



## dRVVT FOR SCREENING AND CONFIRMATION OF LUPUS ANTICOAGULANTS

### SUMMARY

Lupus Anticoagulants (LA) are autoantibodies 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 coagulation pathway. The autoantibodies produced are of IgG class or IgM class or both. As these type of antibodies were first detected in patients with systemic lupus erythematosus (SLE) they were named LA.

LA's prolong phospholipid dependent 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 (Systemic Lupus Erythematosus), 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 XI deficiencies or corresponding inhibitors.

### PRESENTATION

REF	REF	10671010
R1		1 ML
R2		1 ML
R3		2.5
PACKINSERT		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 antithrombophilic 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 the prothrombinase complex. This results in a prolongation of clotting time with SCREEN reagent.

The CONFIRM reagent incorporates additional phospholipids to neutralize LA, thereby achieving a lower clotting time, thus proving the phospholipid dependence of the autoantibodies.

### REAGENTS PROVIDED WITH THE KIT

Tulip's LA Detection System comprises of a 3-reagent set for screening and confirmation of Lupus Anticoagulants. LA SCREEN (Reagent 1) and LA CONFIRM (Reagent 2) are lyophilized preparations containing Russell's Viper Venom enriched with phospholipid at different concentrations, sufficient for performing 10 assays. Both LA SCREEN and LA CONFIRM reagents contain 0.01% thimerosal as preservative. Calcium Chloride (Reagent 3, 0.025M) contains heparin-neutralizing substance, making the reagent system insensitive to the presence of heparin upto 0.4 U/ml. Calcium chloride reagent is intended for use with LA SCREEN and LA CONFIRM reagents, the quantity being 2.5 ml sufficient for performing 20 assays.

### NOTE

Since SCREEN & CONFIRM reagents of the same lot are optimized as a system, it is important that reagents from the same lot are used for accurate and reproducible results.

### STORAGE AND STABILITY

(1) Store the reagent at 2-8°C. DO NOT FREEZE. (2) The shelf life of the reagents is as per the expiry date mentioned on the reagent vial label. (3) Reconstituted reagents can be used for 5 days when stored at 2-8°C, provided it is not contaminated and handled aseptically (4) It is strongly recommended that enough reconstituted reagents should be retrieved for the days use and the unused reagent should be immediately replaced to 2-8°C.

### ADDITIONAL MATERIAL REQUIRED

10 mm x 75 mm glass test tubes, Waterbath at constant temperature of 37°C, Stopwatch, 100 µl precision pipettes, Scrupulously clean and dry micropipette tips, Distilled water, 1 ml precision pipette.

### REAGENT PREPARATION

Bring the LA SCREEN reagent and LA CONFIRM reagent to room temperature (25-30°C) prior to reconstitution. LA SCREEN reagent and LA CONFIRM reagents are reconstituted with 1 ml de-ionized, distilled water each as follows: (1) Add 1 ml of

distilled water to the lyophilized LA SCREEN reagent and LA CONFIRM reagent. (2) Gently mix to dissolve. (3) Keep for 15-20 minutes at room temperature (20-30°C) and mix again gently ensuring complete suspension of the lyophilized material. (4) Thorough mixing should be ensured before withdrawing material every time for test purposes.

#### SAMPLE PREPARATION

Mix nine parts of freshly collected patient's blood with one part of tri-sodium citrate (3.2%). Buffered citrate containing 0.05M HEPES buffer (pH 7.0) in a plastic or siliconized tubes is preferable (such as PROFACT, Cat. No. 10660020). Centrifuge as soon as possible after collection at  $\geq 1500$  g for 15 minutes to obtain PPP. Store in capped tubes at 4°C, and use within 4 hours of collection. If the samples are to be frozen for subsequent testing, the plasma must be centrifuged again or filtered through a 0.2  $\mu$ m filter to remove platelets (to below  $20 \times 10^9/l$ ) as these can otherwise shorten the SCREEN TIME.

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

#### TEST PROCEDURE

Bring all reagents to room temperature (25-30°C) before prewarming at 37°C for testing purpose.

(1) Aspirate enough quantity of reagent from Calcium Chloride reagent vial for immediate testing requirements in a thoroughly clean and dry test tube. Incubate the tube at 37°C for 10 minutes. (2) Place 0.1 ml of LA SCREEN reagent 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°C. (4) Finally to this tube add 0.1 ml of Calcium chloride (prewarmed at 37°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. (6) This is the SCREEN TIME for the plasma specimen. If SCREEN TIME is less than 42 seconds it indicates absence of LA and there is no need to perform CONFIRMATORY test. (7) When the SCREEN TIME is more than 42 seconds repeat the test procedure for the sample using CONFIRM reagent. Repeat the steps 2-6 for testing sample plasma specimen using LA CONFIRM reagent.

It is recommended that sample should be run in duplicate with SCREEN and CONFIRM reagents and the mean time maybe used for arriving at the results.

It is important that SCREEN TIME and CONFIRM TIME on a sample are performed simultaneously for comparative studies.

#### INTERPRETATION OF RESULTS

The normal expected values for SCREEN TIME is 24-42 seconds.

The normal expected values for CONFIRM TIME is 22-38 seconds.

Tulip's LA Detection system is based upon the ratio of clotting time using LA SCREEN and the clotting time of the same sample using LA CONFIRM reagent.

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

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 (Reagent 1)		LA CONFIRM (Reagent 2)		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 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 sample that has LA along with factor deficiencies remains abnormal for 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 show an abnormal PT result also.

#### REMARKS

1. Each laboratory should use known platelet depleted normal and abnormal LA control plasmas available with TULIP, Cat. No. 11030005 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°C±0.5°C, temperature of all equipment must be calibrated daily.
5. Glasswares 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. It is recommended that results of the tests should be correlated with clinical findings to arrive at the final diagnosis.
8. Thorough mixing and homogenization of reconstituted LA SCREEN and LA CONFIRM reagent suspension before use is important to achieve accurate and consistent results.

#### PERFORMANCE CHARACTERISTICS

Precision studies were performed with LADS confirm using normal plasma pool sample, LA high control (n=10) on Hemostar XF coagulometer.

#### Inter/ Intra assay precision

Specimen	Mean ratio	SD	CV (%)
Normal Plasma Control	1.03	0.03	2.9
LA high control	1.87	0.14	7.5








#### WARRANTY

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

#### BIBLIOGRAPHY

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2. 'Anticardiolipin antibodies and Lupus Anticoagulants comprise separate antibody groups with different phospholipid binding characteristics', H. Patrick McNeil, Colin N. Chesterman and Steven A. Krills, British Journal of Haematology 1989, 73, 506-513.
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, 3rd Edition, 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	<b>R1</b>	LA Screen	<b>R3</b>
	Date of Manufacture	<b>REF</b>	Catalogue Number	<b>R2</b>	LA Confirm	Calcium Chloride
<b>LOT</b>	Batch Number/ Lot Number	<b>IVD</b>	<i>In vitro</i> Diagnostic Medical Device	<b>REAGENT</b>		Description of reagent
	This side up	<b>PS</b>	Production Site	<b>EC</b>	<b>REP</b>	Authorised Representative in the European Community

  
**T TULIP DIAGNOSTICS (P) LTD.**

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