TECHNICAL SERIES

# Lupus Anticoagulants

## Basic Concepts and Laboratory Diagnosis

...Setting trends

## General Background

Lupus anticoagulants (LA) were first described in the year 1952 by Conley and Hartmann in two patients suffering from Systemic Lupus Erythematosus (SLE). It was given the name "Lupus" since it was initially recognized in patients with SLE and the name "Anticoagulant" because the two patients diagnosed as SLE developed bleeding complications. It is now clear that Lupus anticoagulant is also encountered in patients without SLE and is associated with thrombosis rather than abnormal bleeding.

Patients with Lupus anticoagulants but without SLE can be categorised as follows:

- Patients presenting with venous and arterial thrombotic events for which no underlying cause may be apparent
- Patients receiving drugs namely procainamide, chlorpromazine, quinidine, hydralazine, interferon
- Patients with a recent acute viral infection
- Patients with HIV infection
- Patients seeking medical attention for other disorders, discovered because of prolonged results with APTT in routine pre-operative evaluation of haemostasis status.

Though the terminology 'Lupus Anticoagulant' is a misnomer because LA invivo cause thrombosis rather than abnormal bleeding, it is still extremely popular and hence ISTH (International Society of Thrombosis and Haemostasis) has recommended the continued usage of the same. Thus LA is neither a syndrome nor a disease but a laboratory phenomenon detected by prolonged results with phospholipid dependent clot based assays.

## Anti-phospholipid Antibody - Lupus Anticoagulants

Anti-phospholipid Antibody is an autoantibody directed against phospholipids, an important extracellular body constituent. Among the various types of Anti-phospholipid antibodies, clinically important are Lupus anticoagulants (LA) and Anti- cardiolipin antibodies (ACL). The clinical importance of LA and ACL is associated with their high frequency of occurrence in patients with unexplained thrombotic events in APS (Anti-phospholipid Syndrome).

Although LA and ACL may be found concurrently in patients with recurrent thromboemblic disease, they are not necessarily connected to each other. Recent reviews have suggested that LA and ACL are separate antibody subgroups with different phospholipid binding characteristics.

**Lupus anticoagulant** is an antibody (IgG or IgM or both) that prolongs phospholipid dependent coagulation assays by binding to the epitopes of the anionic phospholipid portion of the prothrombinase complex.

Prothrombinase is a complex of Factor Xa, Factor Va, anionic phospholipid and calcium, which converts prothrombin to thrombin in the coagulation pathway.

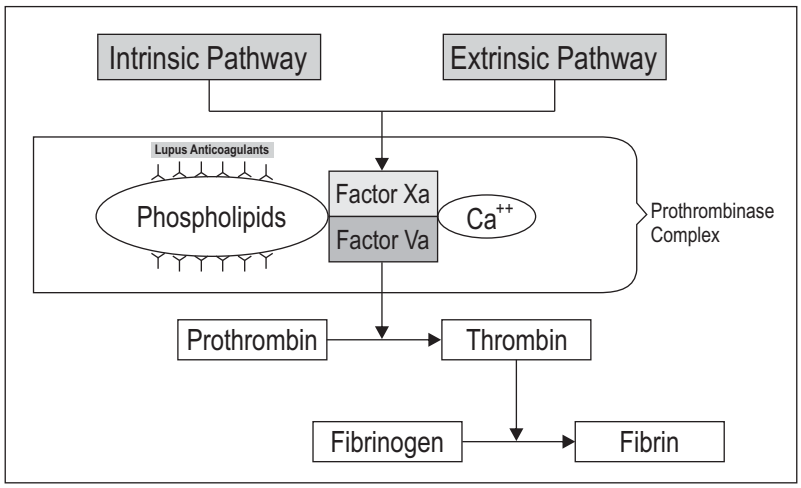


**Fig. 1. LA action on Prothrombinase Complex**

In vitro, Lupus anticoagulants prolong phospholipid dependent coagulation assays and exert their inhibitory effect by:

1. Binding to epitopes of anionic phospholipids on the prothrombinase complex
2. Blocking the calcium mediated binding of Factor Xa and Factor Va to the phospholipids
3. Reduce conversion of prothrombin to thrombin, which leads to prolongation assay results

On the other hand, **anti-cardiolipin antibodies** (ACL) are IgG or IgM or IgA class of antibodies directed against a complex consisting of β-2 Glycoprotein I (β-2 GPI) bound to anionic phospholipids, cardiolipin. The anti-cardiolipin antibodies are usually detected by the ELISA - based methods and employ cardiolipin and β-2 GPI (as a cofactor) on the solid phase.

