Berichrom® Protein C
Berichrom PROTEIN C

Revision bar indicates update to previous version.

CE 0197

Reagents for the determination of protein C activity

Intended Use

Berichrom PROTEIN C 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 chromogenic 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 Factor V 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 a specific snake venom activator. The resulting Protein C_a is assayed in a kinetic test by measuring the increase in absorbance at 405 nm. The assay is based on the following reactions:

$$\text{Protein } C_{\text{sample}} \xrightarrow{\text{Protein C Activator}} \text{Protein } C_a$$

$$\text{p-Glu-Pro-Arg-MNA} \xrightarrow{\text{Protein C}_a} \text{p-Glu-Pro-Arg-OH + MNA}$$

Reagents

Note: Berichrom PROTEIN C 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
Berichrom® Protein C Berichrom PROTEIN C			
ACTIVATOR	Lyophilized reagent containing: • extract from snake venom, Agkistrodon contortrix (reconstituted: ≤0.6 U/mL) • Stabilizer	2–8 °C May be used up to the expiry date indicated on the label if stored unopened.	37 °C: reconstituted, 8 hours; 2–8 °C: reconstituted, 2 weeks; ≤ –20 °C: reconstituted, 4 weeks
SUBSTRATE	Lyophilized reagent containing: • p- Glu-Pro-Arg-MNA (reconstituted: 4 mmol/L)	2–8 °C May be used up to the expiry date indicated on the label if stored unopened.	37 °C: reconstituted, 1 week; 2–8 °C: reconstituted, 6 weeks; ≤ –20 °C: reconstituted, 6 months
ACTIVATOR DILUENT	Ready to use liquid containing: • polyethylene glycol • Caesium chloride • Preservative: • Sodium azide (< 1 g/L) pH 8.25	2–8 °C May be used up to the expiry date indicated on the label if stored unopened.	2–8 °C: once opened, 6 months

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.

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

Caution
Berichrom PROTEIN C 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

Berichrom PROTEIN C ACTIVATOR	Dissolve the contents of the vial with the quantity of Berichrom PROTEIN C ACTIVATOR DILUENT indicated on the label.
Berichrom PROTEIN C SUBSTRATE	Dissolve the contents of the vial with the quantity of distilled water indicated on the label.

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 (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. Please refer to CLSI guideline H21-A5[5] for further details.

Storing the Specimen

Stability of the samples:

≤ −20 °C	1 month
15 to 25 °C	8 hours

Thaw plasma stored at ≤ −20 °C within 10 minutes at 37 °C, and then perform the assay within 8 hours.

Procedure

Materials Provided

REF	Contents	
OUVV15	Berichrom® Protein C Berichrom PROTEIN C	
	Protein C Activator Berichrom PROTEIN C ACTIVATOR	3 × → 10 mL
	Substrate Reagent Berichrom PROTEIN C SUBSTRATE	3 × → 3 mL
	Hepes Buffer Solution Berichrom PROTEIN C ACTIVATOR DILUENT	1 × 30 mL
OUVV17	Berichrom® Protein C Berichrom PROTEIN C	
	Protein C Activator Berichrom PROTEIN C ACTIVATOR	4 × → 5 mL
	Substrate Reagent Berichrom PROTEIN C SUBSTRATE	2 × → 3 mL
	Hepes Buffer Solution Berichrom PROTEIN C ACTIVATOR DILUENT	1 × 30 mL

Materials Required but not Provided

Item	Description
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, or Isotonic Saline Solution
REF ORKL17	STANDARD PLASMA, Standard Human Plasma
–	Acetic acid 20 % (only for two-point method)
Coagulation analyzers[a], 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)

[a] 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

Semi-micro test:

cuvette:	1 cm path length
wavelength:	405 nm
test temperature:	37 °C

Pre-warm the Berichrom PROTEIN C ACTIVATOR and Berichrom PROTEIN C SUBSTRATE as well as the plastic cuvettes/tubes to 37 °C prior to the test.

A. Pipetting scheme for the kinetic method

	Semi-micro test
Plasma sample	100 µL
Berichrom PROTEIN C ACTIVATOR	1 000 µL
Mix and incubate for exactly 5 minutes at 37 °C.	
Substrate Reagent	200 µL
Mix and determine $\Delta A_{405\ nm}$/min After approx. 15 seconds, read the absorbance and start the stopwatch simultaneously. After exactly 60 and 120 seconds, read the absorbance again, calculate the respective ΔA/min values by subtraction, and then calculate the mean value of the two ΔA readings.	

