

## LUPUS ANTICOAGULANT

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## What is a Lupus Anticoagulant ??

Why Test for it?

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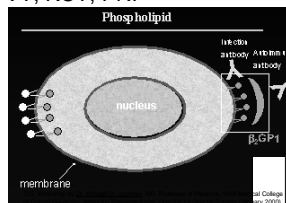
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## Lupus Anticoagulant

•Defined as immunoglobulins which *inhibit* phospholipid-dependent coagulation tests

PT, APTT, dRVVT, KCT, PNP



Triplett, Lupus Anticoagulants: Diagnosis and Management, Current Hematology Reports 2:271-272, 2003.

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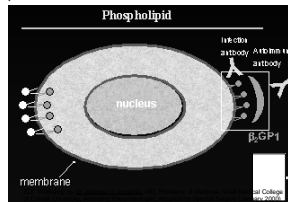
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**Lupus Anticoagulant**

- Defined as immunoglobulins which *inhibit* phospholipid-dependent coagulation tests  
PT, APTT, KCT, dRVVT
- Heterogeneous
- Non-specific



Triplett, Lupus Anticoagulants: Diagnosis and Management, Current Hematology Reports 2:271-272, 2003.

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**Terminology**

	Term	Definition
LA	Lupus Anticoagulant	aPL identified by <i>in vitro</i> phospholipid-dependent clot-based assays; antibodies are against β2GP1 or Prothrombin
aPL	Antiphospholipid Antibodies	IgG, IgM, or IgA antibodies that are directed against target proteins, such as cardiolipin, β2GP1 or Prothrombin, which binds to phospholipids.

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**A True Misnomer .....**

“Lupus Anticoagulant”, i.e. LAC  
 Not a true circulating “anticoagulant”  
*In-vitro* clotting assays are prolonged

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**A True Misnomer .....**

“Lupus Anticoagulant”, i.e. LAC

Not a true circulating “anticoagulant”

*In-vitro* clotting assays are prolonged

Affects the body’s thrombotic (clotting) system

*In vivo*, LAC can be a risk factor for a potential clot in the body (Thrombotic event)

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**Clinical Significance**

Lupus anticoagulants are associated with thrombotic occurrences

- DVT
- PE
- Arterial occlusion
- Pregnancy loss
- Micro vascular thrombi



Triplet, Lupus Anticoagulants: Diagnosis and Management, Current Hematology Reports 2:271-272, 2003.

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**Lupus Anticoagulant**

Autoimmune

- May occur in patients with autoimmune disorders (e.g. HIV infection)
- 20 - 45% incidence seen in SLE patients

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## Lupus Anticoagulant

- Acquired
  - Drug induced:
    - Procainamide, hydralazine, isoniazid, dilantin, phenothiazines, quinidine, and ACE inhibitors
    - Issues at Mental Institutions
  - Infection (bacterial or viral)
    - Kids/Ears
    - Canceled Surgeries

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## Antiphospholipid Syndrome

- Definition:
  - Occurrence of recurrent venous and arterial thrombosis, or recurrent miscarriage or fetal loss associated with the laboratory evidence of antiphospholipid antibodies. (aPL)
  - aPL directed against plasma proteins bound to anionic PL, not against the PL alone
  - The important antigens for aPL are B2-glycoprotein I and prothrombin

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## What is Lupus Anticoagulant?

- Group of immunoglobulins(antibodies) consisting of IgG, IgM, or IgA which interfere with one or more of the in vitro phospholipid dependent steps of coagulation resulting in prolonged results
- LAC is one of the signs of Antiphospholipid Syndrome, not itself a disease

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### History of LAC

- First reported in 1952 with SLE
- 1963 association with thromboses
- 1972 named by Feinstein and Rapaport
- 1975 association with recurrent spontaneous abortions
- Patients exhibited prolonged APTT, leading to the term "Lupus Anticoagulant"
- Extremely heterogeneous set of symptoms and lab results

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### Classification of LAC

- Autoimmune
  - SLE
  - Other connective tissue disease (RA)
  - Primary Antiphospholipid Antibody Syndrome
  - Drugs (procainamide, quinidine)
- Alloimmune
  - post infections (transient)...common in children, AIDS patients
  - malignancies

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### Why detect LA?