## Lupus anticoagulants V/s Anti cardiolipin antibodies

| PARAMETERS | LUPUS ANTICOAGULANTS | ANTI-CARDIOLIPIN ANTIBODIES |
|---|---|---|
| **Family** | **Anti-phospholipid** | **Anti-phospholipid** |
| **Autoantibody class** | **IgG or IgM** | **IgG or IgM or IgA** |
| **Autoantibody directed against** | **Anionic Phospholipids** | **Anionic Phospholipids** |
| **Phospholipid Binding Characteristics** | **Bind with anionic phospholipids on the prothrombinase complex** | **Bind to complex of anionic phospholipid, cardiolipin & β-2 GPI** |
| **Method of Detection** | **Phospholipid dependant coagulation assays** | **ELISA / Immunoassays** |
| **Diagnostic Relevance** | **Thrombosis, Recurrent fetal loss, Thrombocytopenia** | **Thrombosis, Recurrent fetal loss, Thrombocytopenia** |

## APS (Anti-Phospholipid Syndrome)

The term "Anti-phospholipid syndrome" means that a patient has LA and/ or ACL in addition to typical clinical symptoms associated with these antibodies.

Both LA and ACL are associated with:
1. Arterial and venous thrombosis
2. Fetal death/ recurrent abortions
3. Thrombocytopenia (decreased platelet count)

In **"Primary anti-phospholipid syndrome"**, no underlying identifiable disease is present for the thrombotic condition. In primary APS, the antibodies persist in the body regardless of therapeutic intervention.

In **"Secondary anti-phospholipid syndrome"**, an underlying disease is present such as:
- An autoimmune disorder (e.g.: SLE)
- Malignancy
- Infection (bacterial or viral)
- Inflammation
- Drugs (e.g.: chlorpromazine, procainamide, quinidine, hydralazine, interferon)

In the secondary syndrome, the APA's usually decline with successful management of the underlying disease; especially in autoimmune disease such as SLE.

## Clinical findings with LA

Patients with Lupus anticoagulants usually present one of these clinical symptoms:

| CLINICAL MANIFESTATIONS | GENERAL FINDINGS |
|---|---|
| Venous thrombosis | ● Deep venous thrombosis<br>● Pulmonary embolism |
| Arterial thrombosis | ● Myocardial infarction<br>● Gangrene<br>● Stroke |
| Thrombocytopenia | ● Reduced Platelet count |
| Repeated abortions | ● Intrauterine growth retardation<br>● Intrauterine death of fetus |
| Neurologic symptoms | ● Migrane<br>● Chorea |
| Cutaneous symptoms | ● Widespread cutaneous necrosis<br>● Distal cutaneous ischaemia<br>● Livedo reticularis<br>● Leg ulcers |

Clinically recognition of Lupus anticoagulants is important in the management of patients, with or without SLE, who experience unusual thrombotic events or thrombotic events at a younger age than expected.

In patients with SLE or related autoimmune disorders there is a definite increased incidence of thrombocytopenia associated with the presence of the Lupus anticoagulants/ anti-phospholipid antibodies.

Another major clinical manifestation associated with Lupus anticoagulant is fetal loss. Any patient with a history of recurrent first trimester abortion, second or third trimester intrauterine death, should be tested for Lupus anticoagulant . The probable mechanism by which fetal loss occurs can be attributed to thrombosis of placental vessels and placental infarction. The recurrent abortions can occur in patients with or without SLE.

## Pathophysiologic Mechanism of Thrombosis

A strong correlation exists between the occurrence of Lupus anticoagulants and high incidence of thrombotic disease. The exact pathophysiological mechanism accounting for the frequency of thrombosis in patients with Lupus anticoagulants is not clear but various data suggest that there is more than one mechanism causing thrombosis in LA patients.

