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ACTICLOT® C

REF ACC-45

IVD

CE

Σ 90

2°C 8°C

EC REP **Obelis s.a**
Bd. Général Wahis 53, 1030 Brussels, BELGIUM

INTENDED USE

ACTICLOT® C is intended for the measurement of Protein C activity in human plasma via an end-point clotting assay. The assay is for *in vitro* diagnostic use.

EXPLANATION OF THE TEST

Protein C is a vitamin K-dependent anticoagulant protein that normally circulates as an inactive zymogen. Following activation, Protein C inactivates factors V and VIII thus prolonging the clotting time. While Protein C can be activated by thrombin, the rate of activation in vitro is slow. Under such conditions Protein C inhibitor protein inactivates Protein C as fast as it is activated.

PRINCIPLE OF THE METHOD

The venom of the copperhead snake *Agkistrodon contortrix* contains a rapid activator of Protein C.[1] In ACTICLOT C, the Activator reagent, formulated with the Protein C activator isolated from this venom, converts human Protein C to its active protease within 5 minutes.[2] The Activator reagent is also formulated to activate the contact factors of the intrinsic pathway. With this reagent, the clotting time of normal plasma is very long, greater than 100 seconds, while the clotting time of a Protein C deficient plasma is essentially the same as the clotting time of an APTT test, approximately 30-40 seconds. When an unknown test plasma is mixed with Protein C deficient plasma, the Protein C level is proportional to the prolongation of the clotting time.

REAGENTS

The kit contains reagents sufficient to perform 90 tests using an automated coagulation analyzer, 45 tests if a manual end-point method is used.

1. **ACTICLOT Activator**: 3 vials, 1.5 mL (lyophilized)

2. **Protein C Deficient Plasma**: 3 vials, 1.5 mL (lyophilized)

3. **Protein C Control Plasma**: 3 vials, 0.5 mL (lyophilized)

4. **Dilution Buffer**: 3 vials, 5.0 mL, 10X concentrate

WARNING

This product contains *human* source material that has been found to be non-reactive for Hepatitis B Surface Antigen (HBsAg), Hepatitis C Virus (HCV) and Human Immunodeficiency Virus Type 1 and Type 2 (HIV-1, HIV-2) using registered methods. As no known test method can provide complete assurance that products derived from human specimens will not transmit HBsAg, HCV, HIV-1, HIV-2 or other blood-borne pathogens, this product should be handled as recommended for any potentially infectious human specimen.

This product contains *animal* source material. As no known test method can provide complete assurance that products derived from animal specimens will not transmit blood-borne pathogens, this reagent should be handled as recommended for any potentially infectious specimen.

The Dilution Buffer contains sodium azide that may react with lead or copper plumbing to form highly explosive metal azides. Materials discarded into a sink should be flushed with a large volume of water to prevent azide build-up.

Dilution Buffer	Danger		CONT: Imidazole H315, H319, H360, P202, P280, P273, P305 + P351 + P338, P310

Hazard Statements:
H315 Causes skin irritation.
H319 Causes serious eye irritation.
H360 May damage fertility or the unborn child.

Precautionary Statements:
P202 Do not handle until all safety precautions have been read and understood.
P264 Wash thoroughly after handling.
P280 Wear protective gloves/protective clothing/eye protection/face protection.

P302 + P352 IF ON SKIN: Wash with plenty of water.
P332 + P313 If skin irritation occurs: Get medical advice/attention.
P305 + P351 + P338 IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.
P337 + P313 If eye irritation persists: Get medical advice/attention.

REAGENT PREPARATION AND STORAGE

Lyophilized reagents are stable until the expiration date indicated on the label when stored at 2° - 8˚C.

1. ACTICLOT Activator

Reconstitute with 1.5 mL Type I deionised water. Mix/swirl to allow for complete dissolution. Reconstituted Activator is stable for:

	4 hours	48 hours	3 months
	37°C	2° - 8°C	-20°C

2. Protein C Deficient Plasma

Reconstitute with 1.5 mL Type 1 deionised water. Mix/swirl to allow for complete dissolution. Reconstituted Protein C Deficient Plasma is stable for:

	4 hours
	2° - 8°C

3. Protein C Control Plasma

Reconstitute with 0.5 mL Type 1 deionised water. Mix/swirl to allow for complete dissolution. Reconstituted Protein C Control Plasma is stable for:

	4 hours
	2° - 8°C

4. Dilution Buffer

Dilute the Dilution Buffer concentrate to 50 mL with Type 1 deionised water. Working strength Dilution Buffer is stable for:

	1 week	1 month
	18° - 25°C	2° - 8°C

SPECIMEN COLLECTION AND PREPARATION

See "Collection, Transport and Processing of Blood Specimens for Testing Plasma-based Coagulation Assays; Approved Guidelines-Fifth Edition", CLSI Document H21-A5, Vol. 28, No. 5, 2008.[3]

Nine volumes of blood are collected in 1 volume of 0.1M trisodium citrate and centrifuged at 3000 x g for 10 minutes. Plasma should be stored at 2°- 8˚C and assayed within 2 hours. Alternatively, plasma may be stored at -20°C for 1 month and thawed once at 37°C, 30 minutes before use.