- Recurrent spontaneous abortions
  - 15-20% RSA due to APA
- Intrauterine death in the second and third trimesters
- Fetal growth retardation
- Prematurity
- Neonatal thrombosis

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### Why detect LA?

- Most common cause of acquired thrombophilia
  - Arterial:
    - cerebral - account for 30% of strokes under 50
    - coronary & post coronary artery bypass
    - retinal
  - Venous:
    - DVT & PE
    - Renal vein
    - Mesenteric vein

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### Incidence

- LA positive 8.5-10% of DVT
- LA accounts for 30,000 DVT/yr in N.A.

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### Who/why should we be testing for LA?

*Low:* VTE or arterial thromboembolism in elderly patients.

*Moderate:* Accidentally found prolonged aPTT in asymptomatic subjects, early pregnancy loss, provoked VTE in young patients

*High:* Unprovoked VTE and (unexplained) arterial thrombosis in young patients (<50 years of age), thrombosis in unusual sites, late pregnancy loss, any thrombosis or pregnancy morbidity in pts with autoimmune diseases.



ISTH/SSC 2008 Minutes

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# The ISTH/SSC Recommendations for Lupus Testing



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Thrombosis and Haemostasis © F. K. Schmitt: Verlagsgesellschaft mbH (Steiger) 74 (4) 1183-90 (1995)

Scientific and Standardization Committee Communications

## Criteria for the Diagnosis of Lupus Anticoagulants: An Update On behalf of the Subcommittee on Lupus Anticoagulant/Antiphospholipid Antibody of the Scientific and Standardisation Committee of the ISTH

John T. Brandt<sup>1,2,3</sup>, Douglas A. Triplett<sup>1</sup>, Barbara Alving<sup>2</sup>, Inge Scharer<sup>3</sup>

From the Dep. of Pathology, Ohio State University, Columbus, Ohio, USA; <sup>1</sup>Dep. of Pathology, Ball Memorial Hospital, Muncie, Indiana, USA; <sup>2</sup>Dep. of Hematology, Walter Reed Army Research Institute, Washington, D. C., USA; <sup>3</sup>Dep. of Internal Medicine, University Hospital, Frankfurt/Main, Germany

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## ISTH/SSC Recommendations

### Diagnosis of Lupus Anticoagulant

1. A prolonged screening assay which is phospholipid dependent (low phospholipid conc)
2. Lack of correction in mixing study, using normal plasma (pooled)
3. Prove "inhibitor" is phospholipid-dependent (high phospholipid conc.)
4. Rule out other coagulopathies

SSC Subcommittee on Lupus Anticoagulants/Phospholipid Antibodies:  
Brandt JT, Triplett DA, Alving B, Scharer I, 1995.

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## ISTH/SSC Recommendations

### **Diagnosis of Lupus Anticoagulant**

Screen with at least 2 *different* assay principles for negative confirmation (both negative).

LAs. Therefore, more than one screening assay must be performed before the presence of a LA can be ruled out. To enhance the detection of LAs, at least two different types of assays should be used. For example, the combination of an APTT and a dAPTT may not be adequate whereas the combination of a dAPTT and dRVVT may be adequate.

SSC Subcommittee on Lupus Anticoagulants/Phospholipid Antibodies:  
Brandt JT, Triplett DA, Alving B, Scharrer J, 1995.

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## ISTH/SSC 2008 Minutes

The subcommittee recommends performing a dRVVT assay due to its high sensitivity and its relative low variability, also when reagents from different suppliers are used. The subcommittee recommends the use of an APTT as the second test to detect lupus anticoagulant.

The APTT, if performed with silica as activator and low phospholipids content, is the second test of choice because it is sensitive for LA and its CV is within acceptable limits.



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## Testing for LA-Centrifugation

- Obtain platelet poor plasma
  - Less than 10,000/mm<sup>3</sup> (prefer <5,000)
- Spin >1500G's for 15 minutes
  - NCCLS H21-A3
- Do not use fixed-head rotors
  - NCCLS H21-A3

The more platelet free the sample, the greater the sensitivity of most tests to the presence of LA.