Evaluation, kinetic method

Calculation of the factor:

$$F_L = \frac{\text{Assigned value (\% of Norm)}_{\text{reference plasma}}}{\Delta A/\text{min}_{\text{reference plasma}}}$$

Multiply the ΔA/min of the samples by the resulting factor; the protein C content is obtained in % of Norm.

Protein C_{sample} (% of Norm) = $F_L \times \Delta A/\text{min}_{sample}$

B. Pipetting scheme for the two-point method

	Sample	Sample blank
Plasma sample	100 µL	100 µL
Berichrom PROTEIN C ACTIVATOR	1 000 µL	–
Isotonic saline solution	–	1 000 µL
Mix and incubate for exactly 5 minutes at 37 °C.		
Substrate Reagent	200 µL	200 µL
Mix immediately and start the stopwatch simultaneously. After exactly 5 minutes, add:		
Acetic acid 20 %	1 000 µL	1 000 µL
Mix immediately and read the absorbance against the blank within 60 minutes.		

Evaluation, two-point method

Calculation of the factor:

$$F_L = \frac{\text{Assigned value (\% of Norm)}_{\text{reference plasma}}}{A_{\text{reference plasma}}}$$

Multiply the absorbance of the sample by the resulting factor.

Protein C_{sample} (% of Norm) = $F_L \times A_{sample}$

Notes

1. Doubling or halving of volumes used in the test mixtures has no effect on the calculation or laboratoryinternal calculation factor.
2. F_L is a calculating factor established within the laboratory and needs to be determined only once in an assay series. This method of evaluation is basically equivalent to an evaluation via a reference curve.
3. The laboratory-internal factor F_L and/or reference curve must be re-determined for each change of device and for each new lot of Berichrom PROTEIN C.

Internal Quality Control

Normal range: CONTROL N

Pathological range: CONTROL P

Two controls should be measured with each calibration and at least every 8 hours during each testing day (one in the normal range and one in the pathological range). 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 confidence range established in the laboratory. If the measured control value lies outside the confidence range previously established, then the reagents, the laboratory-internal factor F_L and/or the calibration curve and the coagulation analyzer should be examined. Do not release patient results until the cause of deviation has been identified and corrected.

Limitations

False low protein C activity may be obtained in patients being treated with aprotinin[6].

Hemolyzed samples are not suitable for protein C determination.

Some very rare protein C type IIb defects on the substrate binding sites may yield results within the reference range[3].

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

70 to 140 % of Norm[7]

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 measuring range extends from 10 to 150 % 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

For protein C values in the reference range, the coefficient of variation from day to day lies at 1.9 %. For pathological values, the coefficient of variation from day to day lies at 0.4 %.

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

The reproducibility was assessed by the manufacturer for Protein C with Berichrom PROTEIN C based on proficiency testing information in 2019/2020. The overall reproducibility median CV% was found to be <7 % including lot, instrument, laboratory and operator variability factors.

Method Comparison

In a comparison of Berichrom PROTEIN C with immunochemical and other chromogenic methods, the correlation coefficients were equal or better then 0.90[8].

Technical Assistance

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

Current Version of Application Sheets

Berichrom PROTEIN C 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. Dinarvand P, Moser K. Protein C Deficiency. Arch Pathol Lab Med. 2019 Jan 31.
2. Khor B, Van Cott EM. Laboratory tests for protein C deficiency. Am J Hematol 2010;85(6):440-2.
3. Marlar RA, Gausman JN. Laboratory testing issues for protein C, protein S, and antithrombin. Int J Lab Hematol. 2014;36:289-95.
4. Kottke-Marchant K, Comp P. Laboratory issues in diagnosing abnormalities of protein C, thrombomodulin, and endothelial cell protein C receptor. Arch Pathol Lab Med. 2002;126(11):1337-48.
5. CLSI. Collection, Transport, and Processing of Blood Specimens for Testing Plasma-Based Coagulation Assays and Molecular Hemostasis Assays. Approved Guideline – Fifth Edition. CLSI document **H21-A5** [ISBN 1-56238-657-3]. CLSI, 940 West Valley Road, Suite 1400, Wayne, PA 19087-1898 USA, 2008.
6. Espana F, Estelles A, Griffin JH, et al. Aprotinin (Trasylol) is a competitive inhibitor of activated protein C. Thromb Res. 1989; 56: 751-6.
7. Kraus M. Protein C, protein S, factor V Leiden. In: Thomas L, ed. Clinical Laboratory Diagnostics. Frankfurt: TH-Books Verlagsgesellschaft. 1998: 621-5.
8. Sturk A, Morrien-Salomons WM, Huisman MV, et al. Analytical and clinical evaluation of commercial protein C assays. Clin Chim Acta. 1987; 165: 263-70.

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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