PROCEDURE

Materials Provided – See Reagents

Material Required But Not Provided

Type 1 deionised water or distilled water
0.025 M Calcium Chloride solution
clot timer
50 mL graduated cylinder
variable volume pipettor (100-1000 µL)

Assay Calibration

Pooled normal plasma from at least 10 normal donors that has been collected in the same manner as plasma to be tested should be used for preparation of Protein C calibration standards. Alternatively, dilutions of the Protein C Control Plasma may be used to prepare the calibration standards.

Prepare plasma Protein C calibration standards and patient plasma samples as follows. Use the standards and samples immediately after preparing.

Standard/Sample	Volume of Pooled Normal Plasma	Volume of Dilution Buffer
100%	100 µL	400 µL
50%	250 µL	250 µL
25%	250 µL	250 µL
12.5%	250 µL	250 µL
Patient Sample	50 µL	450 µL

Assay Procedure

ACTICLOT C may be performed manually, or by using semi-automated or automated coagulation analysers.

Automated Coagulation Analysers Method

BioMedica Diagnostics offers instrument applications for performing ACTICLOT C on several coagulation analysers. These instrument applications may contain platform specific programming and performance data which differ from that provided in this Instructions for Use. In these cases, the information contained in the instrument application supersedes the information in this Instruction for Use. Please consult the specific manufacturer's instrument manual for complete operating instructions.

Manual End-point Method

Transfer ACTICLOT Activator and 25 mM calcium chloride solution to 37˚C reagent wells.

1. Add 0.1 mL of Protein C Deficient Plasma + 0.1 mL standard dilution or patient sample to a coagulation cuvette.

2. Incubate at 37°C for 2 minutes.

3. Add 0.1 mL of ACTICLOT Activator.

4. Incubate at 37°C for 5 minutes.

5. Add 0.1 mL of 0.025 M Calcium Chloride solution.

6. Start clot timer and note the clotting time.

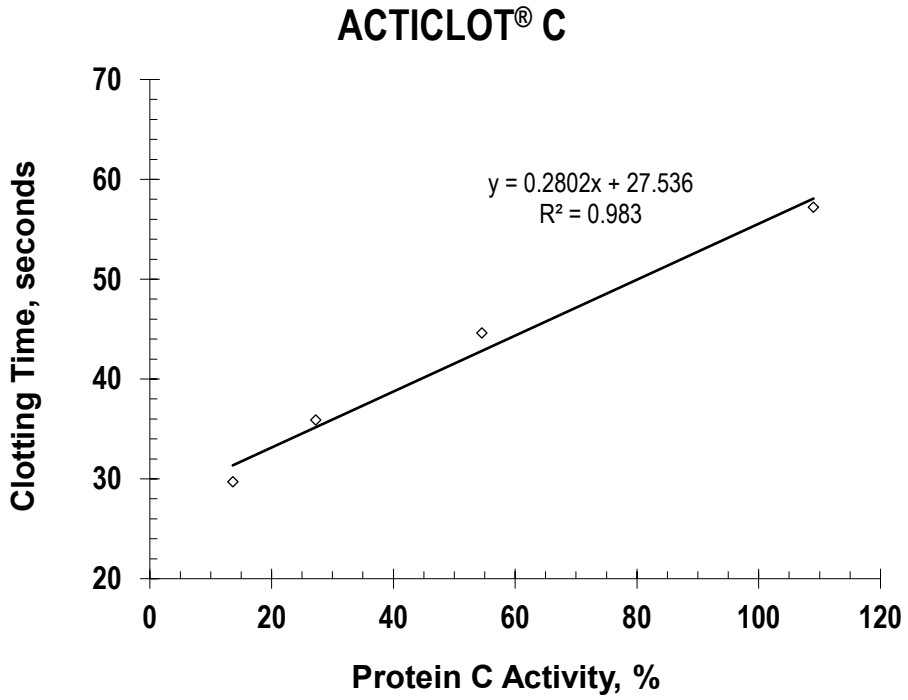
7. Obtain duplicate determinations for each plasma dilution.

