Dilute Russell Viper Venom-like Reagents

dRVT-LS & dRVT-LR

FOR RESEARCH USE ONLY

IMPROVED DILUTE "RUSSELLS VIPER" VENOM - LIKE REAGENTS (dRVT - LS and dRVT - LR)

INTENDED USE

Lupus Anticoagulants (LA) are a diagnostic feature of Anti-Phospholipid Syndrome (APS) an autoimmune condition associated with thrombosis and recurrent pregnancy loss. These dRVT reagents are for detecting and subsequently confirming the presence of LA in patient test plasmas by a clotting test method.

SUMMARY AND EXPLANATION OF THE TESTS

The dilute Russells viper venom clotting time using a low phospholipid concentration is a common screening test for LA (1). The original complex procedure whereby phospholipid, Russells viper venom (RVV) and calcium chloride solution were added sequentially to the test plasma (2) has been simplified so that a combined reagent can be used (3). The LA sensitive (dRVT-LS) reagent contains a low phospholipid concentration whereas the LA resistant (dRVT-LR) reagent contains a higher phospholipid concentration. Procoagulant phospholipid can overcome or reduce the inhibitory effect of LA while having little effect on sensitivity to other clotting abnormalities (4).

RVV-X in the venom converts factor X in test plasmas to Xa and this activates prothrombin to thrombin in the presence of factor Va, calcium and phospholipid. The thrombin then rapidly clots fibrinogen. Both dRVT reagents are adjusted to give results on normal plasma in the range of 30-40 sec. LA interfere with the limited phospholipid surface in the LS reagent and prolong results. Excess phospholipid in the LR reagent bypasses the LA effect thus giving more normal results.

IMPROVEMENT

These new reagents have improved stability compared with conventional products, allowing their supply in liquid "ready for use" form. They are also more specific for LA testing, showing lower sensitivity to coagulation defects other than LA which may otherwise cause false positive LA results or require further investigations (5). The new reagents are based on enzymes from daboia Siamensis venom. This species is distinct from Russells viper (6) although it contains similar procoagulant enzymes giving the same results in clotting tests (5).

REAGENTS

Components in each test kit

dRVT-LS (green) or dRVT-LR (pink) reagents are ready for use, usually in 5 x 10ml vials (also available in a combination box):
Ingredients include vegetable phospholipid, calcium salts, hydrolysed gelatine, purified enzymes from daboia Siamensis venom (<250ng/ml), Polybrene, stabilisers and antimicrobials (<0.02%).

Other materials required for test

Coagulation instrumentation and consumables, or if for manual clotting tests, small test tubes, 37°C water bath, micro-pipette dispensers with tips and stopwatches. Also quality control plasmas and platelet poor normal plasma if mixing tests are to be carried out.

Storage instructions

The dRVT reagents can be stored at 4°C for up to 1 year (or longer at lower temperatures). Do not use the kits beyond the expiry dates shown on packaging. Product deterioration may be indicated by quality control results outside the accepted laboratory range.

SPECIMEN COLLECTION AND PREPARATION

Blood collection

Blood should be collected by clean venipuncture into one ninth its volume of 0.109M sodium citrate (3.2% trisodium citrate dihydrate). Guidelines from the CLSI should be followed (7).

Processing and storage

Citrated blood samples should be centrifuged initially at 2000g for 10min at 20+/-5°C. Supernatant plasma can then be tested fresh. If plasma is to be frozen for storage, it should be removed to a second plastic tube and re-centrifuged for 10 minutes at a higher g force than in the initial step (ie >2500g) for more thorough removal of platelets before transfer to a polypropylene tube for storage at below -50C. In general the latest ISTH (1) and CLSI guidelines (7) should be followed.

DRVT TEST PROCEDURE

Procedural notes and precautions

Carry out tests in duplicate unless the instrument used routinely provides interassay cv below 2% with multiple tests on pooled normal plasma.

Quality control

Normal and abnormal QC plasmas must be included in each batch of 40-50 test samples. These results must be monitored on a regular basis and when values exceed +/-2SD corrective action must be taken.

Test procedure

Screening test:

1. Pre-warm an adequate volume of dRVT-LS reagent at 37°C (0.2ml per manual test; or less if automated).
2. Prewarm 0.2ml of test plasma (or less if automated) in a test tube or cuvette to 37°C for 2 minutes.
3. Dispense an equal volume of the pre-warmed dRVT reagent into the test plasma, mix and time to a clotting endpoint by tilt tube, mechanical or photoelectric sensing.
4. Record the dRVT clotting time as the mean of duplicates. If the duplicates differ by more than 5%, the tests should be repeated.

Confirmatory test:

If the above result is abnormal, repeat the same test procedure but with the dRVT-LR reagent. Proceed to a mixing test if this result is abnormal.

Mixing test:

If the test result with dRVT-LR is abnormal, prepare a 1:1 mix of patient plasma with pooled normal plasma and test with both dRVT-LS and dRVT-LR reagents.
The normal platelet poor plasma should also be tested.
Proportionally smaller volumes can be used throughout.

RESULTS

Expression of results

Clotting time results with each dRVT reagent can be expressed as a ratio relative to the result with PNP. Then the ratio with dRVT-LS can be divided by the ratio with dRVT-LR to yield an overall normalized ratio (8). Most labs use a final cutoff near an overall ratio 1.30.

Interpretation of results

Normal plasmas usually give results of 30-40sec with both dRVT-LS and dRVT-LR reagents. Plasmas which contain LA usually give a prolonged result with the dRVT-LS screening reagent; abnormal being considered as longer than mean normal result plus 3SD (99th percentile according to ISTH, 1). The result with the dRVT-LR reagent on uncomplicated LA plasmas should be relatively normal.

The ratio of dRVT-LS result and LR result on a test plasma is often more meaningful than raw results and can be "normalized" by division with the LS:LR result obtained on pooled normal plasma. A normalized LS:LR ratio greater than 1.3 is strongly suggestive of a LA provided that the dRVT-LR result is normal. However if the dRVT-LR result is abnormal, all tests should be repeated on 1:1 mixes of patient and platelet poor normal plasma (9).

The table below summarizes the value of using combinations of LS and LR tests on neat patient plasma and their mixes with PNP. Note that it is possible that some low affinity LA may be lost in mixing tests (10).

Abnormality	dRVT-LS		dRVT-LR	
	neat	1:1 mix	neat	1:1 mix
Nil (normal)	N	N	N	N
Factor deficiency	Abn	N	Abn	N
LA	Abn	Abn	N	N
LA + defect	Abn	Abn	Abn	N

Safety comments and precautions

Reagents are currently only for in vitro research use. Handle all samples as potentially infectious. Use disposable gloves and contact a doctor immediately if a needle stick injury or unintentional blood person contact occurs. Clean up any spillages with bleach or 70% ethanol and incinerate, sterilise or autoclave waste materials.

Notes:

1. The dRVT reagents contain Polybrene neutralising up to 1.2u/ml heparin and sometimes shortening results on heparin plasmas. Thus differences from APTT, KCT and SACT test results can be expected.
2. New oral antithrombotic agents such as dabigatran, rivaroxaban and apixaban have much less effect on these improved dRVT reagents than on existing reagents. So differences may be observed.
3. It is important to use platelet poor normal plasmas in mixing tests as residual platelets can reduce the prolonging effects of LA.
4. Results from samples which are partially haemolysed, icteric or lipaemic should be interpreted with caution especially when determined using a photoelectric coagulometer.
5. Borderline results should be considered in line with other clotting tests, clinical considerations and markers for APS such as anticardiolipin or anti beta2GPI ELISAs.

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