Echis Clotting Time Test

Catalogue Number: ECTT330

For in vitro diagnostic use only.

Intended Use
Diagen Echis Clotting Time Test (ECT) is suitable for use in monitoring hirudin or hirudin derivative anticoagulant therapy and the effects of other direct thrombin inhibitors (DTIs). It may also be used in parallel with other assays in the screening of patients for lupus anticoagulants (LAs).

Summary and Principle
Both Tiger (Notechis scutatus scutatus) and Taipan (Oxyuranus s. scutellatus) snake venoms convert prothrombin to thrombin; the Saw Scaled Viper (Echis carinatus) venom in saline, buffered and des-carboxy-prothrombin (PIVKA II -protein induced by vitamin K absence/antagonist II) to meizothrombin(1).

Unlike the Tiger (Factor V, phospholipid and Ca²⁺) and Taipan (Phospholipid and Ca²⁺) snakes (2,3), the venom of Echis contains procoagulants able to directly activate prothrombin in the absence of factor V, phospholipid and calcium ions. This process yields meizothrombin, which possesses only a weak coagulant but strong esterase activity.

Because of the lack of a phospholipid requirement, the ECT may also be utilised as a confirmatory test in the screening of patients with lupus anticoagulant (LA). When coupled with the Taipan snake venom time (TSVT) and mixing studies it forms a sensitive assay system for detecting LA(4,5). Please see accompanying LA testing sheet for details.

The characteristics of Echis venom make it a useful tool for monitoring DTIs. The meizothrombin generated by the venom’s action on Prothrombin and the subsequent inhibition by DTIs allows for precise quantification (5,6) and may be used effectively as a drug monitoring method.

Reagent

Echis carinatus venom

A lyophilised dilution of Echis carinatus venom in saline, buffered and stabilised with albumin. The concentration of venom has been titrated to give a clotting time with pooled normal plasma of between 16 and 20 seconds. For reconstitution, remove cap and rubber bung and add 2.0 mL of distilled water to the contents of the vial. Allow 10 - 15 minutes for complete solution. Do not use CaCl₂ with the venom.

Warnings and Precautions

Diagen Echis carinatus venom is for in vitro diagnostic use only. The reagent contains snake venom, which is a poison and may be fatal if it enters the bloodstream. Normal precautions should therefore be taken when handling the reagents. Please refer to the SDS (available on request) for further information. All waste must be disposed of whilst observing all local and national laws.

Collection of Blood Samples

Blood (9 parts) is collected into 1 part of 0.106 M tri-sodium citrate and the plasma obtained by centrifugation at 2500 g for 15 minutes. The plasma is aspirated carefully to avoid cellular contamination and re-centrifuged in a separate, capped container for a further 15 minutes at 2500 g to produce Platelet Poor Plasma (PPP). The plasma should be stored in stoppered tubes.

Procedure

The following section details the products required and procedure used for the Echis carinatus Clotting Time (ECT).

Materials Provided

Cat. No.

ECTT330 – Echis carinatus Venom (10 x 2.0 mL vials).

Materials and equipment required, but not provided:
1. General routine coagulation equipment.
2. Reaction cups or test tubes (12 x 75 mm).
3. Pipette delivering between 100 µL and 200 µL.
4. Imidazole buffer (IMIX600).
5. Distilled water.

Monitoring of Direct Thrombin Inhibitors (DTIs) (6,7)

The ECT test measures the conversion of prothrombin to meizothrombin. Unlike other tests of clotting function, such as the APTT, it is unaffected by the levels of other clotting factors and is therefore a reliable measure of the effect of DTIs on the conversion of fibrinogen to fibrin.

Manual Technique
1. The venom solution is diluted 1 in 5 in saline or Imidazole buffer; this should give a normal control clotting time of 42 ± 3 seconds.
2. 100 µL of plasma is pre-warmed in a tube at 37°C.
3. 100 µL of diluted venom solution is added and the stopwatch started.
4. The resulting clotting time is then recorded.
5. The result is then interpolated from a curve formulated from dilutions of the DTI in normal control plasma. The dose response is linear for Hirudin at 0 – 3000 ng/mL almost linear for Melagatran from 0 – 500 ng/mL and linear for Dabigatran from 0 – 1000 ng/mL.

Automated method

This method can be adapted for any automated method.

Notes:
1. Tubes should be new and scrupulously clean.
2. Water bath temperature should be 37°C.
3. For photo-optical and mechanical instruments, follow the manufacturer’s instructions.

Confirmatory test for the presence of lupus anticoagulants (8)

Manual Technique
1. Add 100 µL of test plasma to test tube and incubate at 37°C for 60 seconds.
2. Add 200 µL of Echis venom and record the clotting time.
3. Repeat steps 1 & 2 using normal control plasma pool.
4. Once both clotting times have been recorded the ECT ratio of test plasma / normal control plasma pool can be calculated.

Please note that normal control plasma pool must be tested in parallel with the patient sample.

Interpretation

In our hands, the normal ECT ratio is defined as 0.90 – 1.11

Samples are considered positive for a LA if the TSVT ratio is greater than 1.10 which is corrected by ≥10% by the ECT ratio. (9,10)

Quality Control

All laboratories should have in place a quality control system that uses normal and abnormal controls to evaluate instrument, reagent and user performance; LA negative and positive controls should be tested alongside patient samples. The controls must be platelet poor, with fewer than 10⁷ platelets/µL. If the controls do not perform within their defined reference ranges, patient results should be considered invalid.

Limitations

Plasma samples from patients receiving therapeutic heparin or contaminated with heparin cannot be reliably tested using the ECT, testing should either be repeated when heparin treatment has stopped or the heparin neutralized with Protamine sulphate or Polyanbrene.

It must be remembered that the ECT is only a supplementary test in the detection of LAs and should be used in conjunction with other detection methodologies.

Venom potency varies from batch to batch, all efforts are made to minimise variation but reference values should be re-established when changing from one lot to another.

Storage and stability

The unopened freeze dried vials are best stored deep frozen, but may be stored for up to 3 years at 2 - 8°C without deterioration.

After reconstitution the venom solution is stable for at least 7 days at 4°C. The solution may also be frozen once at -20°C and thawed for use.

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Packaging
10 x 2.0 mL vials.

References
8. Moore G. W. Combining Taipan snake venom time/Ecarin time screening with the mixing studies of conventional assays increases detection rates of lupus anticoagulants in orally anticoagulated patients. Thrombosis Journal 2007; 5:12

Key guide to symbols

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