factor V Leiden mutation. The plasmas should be pooled on the day of collection and frozen in small aliquots for later use. A positive control may be prepared from aliquots of a known Factor V Leiden positive individual.

Plasma should be frozen at −70 °C for use.

The controls should be prepared and handled in the same manner as the samples.

Acceptable limits should be set for each control plasma by performing precision studies in accordance with CLSI guidelines. Each laboratory should determine its own quality control range for both controls, 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, with each lot of the ProC® Ac R test. This normally amounts to ±2.5 standard deviations from the mean control value.

If the measured control values are outside of the predetermined control range, do not report patient results until the problem has been identified, corrected and documented. Determine which part of the instrument/reagent/control system did not function properly and correct it. After corrective measures are implemented and documented according to *good laboratory practice (GLP)*, retest the controls. If they are now within the confidence range, patient samples can be tested and the results reported.

Results

Results should be expressed as a ratio:

$$\text{ProC® Ac R ratio} = \frac{\text{Clotting time PR3V with activator}}{\text{Clotting time PR3V with saline solution or CA SYSTEM BUFFER}}$$

The ProC® Ac R Test results on pooled normal plasma with Activator Reagent present should be 2 to 3 times longer than results on pooled normal plasma without Activator Reagent (see "Expected Values", page 6).

Limitations

Tests with the ProC® Ac R were carried out with samples from patients on stabilized oral anticoagulant therapy with Vitamin K antagonists, patients on heparin and low-molecular heparin therapy, and patients with lupus anticoagulant.

Samples from such patients may exhibit significantly longer clotting times than samples from normal individuals. However, since the clotting times with saline solution or CA SYSTEM BUFFER are also prolonged, the ratio derived from the clotting time with/without activator remains reliable and the diagnostic significance is maintained. Depending on the instrumentation used, patients under oral anticoagulant treatment with Vitamin K antagonists and levels of Protein C below 50 % may give lower Factor V Leiden assay ratios.

For unfractionated heparin and low molecular weight heparin the reliability of the ratio has been demonstrated up to 1.0 IU/mL and 0.4 IU/mL, respectively. Therapeutic doses of hirudin or other direct thrombin inhibitors and FXa inhibitors may prolong clotting times[7,8] and may affect results expressed as ratio.

Lipoglycopeptide antibacterial drugs (such as oritavancin) may interfere with Dilute Russell's viper venom time (DRVVT) assays. Oritavancin has been shown to elevate Dilute Russell's viper venom time (DRVVT) up to 72 hours after its administration[9].

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.

Interferences

Icteric, lipemic and hemolytic samples and samples with a low hematocrit should not be used with this test, since they may lead to incorrect results (see CLSI H21-A5 for further clarification[6]). Low activities of factors FVII, FVIII, FIX and FXII do not interfere with the ProC® Ac R test. Activities of factors FII and FV of over 10 % and of factor FX of over 20 % also have no effect on the test. At the beginning of treatment with oral anticoagulants with Vitamin K antagonists, i.e. before stabilization, the ProC® Ac R test may provide ratios that are too low. The same applies for Protein C concentrations under 50 %.

The anaesthetic propofol (Diprivan, 2,6-diisopropylphenol, CAS no. 2078-54-8) may induce falsely low clotting times in the activator assay, which may result in ratios below the cut-off (false positive results)[7].

Expected Values

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.

In general, ratios smaller than or equal to 1.5 indicate the Factor V (Leiden) variant. A study with the BCS® System, CA-7000 and CA-1500 systems yielded a decision limit of 1.8 for these assay applications.

Ratios between 1.5 and 2.1 may indicate low concentrations of Protein C and should be further investigated. These investigations could include specific tests for Protein C and DNA tests.

Samples with positive or equivocal results should be confirmed by genetic analysis.

Performance Characteristics

Diagnostic Sensitivity and Diagnostic Specificity

The ProC® Ac R test was validated on a Stago STA instrument against the DNA analysis method and a cut-off value of 1.57 was established by ROC analysis.

A clinical evaluation study was then performed at three independent test centers to evaluate the ability of the ProC® Ac R test to detect the Factor V (Q506) mutation. The ProC® Ac R test was compared with the COATEST APC Resistance V Test and DNA analysis, using a total of 164 samples (82 normal and 82 Factor V (Leiden) positive) from patients ranging in age from 2 to 87 years. The population included patients with the following clinical conditions: untreated (49), DVT/PE or other prothrombotic conditions (58), oral anticoagulants with Vitamin K antagonists (36), heparin/LMW heparin (21), lupus anticoagulant (12) or familial studies (39). 163 of 164 patients were correctly identified using a cut-off value of 1.57 at all sites, giving a sensitivity of 100 % (confidence limits: 95.6 to 100 %) and a specificity of 98.8 % (confidence limits: 93.4 to 99.8 %) relative to the DNA method. Validation was performed on a Stago STA, an Organon Teknika MDA 180 and using the manual tilt tube technique.

An additional clinical evaluation of the ProC® Ac R test with the BCS® System, CA-7000 and CA-1500 systems defined the decision limit by ROC analysis as 1.8. Patients under oral anticoagulant (OAC) therapy with Vitamin K antagonists were excluded in this study. The diagnostic sensitivity and diagnostic specificity for these assay applications were evaluated. A minimum of 198 fresh or frozen samples from unselected volunteer healthy blood donors confirmed negative for Factor V (Leiden) by PCR, and from patients with different disease states confirmed positive for Factor V (Leiden) by PCR were tested. Please refer to Table 1 for the results.

	n	Cut-off	Specificity [%]	Sensitivity [%]	NPV [%]	PPV [%]
CA-1500 System	198	1.8	97.2	98.9	99	96.8
CA-7000 System	214	1.8	98.0	99.1	99	98.2
BCS®	231	1.8	98.1	99.2	99	98.4

Table 1: Results of the diagnostic sensitivity and diagnostic specificity study for ProC® Ac R

The ProC® Ac R test applications with BCS® System, CA-7000 and CA-1500 systems were compared to COATEST APC Resistance V on ACL 9000. A total of 139 fresh or frozen samples from unselected volunteer healthy blood donors confirmed negative for Factor V (Leiden) by PCR, and from patients with different disease states confirmed positive for Factor V (Leiden) by PCR were tested. 138 out of the 139 samples were correctly identified by the ProC® Ac R test with BCS® System, CA-7000 System and COATEST APC Resistance V on ACL 9000. 137 out of 139 were correctly identified by the ProC® Ac R assay with the CA-1500 System.

Note: Sensitivity and specificity of the ProC® Ac R test for non-FV (Leiden) APC resistance screening has not been established.

Note: Due to differences in sample populations, collection techniques and instrument technology, each testing laboratory should determine its own cut-off value.

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.

Precision

The within run precision maximum (intraassay coefficient of variation) was 1.9 %. The total precision maximum coefficient of variation was 8.9 % using two sites, instruments and operators with three plasmas, tested in duplicate for 20 days. Application specific performance data is listed in the respective Reference Guides of the instruments.

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

The reproducibility was assessed by the manufacturer for ProC® Ac R based on proficiency testing information in 2019/2020. The overall reproducibility median CV% was found to be <6 % (valid for samples below normalized ratio 2.0) 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® Ac R 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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