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. After 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 is a rapid activator of Protein C. Under the assay conditions of ACTICLOT C, the ACTICLOT Activator, formulated with the venom from Agkistrodon contortrix, converts human Protein C to its active protease within 5 minutes. The ACTICLOT Activator reagent is formulated to activate both Protein C and 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

1. ACTICLOT Activator: 3 vials each containing 1.5 units of Agkistrodon contortrix venom lyophilized with rabbit brain cephalin and colloidal silica activator.

2. Protein C Deficient Plasma: 3 vials each containing 1.5 mL of lyophilized human plasma depleted of Protein C by immunoabsorption on a column of immobilized antibody immunospecific to human Protein C.

3. Protein C Control Plasma: 3 vials each containing 0.5 mL of lyophilized normal human plasma.

4. Dilution Buffer: 3 vials each containing 5 mL of a 10-fold concentrate. At working strength, the buffer contains 0.12 M NaCl, 0.03M Imidazole, pH 7.35.
WARNINGs AND PRECAUTIONS

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.

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REAGENT PREPARATION AND STORAGE

Unreconstituted 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 purified water.
   - Stability at -20°C: 3 months
     - at 2°-8°C: 48 hours
     - at 37°C: 4 hours

   Some automated equipment may require a larger Activator volume in order to adequately fill the reagent reservoir and pump tubing. In this case, reconstitute all three vials of Activator provided and pool to obtain a reagent volume of 4.5 mL after use. The remaining contents of the reservoir and pump tubing may be returned to the vial, capped, frozen at -20°C and reused. Activator can be frozen and thawed virtually without loss of activity.

2. Protein C Deficient Plasma: Reconstitute with 1.5 mL purified water. Let stand at room temperature for 20 minutes for complete dissolution then swirl gently. Use immediately or store on melting ice until use.

3. Protein C Control Plasma: Reconstitute with 0.5 mL purified water. Let stand at room temperature for 20 minutes for complete dissolution then swirl gently. Use immediately or store on melting ice until use. Use as a quality control reagent when performing the assay.

4. Dilution Buffer: Dilute to 50 mL with purified water.
   - Stability at room temperature: 1 week
     - at 2°-8°C: 1 month

SPECIMEN COLLECTION AND PREPARATION


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

Purified 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 assay calibration standards.

Prepare plasma Protein C calibration standards and patient plasma samples as
follows:

100% Standard: 100 μL pooled plasma + 400 μL Dilution Buffer
50% Standard: 250 μL 100% control + 250 μL Dilution Buffer
25% Standard: 250 μL 50% control + 250 μL Dilution Buffer
12.5% Standard: 250 μL 25% control + 250 μL Dilution Buffer
*Patient samples: 50 μL patient plasma + 450 μL Dilution Buffer

Store dilutions on melting ice and use immediately after preparing.

* Patients with lupus-type anticoagulants should be tested at multiple dilutions
as artifactually high Protein C levels could be inferred from prolonged clotting
times. Similarly, multiple patient plasma dilutions should be used when the
patient plasma has an abnormally high factor VIIIc level. In this case
artifactually low Protein C levels due to shortening of the clotting time of the
Protein C Deficient Plasma may be obtained.

ASSAY PROCEDURE

1. Reconstitute reagents as described.
2. Transfer ACTICLOT Activator and calcium chloride to 37°C reagent wells in
clot timer if the instrument is manual. In the case of automated instruments
prime reagent delivery tubing, set activation time to 5 minutes and maximum
end-point time to 100 seconds.
3. Prepare dilutions as described.
4. To a coagulation cuvette:
   • Add 0.1 mL Protein C Deficient Plasma + 0.1 mL standard dilution
   • Incubate for 2 minutes at 37°C
   • Add 0.1 mL ACTICLOT Activator
   • Incubate for 5 minutes at 37°C
   • Add 0.1 mL Calcium Chloride (0.025 M)
   • Start clot timer and note clotting time
   • Obtain duplicate determinations for each plasma dilution.
5. Repeat step 4 for patients’ plasma dilutions in duplicate.
6. Plot the % Protein C activity of the calibration standards on the x-axis vs.
   mean clotting time on the y-axis.

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

Use Protein C Control Plasma provided in the kit for quality control of the assay.

PERFORMANCE CHARACTERISTICS

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

<table>
<thead>
<tr>
<th>Protein C Level</th>
<th>Intra-Assay C.V.</th>
<th>Inter-Assay C.V.</th>
</tr>
</thead>
<tbody>
<tr>
<td>100%</td>
<td>5.9%</td>
<td>2.4%</td>
</tr>
<tr>
<td>50%</td>
<td>4.7%</td>
<td>3.9%</td>
</tr>
<tr>
<td>10%</td>
<td>9.1%</td>
<td>9.3%</td>
</tr>
</tbody>
</table>

Correlation between ACTICLOT C and ELISA (coumadin patients on coumadin not included):

<table>
<thead>
<tr>
<th>Regression Line</th>
<th>Correlation Coefficient</th>
<th>Standard Error of Estimate</th>
</tr>
</thead>
<tbody>
<tr>
<td>y = 0.93x + 0.0014</td>
<td>0.952</td>
<td>0.086</td>
</tr>
</tbody>
</table>

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

REFERENCES


DEFINITIONS OF SYMBOLS