RESULTS

Representative Standard Curve

A standard curve is constructed by plotting the mean clotting time for each Protein C standard versus its corresponding activity in percent. A standard curve should be generated each time the assay is performed. Draw the line of best fit between the points, typically a linear equation, for data analysis.

The following standard curve is for demonstration purposes only.



CALCULATION OF RESULTS

Determine the % Protein C in the test sample by interpolating from the standard curve and multiplying the result by two to correct for dilution. In the case of patients with lupus anticoagulants or abnormally high Protein C activity, where multiple patient dilutions were assayed, correct the Protein C level for the dilution. Corrected Protein C levels from at least two dilutions must agree.

QUALITY CONTROL

The Protein C Control plasma included in the kit has been evaluated against an internationally recognized reference material and may be assayed as part of a laboratory's quality control program. Control plasma should be included in the run whenever freshly reconstituted reagents are used. The level obtained for the control plasma should fall within the range specified by the vendor or laboratory. If the control plasma fails to yield Protein C levels within the range specified, the run should be repeated. Contact BioMedica Diagnostics if repeated assaying of the control fails to yield Protein C levels within acceptable limits.

LIMITATIONS OF THE PROCEDURE

Patients with Lupus Anticoagulants should be tested at multiple dilutions as artifactually high Protein C levels could be inferred from prolonged clotting times.

Patients with abnormally high FVIII:C levels may present with inaccurately low Protein C levels as shorter clot times of the Protein C Deficient Plasma may be obtained. These samples should be tested at multiple dilutions.

INTEFERRING SUBSTANCES

The following substances at the indicated levels may interfere with ACTICLOT C.

Substance	Concentration
Unfractionated Heparin (UFH)	> 4 units/mL
Low Molecular Weight Heparin (LMWH)	> 2 units/mL
Hemoglobin	> 500 mg/dL
Bilirubin	> 21 mg/dL
Triglycerides	> 900 mg/dL

PERFORMANCE CHARACTERISTICS

Accuracy

In a clinical study comparing ACTICLOT C to a Protein C ELISA, the following results were obtained:

Plasma Protein C Concentration (% of Normal, mean ± S.D.)

Pathological Condition	N	ACTICLOT C	Protein C ELISA
Normal	40	89.0 ± 17.0	94.0 ± 16.0
DIC	10	29.4 ± 11.9	34.2 ± 13.0
Liver Disease	10	18.6 ± 9.6	20.1 ± 14.6
Protein C Deficiency, Congenital	10	37.9 ± 7.1	45.0 ± 8.1

Neonates*	12	21.9 ± 5.3	19.3 ± 8.0
Heparinized	10	93.7 ± 16.1	93.5 ± 14.2
Warfarin	20	23.2 ± 9.0	57.7 ± 15.7

* e.g. respiratory distress syndrome, sepsis, thrombosis, renal failure

Correlation between ACTICLOT C and Protein C ELISA (warfarin patients not included):

Regression	Correlation Coefficient	Standard Error of Estimate
y = 0.93x + 0.0014	0.952	0.086

Precision

The coefficient of variation of ACTICLOT C has been determined using samples prepared by mixing plasma immunodepleted of Protein C with normal pooled plasma to obtain Protein C levels of 10%, 50% and 100%.

Protein C Level	Intra-Assay Variation	Inter-Assay Variation
100%	5.9%	2.4%
50%	4.7%	3.9%
10%	9.1%	9.3%

REFERENCES

1. Stocker, K., Fischer, H., Mejer, J., Brogil, M. and Svendsen, L: Characterization of the protein C activator Protac from the venom of the Southern Copperhead (Agkistrodon contortrix) snake. *Toxicol.* 1987, 25: 239-252.

2. Martinoli, J. L. and Stocker, K. Fast functional protein C assay using Protac, a novel protein C activator. *Thromb. Res.* 1986, 43: 253- 264.

3. *Collection, Transport and Processing of Blood Specimens for Testing Plasma-based Coagulation Assays and Molecular Hemostasis Assays; Approved Guidelines-Fifth Edition*, CLSI Document H21-A5, Vol. 28, No. 5, 2008.

DEFINITIONS OF SYMBOLS

	Consult instructions for use		Warning
IVD	In vitro diagnostic medical device	2°C 8°C	Temperature limitation Store at 2°C to 8°C
LOT	Lot Number	REF	Catalog Number
	Expiration Date		Manufacturer
	Contains sufficient for <n> tests	CONT	Contains...
CE	CE mark	EC REP	Authorized representative in the European Union