The probable mechanisms of thrombosis can be attributed as follows:

1. Since a number of patients with Lupus anticoagulants and thrombosis have some degree of thrombocytopenia, platelet activation is thought to play an important role in arterial thrombosis. In patients with Lupus anticoagulants and concomittant Anti-thrombin III or Protein–C deficiency, there is an increased risk of venous thrombosis.
2. In patients with SLE, the mechanism of thrombosis is probably mediated by auto antibodies that affect the endothelial cell function.
3. In normal population, the vessel wall provides a non-thrombogenic surface, as PGI2 (Prostacyclin) secreted by the endothelial cells is a potent vasodilator and inhibits platelet aggregation. In some patients it has been observed that the Lupus anticoagulant interferes with the secretion of PGI2 from the vessel walls. Thus an impaired synthesis or deficient release of PGI2 from endothelial cells could account for an increased risk of thrombosis.
4. Other possible mechanisms by which Lupus anticoagulants might mediate thrombosis include,
   - Inhibition of Protein C activation thereby resulting in impaired degradation of Factor Va by activated Protein C
   - Decreased fibrinolytic Activity
   - Prekallikrein inhibition
   - Inhibition of Antithrombin III activity

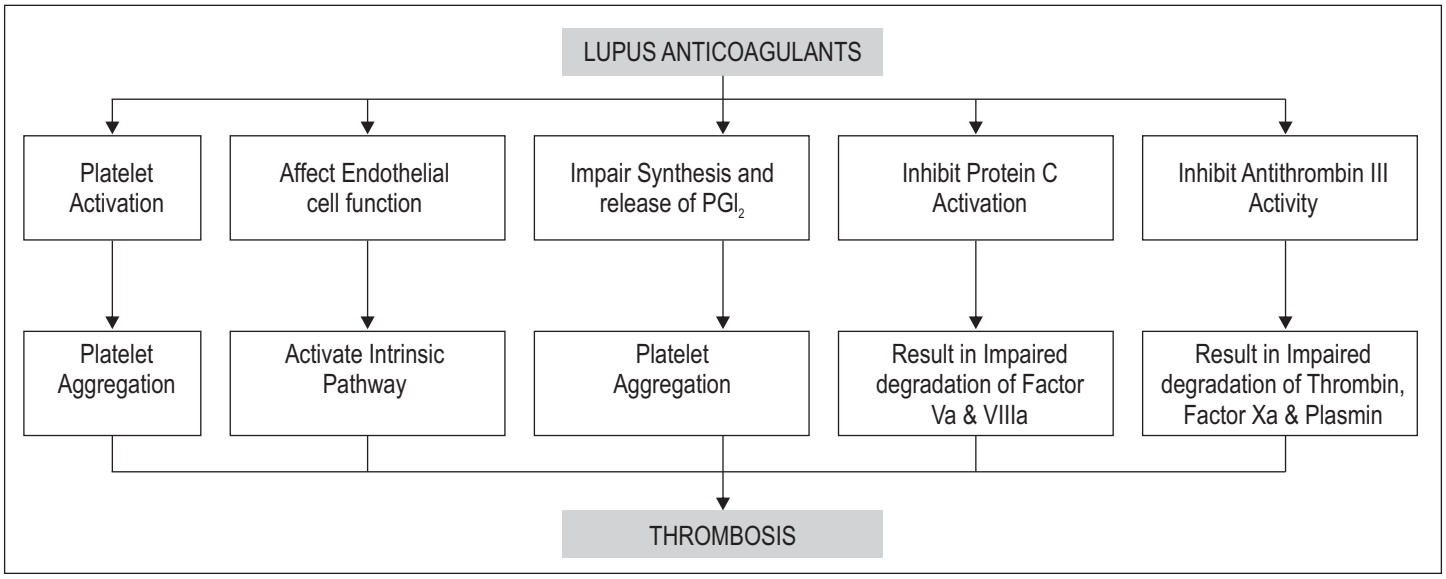


**Fig. 2. LA and Thrombosis**

## Lupus Anticoagulants and Bleeding Complications

Bleeding complications in patients with Lupus anticoagulants is a rare phenomenon. But recent reviews indicate that a subset of patients with LA were associated, interestingly, with bleeding complications. A reason for this rare phenomenon remained obscure until some workers demonstrated that LA patients with bleeding complications also showed prothrombin deficiency.

