CE

L A CONFIRM

dRVVT FOR CONFIRMATION OF LUPUS ANTICOAGULANTS

SUMMARY

Lupus Anticoagulants (LA) are auto antibodies against the anionic phospholipid portion of prothrombinase. Prothrombinase is a complex of factor Xa; factor Va, phospholipid and calcium ions involved in the conversion of prothrombin to thrombin in the common pathway. The autoantibodies produced are of IgG class or IgM class or both. Since these antibodies were first detected in patients with systemic lupus erythematosus (SLE) they were named LA.

LA's prolong phospholipid dependant tests such as the activated partial thromboplastin time (APTT) and kaolin clotting time (KCT). The name anticoagulant is a misnomer since patients do not have a bleeding tendency. Instead there is a clear association of thrombo-embolism. LA is also an important cause of recurrent abortions in women. Since these antibodies are also found in patients with SLE, detection of LA is important in management of patients with or without SLE experiencing unusual thrombotic events and habitual abortions.

The Dilute Russell's Viper Venom Test (dRVVT) was first introduced by Thiagarajan et. al. in 1986. dRVVT is a simple, sensitive and specific assay for detection of LA. Since Russell's Viper Venom activates factor X directly, dRVVT is more specific for LA than APTT. Results are affected neither by contact factor abnormalities nor by factor VIII, factor IX deficiencies or corresponding inhibitors.

PRESENTATION

REF REF	10673005
R1	0.5 ml
R2	0.5 ml
Pack insert	1

PRINCIPLE

Russell's viper venom directly activates factor X in the presence of phospholipid and calcium ions, bypassing factor VII of the extrinsic pathway and the contact and antihaemophilic factors of the intrinsic pathway. In normal plasma in the absence of lupus anticoagulants, factor X is directly activated by Russell's viper venom, which in presence of phospholipid and calcium ion leads to clot formation. In patients with LA, autoantibodies bind the epitopes of reagent phospholipids thereby preventing the activation of prothrombinase complex. This results in a prolongation of clotting time with LA SCREEN reagent.

REAGENTS PROVIDED WITH THE KIT

LA CONFIRM Reagent 1 contains lyophilized preparation of Russell's viper venom and phospholipid. The reagent also incorporates 0.01% thimerosal as preservative.

LA CONFIRM Reagent 2, Calcium chloride reagent (0.025M).

STORAGE AND STABILITY

1. Store the reagent at 2-8^{0}C. DO NOT FREEZE.
2. The shelf life of the reagent is as per the expiry date mentioned on the reagent vial label.
3. Reconstituted reagent can be used for 5 days when stored at 2-8^{0}C, provided it is not contaminated.

ADDITIONAL MATERIAL REQUIRED

10 mm x 75 mm glass test tubes, waterbath at constant temperature of 37^{0}C, stopwatch, 100 µl precision pipettes, scrupulously clean and dry micropipette tips, distilled water, 1 ml precision pipette.

REAGENT PREPARATION

Bring the LA CONFIRM Reagent 1 to room temperature (25-30^{0}C) prior to reconstitution. Add 0.5 ml of distilled water to the lyophilized LA CONFIRM Reagent 1. Gently mix to dissolve. Keep for 15-20 minutes at room temperature (25-30^{0}C) and mix gently ensuring complete resuspension of the lyophilized material. Thorough mixing should be ensured before withdrawing material every time for test purpose.

SAMPLE PREPARATION

Mix nine parts of freshly collected patient's blood with one part of tri sodium citrate (3.2%). Centrifuge as soon as possible after collection at 1500 g for 15 minutes to obtain PPP (Platelet Poor Plasma). Store in capped tubes at 4^{0}C, and use within 4 hours of collection. If the samples are to be frozen for subsequent testing, the plasma must be centrifuged again as these can otherwise shorten the CONFIRM TIME.

- Clotted samples should be discarded
- Erroneous results may occur in patients with abnormal haematocrits, as the plasma to citrate concentration in these samples is not optimal.

TEST PROCEDURE

Bring all the reagents to room temperature (25-30^{0}C) before prewarming at 37^{0}C for testing purpose.

1. Aspirate enough quantity from Calcium chloride (Reagent 2) vial for immediate testing requirements in a thoroughly clean and dry test tube. Incubate the tube at 37^{0}C for 10 minutes.
2. Place 0.1 ml of LA CONFIRM (Reagent 1) in a clean and dry test tube (ensure thorough mixing before withdrawing material for testing purpose).
3. To this tube add 0.1 ml of plasma (PPP). Shake the tube gently to mix the contents and incubate for 1-2 minutes at 37^{0}C.
4. Finally to this tube add 0.1 ml of Calcium chloride (prewarmed at 37^{0}C for 10 minutes) and simultaneously start the stopwatch.
5. Stop the stopwatch as soon as the clot formation begins. Record the time in seconds. This is the CONFIRM TIME for the plasma specimen.