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**Screening Tests for LAC**

- APTT - very reagent dependent, use sensitive reagent for screening
- dRVVT - variable depending on source of phospholipid, can also be used as a confirmatory test by adding phospholipid
- Kaolin Clotting Time - exquisitely sensitive to residual platelets, requires a lot of plasma, laborious technique

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**Screening reagents:**

- The most common Lupus sensitive reagents are:
  - IL APTT-SP
  - OT Auto APTT
  - Actin FSL
  - Hemoliance SynthAFax & SynthASil

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**Testing for LA**

- Ruling Out Heparin:
- Thrombin Time/Reptilase Time
  - Heparin neutralizing products
- Factor Assays:
- R/O factor deficiency
  - Look for parallelism

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### Establishing an Inhibitor

- Mixing Studies
  - The purpose of mixing studies is to differentiate between a factor deficiency and the presence of an inhibitor
- Source of normal PPP critical\*\*\*platelets
- Set up your own criteria for mixing study decisions. Most have a cutoff 5 seconds above the value obtained for the PPP.

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### Is it Inhibitor or Factor Deficiency?

- 1:1 mix with normal PPP
- 4:1 mix with normal PPP
- Do aPTT and compare to aPTT on PPP
- Time dependency (immediate & after 1-2 hrs incubation at 37°C)
- Patient:           45           45
- 1:1                 36           32
- PPP:             30           30
- Likely Dx:       Inh           Factor Def

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### Is it Inhibitor or Factor Deficiency?

- Factor Assay Parallelism
  - do at least 3 dilutions on the patient plasma
  - plot against the standard curve for the factor
  - if lines are parallel, then LAC unlikely or very weak
  - if patient line crosses std line, possible LAC inhibitor present
- Preferentially affects contact factors but can affect other factors
- Only report results from the parallel part of the curve

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**Is It Specific Inhibitor?**

- Look at parallelism - specific inhibitor will have line parallel to the standard line
- If LAC is too strong and interferes with Factor VIII or IX assays, use chromogenic assay
- Some hemophiliacs (particularly those with active infections) may have both Factor Deficiency and LAC, but not a specific factor inhibitor

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**Is Inhibitor Phospholipid Dependent?**

- According to the ISTH Standardization Committee, at least two different methodologies are required to confirm the presence of LAC
  - Decrease amount of PL to accentuate the inhibitor
  - Increase amount of PL to inhibit or bypass the LAC
  - Test for the altered configuration of PL
  - Snake venom assay systems

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**Decrease Amount of PL**

- Tissue Thromboplastin Inhibition (TTI)
  - Diluted prothrombin reagent plus extra calcium...very sensitive but non-specific, 30% of normals will be positive
- Dilute Russell Viper Venom Time(dRVVT)
  - Initiates coagulation by activating factor X and utilizes a dilute source of phospholipid which will further prolong the clotting time in the presence of LA. It can have a confirmatory test by the addition of extra PL. (IL Test LAC Screen and LAC Confirm)

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### Increase Amount of PL

- Platelet Neutralization Procedure (PNP)
  - Increases the amount of phospholipid which neutralizes the LA and corrects the clotting time - used as confirm test when sensitive APTT is the screening test
- Rabbit Brain Neutralization Procedure
- Phosphatidylserine Liposomes
- Platelet Derived Vesicles

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### Other Methods

- Altered Configuration of Phospholipids
  - Hexagonal Phase Phosphatidylethanolamine
- Snake Venom Assay Systems
  - Textarin/Ecarin Ratio
    - ecarin snake venom which activates prothrombin in absence of PL and calcium ions
  - Taipan Venom Time
    - similar to dRVVT, uses a dilute PL reagent and a platelet neutralization procedure but with snake venom which activates prothrombin directly without needing Factors V or X.