As per their observations, the probable mechanism of LA interaction with prothrombin molecule can be summarized as follows,

1. Lupus anticoagulants bind to epitope of prothrombin molecule not actively involved in the coagulation pathway.
2. The LA – prothrombin complex so formed is cleared rapidly from the circulation giving rise to prothrombin deficiency, resulting in bleeding tendency.

The above mentioned phenomenon was coined as “Hypoprothrombinemia- Lupus anticoagulant” syndrome. Studies point out that clinically it may also be important to evaluate the prothrombin time when screening patients for LA.

## Phospholipid dependence of Lupus Anticoagulants

It is now clear that Lupus anticoagulants are immunoglobulins of IgG class or IgM class or both that prolong phospholipid dependant clot based assays. The phospholipid dependence of Lupus anticoagulants in invitro assay systems has been extensively studied and convincing evidence of the same may be summarised as follows:

1. The Lupus anticoagulants react against negatively charged phospholipids and are able to inhibit the binding of Factor X and Prothrombin to the procoagulant phospholipid surface, thereby accounting for prolongation of phospholipid dependent clot based assays.
2. Reducing the phospholipid component of clotting mixture potentiates the Lupus anticoagulants effect. Thus the most sensitive tests for detecting the phospholipid inhibitor contain limited amount of phospholipids.
3. Increasing the procoagulant phospholipid in clotting mixture diminishes the effect of anticoagulant (neutralization effect) and corrects the abnormal clotting time so observed initially. This confirms the presence of phospholipid dependent Lupus anticoagulants.

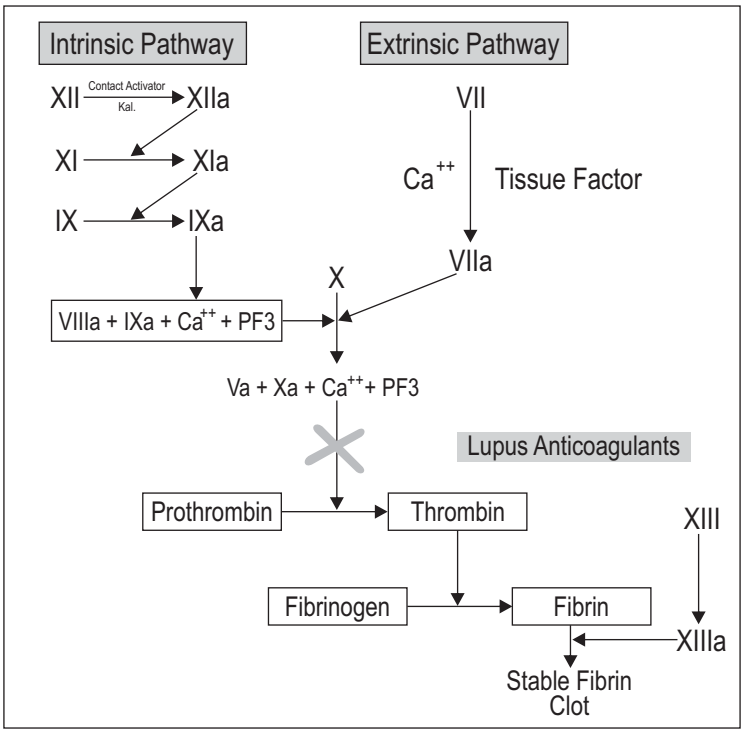


**Fig. 3. In vitro LA action**

## Laboratory Diagnosis of Lupus Anticoagulants

In the past a number of different tests have been proposed and used for the diagnosis of Lupus anticoagulants.