INTERPRETATION OF RESULTS

The normal expected values for CONFIRM TIME is 22-38 seconds.
For confirming the phospholipid dependence of LA the results should be interpreted as ratio,

$$\text{Ratio (R)} = \frac{\text{Mean Screen Time}}{\text{Mean Confirm time}}$$

Ratio (R)	R < 1.3	R = 1.5-1.8	R= 1.8-2.4	R>2.4
Interpretation of results	Normal	Moderate LA	High LA	Very High LA

If results are borderline, (ratio 1.3-1.4) mixing studies may be done further with the sample specimen. These tests should be carried out on a 50:50 mixture of test plasma and normal plasma.

INTERPRETATION OF RESULTS WITH MIXING STUDIES

LA SCREEN		LA CONFIRM		Interpretation of results
Patient plasma	50:50 mixture of patient and normal plasma	Patient plasma	50:50 mixture of patient and normal plasma	
N	N	N	N	LA absent
Ab.N	Ab.N	N	N	LA present
Ab.N	N	Ab.N	N	Factor deficient
Ab.N	Ab.N	Ab.N	N	LA + factor deficient
Ab.N	Ab.N	Ab.N	Ab.N	Other inhibitor

N-Normal, Ab.N - Abnormal

- Prolonged SCREEN TIME and CONFIRM TIME are also obtained with plasma samples of patients with factor II, V and X deficiencies as well as on warfarin therapy. These defects correct on addition of normal plasma. In such cases individual assays of factor II, V and X should be performed.
- Plasma samples that have LA along with factor deficiencies remains abnormal in SCREEN TIME, showing only partial correction of the defect on mixing. Such plasma yields abnormal CONFIRM TIME only in neat plasma due to factor deficiency and not the LA.
- If normal plasma corrects neither the SCREEN TIME nor the CONFIRM TIME then an inhibitor against any of the factors II, V and X may be suspected and should also show an abnormal PT result.

REMARKS

1. Each laboratory should use known platelet depleted normal and abnormal LA control plasmas with each test series for validation of results.
2. Each laboratory should establish the acceptable control values and normal range.
3. Incorrect mixture of blood and tri sodium citrate, insufficient prewarming of plasma and reagent, contaminated reagents, glassware etc. are potential sources of error.
4. Since the LA test functions optimally at 37^{0}C +/- 0.5^{0}C, temperature of all equipment must be calibrated daily.
5. Glassware's and cuvettes used in the test must be scrupulously clean and free from even traces of acids/alkalies or detergents.
6. Since the test uses platelet poor plasma, each laboratory must calibrate the necessary force and time required during centrifugation to yield PPP. Contamination of plasma with excess platelets could lead to erroneous results.
7. Thorough mixing and homogenization of reconstituted LA CONFIRM (Reagent 1) reagent suspension before use is important to achieve accurate and consistent results.
8. It is recommended that test results should be correlated with clinical findings to arrive at the final diagnosis.

PERFORMANCE CHARACTERISTICS

Precision studies were performed with LA confirm using normal plasma pool sample, LA high control (n=15) and factor deficient plasma on Hemostar XF coagulometer.

Inter/ Intra assay precision

Samples	Mean ratio	SD	CV (%)
Normal plasma control	1.01	0.06	6.2
LA high control	1.89	0.15	7.9

SPECIFICITY

Specificity studies were performed in known LA high control and LA low control, 50 normal plasma samples, and Factor deficient plasmas that were screened with LA Screen.

WARRANTY

This product is designed to perform as described on the label and package insert. The manufacturer disclaims any implied warranty of use and sale for any other purpose.

BIBLIOGRAPHY

1. 'The Use of Dilute Russell Viper Venom Time for the Diagnosis of Lupus Anticoagulants', Perumal Thiagarajan, Vittorio Pengo, Sandor S. Shapiro, Blood, 1986, Vol.63 (No.4), 869-874.
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3. 'Criteria for the diagnosis of Lupus Anticoagulants: An update', J. Brandt, D.A. Triplett, B. Alving, Thrombosis and Haemostasis, 1995, 74(4), 1185-1190.
4. Haemostasis and Thrombosis, J. Hirsh, H. Colman, 1994, J.B. Lippincott and Company.
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SYMBOL KEYS

 Temperature limitation	 Manufacturer	 Contains sufficient for <n> tests
 Use by	 Consult Instructions for use	R1 LA Confirm
 Date of Manufacture	REF Catalogue Number	R2 Calcium Chloride
LOT Batch Number/ Lot Number	IVD *In vitro* Diagnostic Medical Device	REAGENT Description of reagent
 This side up	PS Production Site	EC REP Authorised Representative in the European Community

 **TULIP DIAGNOSTICS (P) LTD.**

PS

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