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### Associated disease states of Lupus Anticoagulant:

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|-----------------------------------|------------------------------|
| • Cardiovascular:                 | • Immunologic:               |
| Myocardial Infarction             | AIDS                         |
| Peripheral Vascular disease       | Autoimmune Hemolytic-Anemia  |
| Rheumatic Heart disease           | Pulmonary Vasculitis         |
| • Gynecologic:                    | Rheumatoid Arthritis         |
| Eclampsia                         | Systemic Lupus Erythematosus |
| Elective abortion                 | Immune Thrombocytopenia      |
| Fibrocystic disease of the breast | Behcet's syndrome            |
| Multiple miscarriages             | Kawasaki disease             |
|                                   | Viral Infections             |

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**Associated disease states of Lupus  
Anticoagulant:**

- **Neurologic:**  
Acute Guillian-Barre syndrome  
Hydrocephalus  
Acromegaly  
Chorea  
Stroke
- **Urologic:**  
Benign Prostatic Hypertrophy
- **Oncologic:**  
Adenocarcinoma  
Multiple Myeloma  
Prostatic cancer  
MyeloFibrosis  
Hairy Cell Leukemia  
Esophageal cancer  
Hodgkin/non-Hodgkin lymphomas  
Cervical cancer  
CML  
CLL

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**Associated disease states of Lupus  
Anticoagulant:**

- **Other:**  
Von Willebrand's disease  
Gaucher disease  
Liver Cirrhosis  
Drug Induced Lupus  
Infections  
Epithelial malignancies

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**Transient LA:**

- **DRUG EXPOSURE**
  - Chlorpromazine
  - Procainamide
  - Hydralazine
  - Quinidine
  - Antibiotics
  - Phenytoin
- **INFECTION**
  - Bacteria
  - Protozoan
    - Pneumocystis carinii
  - Viral

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**Treatment for LA:**

- Heparin / Oral Anticoagulant
- Low dose aspirin (75mg/day) with or without Prednisone (40-60mg/day)
- Low dose aspirin with LMWH with or without IVIG
- Treat underlying disorder (infection, lymphoma, change drugs, etc)

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**Results Post-Treatment**

- Post heparin...immediate increase in aPTT, slowly declining until next dose(std unfract hep)
- If aPTT was originally high, may have to monitor with heparin assay, at least until appropriate aPTT level is attained
- LMWH....no affect on aPTT, must monitor with Anti-Xa Heparin assay
- Coumadin will affect both PT and aPTT when therapeutic

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**Results Post-Treatment**

- Low dose aspirin with LMWH and IVIG has achieved a higher rate of live newborns but with a high rate of severe side effects (preeclampsia, preterm delivery, low birth weight)

Von Tempelhoff, G.F. Thromb and Haemost, Supplement July 2001

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**Risk of Thrombosis**

- Increased risk:
  - Hyperhomocysteinemia with aPL
  - aCL IgG and /or IgM >40 U/mL with positive dRVVT
- No increased risk:
  - Abn APCR with aPL
  - aCL IgG or IgM high with negative dRVVT

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**Low Risk of Thrombosis w/ LAC**

- ICU
  - Transient rise of aPL is frequent
  - Not associated with thrombosis/bleeding
  - Sepsis and/or catecholamine treatment shows strong association with LAC
  - Probability of spontaneous disappearance 100% at 4 weeks
  - Overall mortality not associated with LAC

Wenzel, C. Thromb and Haemost, Supplement July 2001

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**Low Risk of Thrombosis w/ LAC**

- Haematological Disorders
  - Leukemia, lymphoma, myeloma, myelodysplastic syndrome, myeloproliferative syndrome
  - No association between presence of LAC and development of thrombosis

Grosso, S. Thromb and Haemost, Supplement July 2001

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**Summary**

- LACs are Heterogeneous
- No single test that is 100% sensitive or specific
- Increasing evidence for LAC/aCL having a causative role in thromboembolic events
- Transient LAC are not associated with clinical complications
- LAC and aCL are members of the APA family but they are not the same antibodies

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**Summary**

- Antigenic targets for APA are a variety of protein/phospholipid complexes
- LAC/aCL are associated with a variety of maternal and fetal complications
- Lab diagnosis requires an organized sequential approach
- Repeat testing is required to demonstrate persistent LAC or transient

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**4 Step Approach**

- Screening tests
- Is it an Inhibitor?
- Is the Inhibitor Phospholipid Dependent?
- Rule Out Other Coagulopathies or Heparin

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**Any Questions???**



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