Diagnostic Reagents Limited

UK CA

Taipan Venom

Catalogue Number: TAVX320 (2.0 mL)

For *in vitro* diagnostic use only.

Intended Use
Diagen Taipan venom is suitable for use in the *in vitro* detection of lupus anticoagulants (LAs) and particularly 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 [(2)], 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 when trying to detect the presence of lupus anticoagulants in patients receiving VKA therapy 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. When taking all of these points into consideration, the TSVT can be considered a useful **additional** assay in the diagnosis of LA[(3)] (particularly when performed in parallel with the Echis Clotting Time (ECT) [(4)] - see accompanying Lupus testing sheet for details), along with the most frequently used assays, the DRVVT and a variety of Activated Partial Thromboplastin Time (APTT) based tests.

Reagent
Taipan Snake Venom **10 vials**
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.

Warnings and Precautions
Diagen Taipan 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 Taipan Snake Venom Time (TSVT).

Materials Provided
Cat. No.
TAVX320 – Taipan 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 and 2 mL.
4. Bell and Alton Platelet Substitute (BAPS040).
5. Imidazole Buffer (IMBX600).
6. Distilled water.

TSVT - 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.**

Technique
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 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 the 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, a **TSVT ratio greater than 1.10 suggests the presence of LA**. This can be confirmed by using a higher concentration of platelet substitute or washed platelets; this should shorten the ratio (correct the clotting time) in the presence of LA. Standard mixing tests using test plasma & normal control plasma can also help in confirming the presence of LA.

Additionally, the test can be performed in conjunction with the *Echis Carinatus* clotting time (ECT) test which should give a normal ratio in the presence of a lupus anticoagulant. – **Please see accompanying LA testing sheet for details.**

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 used. 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.**

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

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
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.

The contents of the vial should be kept between 2 - 8°C once reconstituted, and are then stable for up to 24 hours; the venom loses potency at elevated temperatures and should not be kept at room temperature for prolonged periods between use.

After reconstitution the venom should **not** be refrozen.

TAVXIFU-007 Revision -

Packaging
10 x 2.0 mL.

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. 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.
4. 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

REF	Manufacturers catalogue number.		Consult instructions for use.
LOT	Manufacturers batch number.	Recon.	Requires reconstitution.
IVD	For *in vitro* diagnostic use only.		Product expiry date.
	Biological risks.	+4°C -20°C	Store at 4°C or below. Best stored deep frozen.

Manufacturer.

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