The traditional screening methods used for the laboratory diagnosis of Lupus anticoagulants can be broadly classified as follows:

A. Immunological assays
B. Clot based assays incorporating phospholipids in the reagent sysytem

**A. Immunological Assays**

Individuals with Lupus anticoagulants may also show the presence of other anti phospholipid antibodies. The most frequent finding is the presence of Anti- cardiolipin antibodies. The commonly employed method is the ELISA technique where the solid phase is coated with cardiolipin and b-2 GPI (as a cofactor). The ELISA method detects IgM, IgG and IgA class of anti-cardiolipin antibodies. An important point to note is that Lupus anticoagulants and anti-cardiolipin antibodies differ in their phospholipid-binding characteristic hence detection of anti cardiolipin antibodies is not specific for the presence of Lupus anticoagulants though they may be present in the same patient.

**B. Clot based assays**

**1. APTT (Activated Partial Thromboplastin Time)**

Since Lupus anticoagulants bind to the phospholipid complex they do prolong phospholipid based coagulation assays. Logically the activated partial thromboplastin time (APTT) is prolonged and this property has been used for the detection of LA using APTT reagents.

In the context of LA detection, the APTT test has certain shortcomings:

- An important variable related to the suitability of APTT reagents in the detection of Lupus anticoagulants is the composition of phospholipids used in the reagent system. Different reagents have varying sensitivity for the presence of Lupus anticoagulants on the basis of their phospholipid composition.
- The APTT test is affected by the presence of inhibitors to Factor VIII, IX and XI. Inorder to rule out the presence of inhibitors ususally mixing studies are performed. In mixing studies a 1:1 or 1:4 ( Normal plasma: Patient plasma) is used. Failure to correct the prolongation of clotting time using the mixing studies indicates the presence of Lupus anticoagulants.

- The APTT test is also the test of choice for monitoring heparin therapy. This reduces the specificity of the APTT to LA. The presence of heparin can be ruled out by using a thrombin time test. If thrombin time test shows normal values, then the sample does not contain heparin. But if the thrombin time test is abnormal, then heparin neutralization test is used which includes protamine sulphate. In this test, various concentrations of protamine sulphate are added to plasma before the addition of thrombin reagent. When protamine sulphate neutralizes all the heparin present, the clotting time reverts to normal value.

2. **TTI (Tissue Thromboplastin Inhibition test)**
   The Tissue Thromboplastin Inhibition test utilizes a diluted PT reagent. The results are expressed as ratio of patient values to normal control values.

   The TTI test is affected by numerous variables:
   - Species and tissue origin of thromboplastin can affect the test results as different sources of thromboplastin have varying sensitivity and responsiveness.
   - Choice of "Normal" reference plasma is the most critical variable, because depending on the laboratory the choice of reference plasma could be lyophilized plasma, a frozen plasma pool or fresh plasma. The ratio of patient to normal can therefore change according to the choice of "normal" plasma.
   - Some IgM Lupus anticoagulants do not prolong the TTI test.

3. **KCT (Kaolin Clotting Time)**
   KCT is similar to APTT, the difference being that KCT reagent is devoid of phospholipids and incorporates Kaolin as contact activator. The test is performed on a range of mixture of normal and patient's plasma. Different patterns of response are obtained indicating the presence of Lupus anticoagulants or the deficiency of one or more coagulation factors.

   The KCT test though sensitive is not specific for LA, additionally:
   - It cannot be automized
   - The test shows prolonged results with factor VIII, IX, XI & XII deficiency or corresponding inhibitors
   - The test is also highly sensitive for the presence of heparin.

4. **PNP (Platelet Neutralization procedure)**
   When platelets are used instead of phospholipid reagents in clotting tests, the test becomes insensitive to Lupus anticoagulants.
   The platelets have the ability to adsorb the Lupus anticoagulant and this property is used in the PNP test.
   The platelet preparations substitute the standard phospholipid extract in the dAPTT (dilute APTT) reagent.
   If a prolonged result is obtained with APTT reagent, the sample is again tested with diluted APTT reagent with platelet substitute. If the sample shows reduced clotting time then the inhibitory effect is due to Lupus anticoagulants. But if an inhibitor is directed against specific coagulation factor, the clotting time is not shortened.

   The PNP test though useful did not gain wide usage :
   - Due to limited stability the platelet preparations loose their activities on storage hence do not show reproducible results.
   - They cannot differentiate between Lupus anticoagulants and Factor VIII inhibitors.

