Dilute Russell’s Viper Venom Time (DRVVT)

PRINCIPLE
Russell’s viper venom (RVV) contains an enzyme that directly activates FX, which then activates prothrombin in the presence of phospholipid, calcium ions, and FV. Dilution of the venom and the phospholipid make the test particularly sensitive to the presence of LAC/APA. A prolonged DRVVT may thus be caused by inhibitors to phospholipids, but may also be caused by deficiency or abnormality of factors II, V, X, or fibrinogen. If prolonged, compared with a locally determined normal range, the DRVVT is repeated with washed freeze/thaw fractured platelets replacing phospholipids. Correction of the abnormal result, when platelets replace phospholipid, indicates the presence of anti-phospholipid antibodies.

REAGENTS
- Imidazole (glyoxaline buffer) - 0.05M pH 7.3
  - 3.4 g imidazole
  - 5.85 g sodium chloride
  1. Dissolve in 800 ml distilled water.
  2. Adjust pH to 7.3 using 1M HCL.
  3. Make up to 1 litre with distilled water.
- Russell’s viper venom (Diagnostic Reagents, Chinnor Road, Thame, U.K.)
  1. Add 2 ml distilled water to 0.2 mg venom to give 0.1 mg/ml.
  2. Freeze at -35°C or lower in 20 µl amounts.
  3. For use, thaw and dilute approximately 1 in 500 in imidazole buffer (10 µl in 5 ml) to give dilute RVV (DRVV).
- Phospholipid (PL; Diagen Bell and Alton Platelet substitute, Diagnostic Reagents, Chinnor Road, Thame, U.K.)
  Reconstitute with distilled water according to manufacturer’s instructions.
- 25mM calcium chloride solution
- Washed freeze/thaw lysed platelets
  Normal human platelets are washed and lysed by freeze/thaw to expose procoagulant phospholipid. A full method is given at the end of this section (see “Preparation of Washed Platelets for DRVVT” on page 123).
Pooled normal plasma
This should be carefully prepared to ensure the minimum number of residual platelets is present, as described under sample preparation, above. It may be convenient to use the pooled normal plasma described in Section 7, provided that the pooled plasma is centrifuged twice prior to deep freezing.

METHOD

1. To a glass test tube at 37°C, add 0.1 ml phospholipid and 0.1 ml of pooled normal plasma.

2. Warm to 37°C for 1–2 minutes.

3. Add 0.1 ml diluted RVV (RVV at room temperature). Mix and leave for exactly 30 seconds at 37°C.

4. Add 0.1 ml pre-warmed 25 mM calcium chloride. Mix and start stopwatch.

5. Time clot formation. Perform all tests in duplicate.

   The pooled normal plasma should give a clotting time of 30–35 seconds. If <30 seconds, further dilute the RVV solution by adding more imidazole buffer. If >35 seconds, add more stock RVV solution. Repeat test on pooled normal plasma until a time of 30–35 seconds is obtained.

6. Prepare a dilution of 1/8 of PL in saline; for example, by adding 0.1 ml PL to 0.7 ml saline.

7. Repeat steps 1 to 5 with platelet substitute 1/8 replacing neat PL.

   If the clotting time (previously 30–35 seconds) is now prolonged to 35–40 seconds, this indicates that the PL concentration is sufficiently dilute to make the test sensitive to antibodies to that phospholipid.

   If the clotting time exceeds 40 seconds, repeat steps 1-5 using PL diluted 1 in 4. If the clotting time remains 30–35 seconds, repeat steps 1-5 using PL diluted 1 in 16. If the time is 35–40 seconds, proceed to the next step.

8. Using this RVV solution (giving 30–35 seconds with neat PL and pooled normal plasma) and the dilution of phospholipid associated with a time of 35–40 seconds, repeat 1-5, substituting patient plasma for pooled normal.

9. Calculate the ratio of DRVV time (DRVVT) for patient plasma over DRVVT for pooled normal plasma to give the DRVVT ratio.
Steps 1-5 are now repeated using pooled normal plasma, and again using test plasma, but where washed freeze/thaw lysed platelets are used in place of PL. This is the platelet neutralization procedure (PNP). In step 2, the mixture is incubated for 10 minutes. The ratio of test to pooled normal is calculated for PNP.

**INTERPRETATION**

The normal range for DRVVT ratio should be established locally using plasma from normal subjects, as described in Section 8. The normal range (mean ± 2 SD) of DRVVT ratio with PL is typically 0.90–1.10. A prolonged DRVVT ratio indicates either deficiency of factors II, V, X, or fibrinogen, or it indicates the possible presence of anti-phospholipid antibody.

A DRVVT ratio that is prolonged with phospholipid but decreases or corrects in the PNP ratio is suggestive of anti-phospholipid antibody.

Note that deficiency of the clotting factors mentioned above would be associated with prolonged ratios in both DRVVT with phospholipid and in the PNP.

The presence of heparin in the sample can lead to results similar to those when anti-phospholipid antibody is present.

**CORRECTIONS IN THE DRVVT IN THE PNP TEST**

Corrections or a decrease of the prolonged DRVVT ratio to within the normal range is strong evidence for the presence of LAC.

Often, the PNP ratio is not corrected to within the normal range. The degree of correction required to indicate the likely presence of LAC is not known for certain. One approach is to calculate the percentage correction as:

\[
\text{Percentage correction} = \left( \frac{\text{DRVVT ratio with PL} - \text{PNP ratio}}{\text{DRVVT ratio with PL}} \right) \times 100
\]

A result above the normal range that corrects by >10% in the PNP is considered indicative of LAC.
38.1 Preparation of Washed Platelets for DRVVT

**REAGENT**

Tyrode’s buffer pH 6.5
- 8.0 g NaCl
- 0.2 g KCl
- 0.065 g NaH₂PO₄·2H₂O
- 0.415 g MgCl₂·6H₂O
- 1.0 g NaHCO₃

Dissolve in 900 ml distilled water and adjust pH to 6.5. Make up to 1 litre with distilled water.

**METHOD**

1. Prepare platelet-rich plasma (PRP) from fresh citrated blood by centrifugation at 170 g (1200 rpm) for 10 minutes at room temperature.

   OR

   Use platelet concentrates as for treatment of patients.

2. Dilute PRP 1 in 2 with Tyrode’s buffer.

3. Place the dilute platelets in plastic conical-bottomed centrifuge tubes and centrifuge for 10 minutes at 850 g at room temperature.

4. Discard the supernatant using a plastic Pasteur pipette and resuspend the pellet of platelets in Tyrode’s buffer. (Add the same amount of buffer as the original volume of PRP.) Centrifuge at 850 g, as before. This process is repeated twice.

5. Discard the supernatant and resuspend in Tyrode’s buffer, using the same amount of buffer as 25% of the original volume of PRP.

6. Aliquot the final washed platelets into 1.0 ml amounts in plastic tubes and freeze and thaw twice at -20°C prior to use.