Diagnostic Reagents Limited

UK CA

Russell's Viper Venom

Catalogue Number: RVVX310 (0.2 mg per vial)

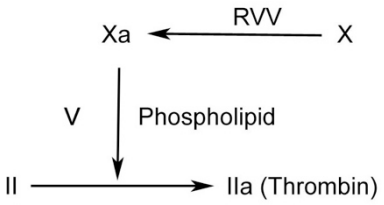
For *in vitro* diagnostic use only.

Intended Use

Diagen Russell's Viper Venom (RVV) is suitable for use in the *in vitro* detection of Lupus anticoagulants (LA) in patients. It may also be useful for indicating deficiencies in Factor V, Factor X, prothrombin, and platelets.

Summary and Principle

It has been shown that plasma samples from patients deficient in prothrombin, factor V or factor X [1, 2] all give abnormally long RVV clotting times compared with normal plasma. The venom is a powerful enzyme and in the presence of calcium ions can convert factor X into an activated form (factor Xa). The activated factor X, together with phospholipid and factor V, converts prothrombin to thrombin in a few seconds [3,4]. Thus, if factor X, factor V, platelets or prothrombin are reduced in a plasma sample, the clotting time with RVV clotting time should be prolonged.



The Dilute Russell's Viper Venom Time (DRVVT) may be used in the detection of LA. International guidelines recommend that haemostasis laboratories should use only DRVVT and Activated Partial Thromboplastin Time (APTT) assays in the detection of LA [5]. LAs comprise part of the heterogeneous spectrum of acquired autoantibodies named antiphospholipid antibodies (APA) [6]. The occurrence and persistence of APA can be associated with a wide range of clinical signs and symptoms, most commonly arterial and venous thrombosis, recurrent foetal loss, and thrombocytopenia. The DRVVT is widely used to diagnose the presence of LA as it is a phospholipid dependent assay. However, prolonged clotting times can also be caused by deficiencies or inhibition of Factors II, V and X. Deficiencies in factors may be corrected using mixing studies. No single LA assay can absolutely identify the presence of all LAs, therefore we recommend that at least one other coagulation-based test be used in conjunction with the DRVVT.

Reagent

Diagen Russell's Viper Venom 10 vials

A lyophilised dilution of Russell's Viper venom in saline, giving each vial a final concentration of 0.2 mg. For reconstitution, remove metal cap and rubber bung and add 2.0 mL of distilled water to the contents of the vial for a 1 in 10,000 dilution. Allow 10 - 15 minutes for complete solution.

Warnings and Precautions

Diagen Russell's Viper 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. Re-centrifuging the plasma for a further 15 minutes at 2500g produces Platelet Poor Plasma (PPP). The plasma should be stored in stoppered tubes.

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Procedure

The following section details the products required and procedure used for the Dilute Russell's Viper Venom Time (DRVVT).

Materials Provided

Cat. No.

RVVX310 – Russell's Viper Venom (10 x 0.2 mg vials).

Materials and equipment required, but not provided:

1. General routine coagulation equipment.
2. Diagen Bell and Alton Platelet Substitute (BAPS040).
   or Diagen Micronized Silica APTT Reagent (MSPS060/1)
3. Imidazole Buffer (IMBX600) for dilutions.
4. 25 mM $CaCl_2$.
5. Reaction Cups or test tubes (12 x 75 mm).
6. Pipette delivering between 100 µL, 200 µl and 2.0 mL.
7. Distilled water.

Manual Technique

Preparation

1. Dilute the RVV a further 1/100 in Imidazole buffer to give a final concentration of 1/1,000,000.
2. Dilute the Bell and Alton platelet substitute or Micronized Silica a further 1/16 in Imidazole buffer.

Technique

1. Add 100 µL of dilute RVV to 100 µL of test plasma.
2. Add 100 µL of 1/16 platelet substitute dilution and incubate at 37°C for 30 seconds.
3. Add 100 µL of 25mM $CaCl_2$ and record the clotting time.
4. Repeat steps 1 & 2 using normal control plasma pool.
5. Once both clotting times have been recorded, the DRVVT ratio of test plasma / normal control plasma pool can be calculated

Please note that normal control plasma 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 and at the recommended dilutions, we have found the DRVVT normal range to be 28 – 35 seconds. This range will vary for different dilutions and for mechanical or photo-optical instruments. A DRVVT ratio of greater than 1.16 is considered raised and requiring further investigation.

A prolonged DRVVT may be due to reasons other than the presence of LAs. In such cases, it is useful to perform mixing studies by re-testing the test sample mixed 1:1 with **normal** plasma. Generally, the DRVVT remains prolonged with LAs (although it is possible to dilute out weakly positive LAs) but is corrected if the prolongation is due to a reduction of clotting factors II, V, and X or a combination of these.

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 LA 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, it indicates a failure in the test system. In such cases patient results should be reinvestigated.

Limitations

Plasma samples from patients receiving or contaminated with heparin cannot be reliably tested using the DRVVT, 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.

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Storage and stability
The unopened freeze-dried vials are **best stored deep frozen** but may be stored for up to 5 years at 2 - 8°C without deterioration. Once reconstituted, the contents of the vial are then stable for up to 24 hours when held at 4°C.

Packaging
10 x 0.2 mg vials.

References
1. Telfer, T.P., Denson , K.W., and Wright. D.R (1956). *Brit J. Haemat* 2, 308.
2. Hougie, C., Barrow, E. M., and Graham, J. B. (1957). *J. Clin invest.,*36, 485
3. Macfarlane, R. G (1961). *Brit J. Haemat.,*7, 496
4. Williams, W. J., and Esnouf, M. P (1962). *Biochem J.* 84, 52
5. Arnout, J., (2001) Antiphospholipid syndrome: Diagnostic aspects of lupus anticoagulants. Thromb Haemost, 86:83-91.
6. Brandt, J. T., Triplett, D. A., Alving, B. and Scharrer, I. Criteria for the diagnosis of lupus anticoagulants, an update: on behalf of the Subcommittee on Lupus Anticoagulants/Antiphospholipid Antibodies of the ISTH *Thromb Haem* 1995, 74(6):1597-1603.

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

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