5. **Hexagonal Phase Phospholipids**
   The hexagonal phospholipid neutralization assay is a dilute APTT reagent, which contains phospholipids in hexagonal steric configuration. The principle of this method is same as PNP test.
   The major drawback of this test being hexagonal phospholipids do not occur *in-vivo* hence the authenticity of this test principle for detecting the presence of Lupus anticoagulants has been rightly challenged.

## Current Criteria for the Diagnosis of Lupus Anticoagulants

The Lupus Anticoagulant/ Anti-phospholipid Antibody sub-committee of ISTH (International Society of Thrombosis and Haemostasis) first published its recommendations and criteria for diagnosis of Lupus anticoagulants in 1991.

Subsequently, the sub-committee further reviewed:
- Important advances in the understanding of Lupus anticoagulants
- New assays for the identification of Lupus anticoagulants
- New information concerning the technical aspects of test performance.

In 1995, the sub-committee published the revised recommendations and criteria for the laboratory diagnosis of Lupus anticoagulants.

## Current Recommendations of the ISTH

1. Both patient and normal plasma should be as platelet free as possible; the platelet count should be less than 10 x 109/l. This applies for testing on fresh plasmas, preparation of frozen samples and preparation of pooled normal plasma. Filtration appears to be an excellent method for the preparation of platelet free plasma for LA testing but may interfere with testing of other coagulation factors. Ultra- centrifugation must be performed carefully to avoid formation of platelet debris. Alternately atleast a two-step centrifugation of properly collected blood at the correct speed is also acceptable.

2. Two or more tests should be used to screen for diagnosis of LA . At least one of these tests should be based on a low phospholipid concentration,
   - KCT
   - dRVVT
   - dAPTT
   - dPT

   The assays should represent different assay principle for example, a dAPTT and a dRVVT.
3. Inhibitor activity should be documented by the effect of patient plasma on pooled normal plasma. This step can be incorporated into the initial screening procedure. The sensitivity and specificity of the ratio of patient to normal plasma should be established before it is used routinely.
4. A diagnosis of a LA should not be made on the basis of multiple abnormal screening assays and mixing studies alone. Confirmatory studies need to be performed to document the phospholipid dependence of the inhibitor.
5. Confirmatory assays should be based in the method giving an abnormal screening assay. For example if the dRVVT is abnormal, then a confirmatory assays based on the dRVVT should be used.
6. Routine clotting tests, such as PT and APTT, should be performed to evaluate the possibility of other coagulation disorders that may interfere with the LA methodology. If the chosen method for screening or confirmation is known to be sensitive to heparin, a thrombin time may be helpful in detecting its presence.
7. Solid phase assays for anti- phospholipid antibodies such as ELISA (e.g. anti- cardiolipin antibodies) although frequently positive in patients with LA's should not be considered as a confirmatory procedure for LA activity.
8. Factor assays should be performed whenever there is suspicion of a specific factor deficiency or inhibitor. Two or more dilutions of the patient plasma should be evaluated in any factor assay.
9. The term Lupus anticoagulant should be retained until the pathophysiology of these inhibitors is more fully delineated.

Based on these recommendations, a flow chart (Fig.: 4) was established thereby assisting the laboratorians to establish a routine approach in the diagnosis of LA's and improve testing standards in the detection of LA's.

