Diagnostic Reagents Limited

UK CA

Taipan/Echis venom clotting time Ratios for the detection of Lupus Anticoagulants

For *in vitro* diagnostic use only.

Intended Use
Diagen Taipan and Echis venom clotting times are suitable for use in the *in vitro* detection of lupus anticoagulants (LA) and particularly useful in patients receiving vitamin K antagonist (VKA) therapy.

Summary and Principle
LAs comprise part of the heterogeneous spectrum of acquired autoantibodies named antiphospholipid antibodies (APA) [1]. The occurrence and persistence of APA can be associated with a wide range of clinical signs and symptoms, most commonly arterial and venous thrombosis and pregnancy morbidity.

When in the **presence of phospholipid** and calcium ions, Taipan (*Oxyuranus s. scutellatus*) venom is a direct activator of both native prothrombin [1], and that produced when a patient is anticoagulated with VKA (des-carboxy-prothrombin). This is also true in the absence of clotting factors V, VII and X; which makes it of value in the detection of the presence of LAs in patients receiving oral anticoagulant therapy (OAT), where factors II, VII and X are reduced. The diluted Prothrombin Time (DPT) is profoundly affected by reduction in factors II, VII and X; whereas the Dilute Russell's Viper Venom Time (DRVVT) is affected by a reduction in factors II and X. The Taipan snake venom time (TSVT) however, is only affected by a reduction of factor II. Echis (*Echis Carinatus)* venom similarly activates prothrombin & des-carboxy-prothrombin in the absence of clotting factors V, VII and X but importantly**, without the requirement of Phospholipid or Calcium ions**.

Taking all of these points into consideration, the TSVT performed in parallel with the Echis clotting time (ECT) [3] can be considered a useful **additional** assay in the diagnosis of LA [4] along with the most frequently used assays, the DRVVT and a variety of Activated Partial Thromboplastin Time (APTT) based tests.

Samples are considered positive for LA if the TSVT is prolonged, but the ECT is normal – see interpretation.

Reagent
Taipan Snake Venom - Catalogue Number TAVX320
A lyophilised dilution of Taipan venom extract in calcium chloride, stabilised and buffered. For reconstitution remove cap and rubber bung, and then add **2.0 mL of distilled water** to the contents of the vial. Allow 10 - 15 minutes for complete solution.

Echis Snake Venom – Catalogue Number ECTT330
A lyophilised dilution of Echis venom extract stabilised with and buffered. For reconstitution remove cap and rubber bung, and then add **2.0 mL of distilled water** to the contents of the vial. Allow 10 - 15 minutes for complete solution.

Warnings and Precautions
Both Diagen Taipan and *Echis carinatus* venom are for *in vitro* diagnostic use only. The reagents contain snake venoms, which are poisons and may be fatal if they enter the bloodstream. Normal precautions should therefore be taken when handling. 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.

TAECIFU-003 Revision -

Procedure
The following section details the products required and procedure used for the Taipan Snake Venom Time (TSVT) & Echis Clotting Time (ECT).

Materials Required
Cat. No.
TAVX320 - Taipan Snake Venom (10 x 2.0 mL vials).
ECTT330 - Echis Snake Venom (10 x 2.0 mL vials).

Materials and equipment required, but not provided:
1. General routine laboratory coagulation equipment.
2. Reaction cups or test tubes (12 x 75 mm).
3. Pipette delivering between 100 µL, 200 µL, 2.0 mL.
4. Bell and Alton Platelet Substitute (BAPS040).
5. Imidazole Buffer (IMBX600).
6. Distilled water.

Manual Technique
Preparation
1. After reconstitution (2.0 mL distilled water), the titrated Taipan venom is ready for use
2. Dilute the Bell and Alton platelet substitute 1/4 to 1/8 in Imidazole buffer. **Our calibration has been performed with platelet substitute diluted 1/6 in imidazole buffer.**

Taipan Snake Venom time (TSVT) - manual method
1. Add 100 µL of test plasma to 100 µL of diluted platelet substitute and incubate at 37°C for 60 seconds.
2. Add 200 µL of diluted Taipan venom and record the clotting time.
3. Repeat steps 1 & 2 using platelet poor normal control plasma pool.
4. Once both clotting times have been recorded the TSVT ratio of test plasma / normal control plasma pool can be calculated.
Please note that normal control plasma pool should be tested in parallel with the patient sample.

Echis Clotting time (ECT) - manual method
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 can be calculated.
Please note that normal control plasma pool must be tested in parallel with the patient sample.

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.**

Interpretation
In our hands, the normal **TSVT** ratio is defined as 0.93 – 1.10 **ratios greater than 1.10 suggests the presence of LA**. The cut off ratio of 1.10 is dependent, in part, by the sensitivity of the test system and the choice of Phospholipid (Platelet substitute) dilutions. Our in house method aims to achieve a normal plasma clotting time of approximately 28 seconds. However, **it is most important that each laboratory determines appropriate dilutions and cut off value for each lot of reagents.**

In our hands, a 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.** [3, 5]

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 10^4 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 TSVT

and ECT, testing should either be repeated when heparin treatment has stopped or the heparin neutralized with Protamine sulphate or Polybrene.

It must be remembered that the TSVT and the ECT are supplementary in the detection of LAs. The tests should be used in conjunction with other detection methodologies.

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

Storage and stability

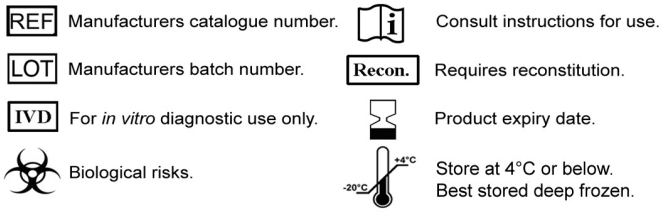
Taipan and Echis venom in the lyophilised state are best stored deep-frozen.

After reconstitution: **See Individual IFUs.**

References

1. Arnout, J., (2001) Antiphospholipid syndrome: Diagnostic aspects of lupus anticoagulants. Thromb Haemost, 86:83-91.
2. Speijer, H., Grovers Reimslag, JPW., Zwaal, RFA., Rosling, J. Prothrombin activation by an activator from the venom of *Oxyurannus scutellatus* (Taipan Snake). J Biol Chem 1986; 261:13258-67.
3. 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.
4. Rooney, AM., McNally, T., Mackie, IJ., Machin SJ. The Taipan snake venom time: a new test for lupus anticoagulant. *J Clin Pathol* 1994;47:497-501.
5. Moore GW, Smith MP, Savidge GF. The Ecarin time is an improved confirmatory test for the Taipan snake venom time in warfarinised patients with lupus anticoagulants. Blood Coagul Fibrinolysis. 2003; 14:307-312.

Key guide to symbols




Manufacturer.


Diagnostic Reagents Ltd.
Thame
Oxon, OX9 3NY
UK
Tel:+44(0)1844 212426
Email: sales@diagen.co.uk
Website: www.diagen.co.uk

Diagnostic Reagents Limited is a BS EN ISO13485:2016 certified company

TAECIFU-003 Revision -

© Diagnostic Reagents Ltd 2022