Meridian Healthcare®

LUPUS ANTICOAGULANTS(LA)

PRODUCT CODE: COAG106A

COAG106A
2x1ml; 1x2.5ml
STORE AT 2-8°C
INSTRUCTIONS FOR USE
FOR IN-VITRO DIAGNOSTIC USE ONLY

IVD For In Vitro Diagnostics Use Only
LOT Lot Number
REF Catalogue Number
Storage Temperature
Expiry Date (Year / Month)
Warning, Read Enclosed Documents
Instructions For Use
Manufactured By

Lupus Anticoagulants (LA) Kit

This test is used for the screening and confirmation of Lupus Anticoagulants (LA).

Summary and Explanation of the Test:
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 (dRV VT) was first introduced by Thiagarajan et. al. in 1986. dRV VT is a simple, sensitive and specific assay for detection of LA. Since Russell's Viper Venom activates factor X directly, dRV VT 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.

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 reagent.

REAGENTS PROVIDED WITH THE KIT:

Fortresss **LA kit** comprises of a 3-reagent set for screening and confirmation of Lupus anticoagulants. Reagent 1 (R1) and Reagent 2 (R2) are lyophilised preparations containing Russell's Viper Venom enriched with phospholipid at different concentrations, sufficient for performing 10 assays. Both R1 and R2 reagents contain 0.01% thimerosal as preservative.
Calcium Chloride (Reagent 3 (R3), 0.025M) contains heparin neutralizing substance, making the reagent system insensitive to the presence of heparin up to 0.4U/ml. Calcium Chloride reagent is intended for use with R1 and R2 reagents.
In summary:

Kit Contents:		**COAG106A**
R1	LA Screen Reagent	1 x 1 ml
R2	LA Confirm Reagent	1 x 1 ml
R3	Calcium Chloride, 0.025 M	1 x 2.5 ml

Note:
The R1 and R2 reagents supplied in this kit for screening & confirmation of Lupus Anticoagulants are optimised as a system, therefore, it is important that reagents from the supplied lots within a kit are used for accurate and reproducible results.

MATERIALS REQUIRED but not Supplied:
100 µl & 1 ml precision pipettes,
Stopwatch
Fortress Plasma Control level I (COAG108A)
Fortress Plasma Control level II (COAG109A)

Storage and Stability:
1. Store the reagents at 2-8°C. DO NOT FREEZE.
2. The reagents are stable up until the expiry dates shown on the reagent vials.
3. Reconstituted reagents are stable up to 5 days when stored at 2-8°C. Avoid reagent contamination.
4. It is strongly recommended that enough reconstituted reagents should be retrieved for the days use and the unused reagent should immediately be placed at 2 – 8°C.

REAGENT PREPARATION
Bring the reagents to room temperature (about 25°C) prior to reconstitution.
(1)Add 1 ml of distilled water to the lyophilised R1 (**LA SCREEN)** and R2 (**LA CONFIRM)** reagent.
(2) Gently mix to dissolve and Keep for 15-20 minutes at room temperature. Mix again gently ensuring complete re-suspension of the lyophilized material.
(3)Mix well before withdrawing material every time for testing.

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 siliconized tube. Centrifuge immediately after collection at ≥ 1500 g for 15 minutes to obtain Platelet Poor Plasma.
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 µm filter to remove platelets (to below 20 x 10) as these can otherwise shorten the SCREEN TIME.
Note:
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 (about 25°C) before prewarming at 37°C for testing.
1. Aspirate the required quantity of **Calcium Chloride** in a test tube. Incubate the tube at 37°C for 10 minutes.
2. Place 0.1 ml of R1 (**LA SCREEN**) in a clean and dry test tube, mix well before withdrawing material for testing
3. To this tube add 0.1 ml of platelet poor plasma as prepared in the sample preparation above. 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 then **45 seconds,** it indicates absence of LA and there is no need to perform **CONFIRMATORY** test.
7. When the **SCREEN TIME** is more than 45 seconds repeat the test procedure for the sample using R2 (**LA CONFIRM)** reagent.
Repeat the steps 2 – 6 for testing sample plasma specimen using R2 (**LA CONFIRM)** reagent.

It is recommended that sample should be run in duplicate with SCREEN and CONFIRM reagents and the mean time may be 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 28-45 seconds.
The normal expected values for CONFIRM TIME is 28-40 seconds.
FORTRESS's LA kit is based upon the ratio of clotting time using LA SCREEN reagent and clotting time of the same sample using LA CONFIRM reagent.

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

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	High LA

If results are borderline, (ratio of 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 (R 1)		LA CONFIRM (R 2)		
Patient plasma	**50:50 mixture of patient and normal plasma**	**Patient plasma**	**50:50 mixture of patient and normal plasma**	**Interpretation of results**
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 (also available with FORTRESS) and abnormal LA control plasmas(also available with FORTRESS) 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. 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 Platelet Poor Plasma. Contamination of plasma with excess platelets could lead to erroneous results.
7. It is recommended that test results 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.

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

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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, 1994, J.B. Lippincott and Company Data on file: Fortress Diagnostics Ltd.

Meridian Healthcare srl
Via Caronda, 446 SC/A - 95129 Catania - Italy
Tel. +39 095 725 68 69 - Fax:. +39 095 725 44 54
info@meridianhealthcare.it
www.meridianhealthcare.it