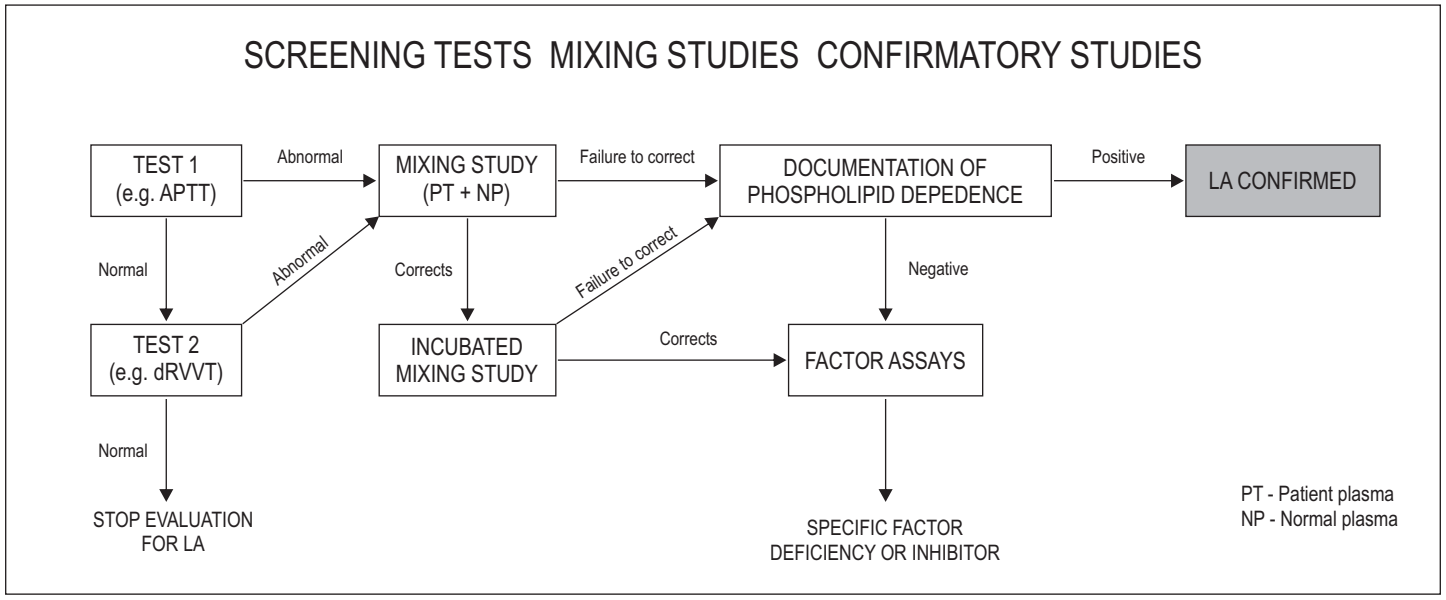


**Fig. 4. Flow Chart for laboratory diagnosis of LA**

## Scientific Sub - comittee criteria for the laboratory diagnosis of Lupus anticoagulants

In order to make a diagnosis of LA; sample should show each of the following:

1. Prolongation of at least one phospholipid – dependent clotting assay.
2. Evidence of inhibitory activity shown by the effect of patient plasma on pooled normal plasma.
3. Evidence that the inhibitory activity is dependent on concentration of phospholipid. This may be achieved by addition or alteration of phospholipids/ hexagonal phase phospholipids/ platelet/ platelet vesicles in the test system.
4. LA's must be carefully distinguished from other coagulopathies that may give similar laboratory results or may occur concurrently with LA's. Specific factor assays and the clinical history may be helpful in differentiating LA's from these other possibilities.

## dRVVT : The test of choice for screening and confirmation of LA

The dilute Russell's viper venom time test (dRVVT) first became popular following publication by Thiagarajan et al in 1986. Dr. Exner further simplified and standardized this method subsequently.

In general dRVVT based tests comprise of:

- SCREENING REAGENT, containing limited amount of phospholipids with RVV (Russell's Viper Venom)
- CONFIRMATION REAGENT, containing additional phospholipids with same amount of Russell's Viper Venom, to confirm the presence of phospholipid dependent Lupus anticoagulants.

## Principle of dRVVT for LA detection

Russell's Viper Venom directly activates Factor V and X in 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 V and 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 SCREENING reagent. The CONFIRMATION reagent incorporates additional phospholipids to neutralize the LA. Once LA are neutralized clot formation proceeds relatively uninterrupted achieving a lower clotting time, to prove the phospholipid dependence of the autoantibodies.

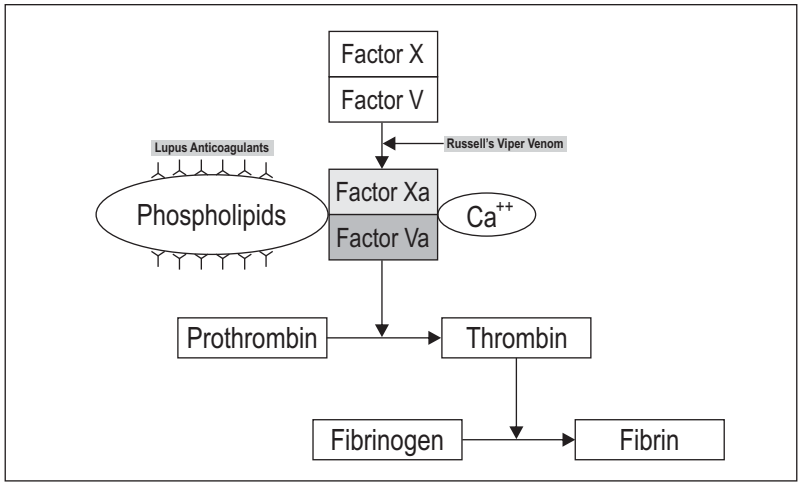


**Fig. 5. Principle of the dRVVT Test**

## Interpretation of results with dRVVT test

If SCREEN TIME is prolonged, to confirm the presence of lupus anticoagulants the plasma sample is tested with CONFIRMATION REAGENT.

If CONFIRM TIME results shows a lower clotting time as compared to SCREEN TIME, it indicates the presence of phospholipid dependant Lupus Anticoagulants.

Also the results can be expressed as ratio,

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

The results expressed, as ratio is further useful in classifying the patient as normal, moderate, high and very high LA.

If results of the ratio are borderline, mixing studies may be done further with the sample specimen. The mixing studies should be carried out with a 50:50 mixture of test plasma and normal plasma.

## dRVVT as positive predictor of thrombotic events in APS

As suggested by ISTH in the criteria for the diagnosis of LA, the sample should show prolongation of results with phospholipid dependent clot based assays. Also, the inhibitory activity should be evident by altering the phospholipid concentration to confirm the phospholipid dependence of LA.

The dRVVT test is universally being accepted as the coagulation assay of choice for LA detection because the assay incorporates, SCREENING and CONFIRMATION reagent thereby,

- Indicating the phospholipid dependence of LA
- Achieving maximum sensitivity for the presence of LA's.

In an independent study conducted, an important information with respect to the positive predictive value (PPV) of dRVVT test viz. a viz. ACL-ELISA in presumed thrombotic events in APS was revealed. As per their findings, dRVVT proved to be more specific test (100%) for presumed thrombotic events, miscarriage and pulmonary hypertension than ACL-ELISA method (83%) for the presence of LA in APS.

## Therapeutic measures for patients with LA

For the therapeutic management of patients with Lupus anticoagulant, different regimens in uncontrolled trials has resulted some success in treatment. However no random prospective trial has been conducted in LA patients to determine the optimum therapeutic regimen.

Therapeutic measures for LA patients can be broadly classified as,

1. Management of patients with Primary Anti-phospholipid Antibody syndrome
2. Management of patients with Secondary Anti-phospholipid Antibody syndrome

**1. Management of patients with Primary 'APS'**

- When the Lupus anticoagulant is associated with evidence of severe prothrombin deficiencies or thrombocytopenia treatment with adrenal corticosteroids is indicated.
- Presence of Lupus anticoagulant and thrombosis is guided by the fact that recurrence is common. Hence for patients presenting with venous thrombosis long term anticoagulation (probably for life) with oral anticoagulants is indicated. For arterial thrombosis low dose aspirin is indicated.
- In management of pregnant patients with or without SLE and Lupus anticoagulants the exact regimen to be used till date is not clear. Initially some workers reported successful pregnancy out comes in patients with LA by treating with prednisolone and low dose aspirin. However long term, high dose corticosteroids during pregnancy are associated with significant side effects namely
  - severe preeclampsia
  - infections
  - gestational diabetes
  - osteoporosis.

  Recently some workers have suggested low dose aspirin and low dose heparin with some success in pregnancy outcomes. Thus, different therapeutic regimens in uncontrolled trials have resulted in some success, but no careful randomized trials have yet been conducted to arrive at an optimum and safe therapeutic regimen that can be universally adapted.

**2. Management of patients with Secondary 'APS'**

- When the Lupus anticoagulant is present in patients with an underlying autoimmune disease, management with appropriate immunosuppressive therapy is indicated.

## References and Suggested readings


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