LA-APL: Testing Guidelines Update

Disclosures (Nichols)

• Relevant Financial Relationships: NONE

• Off-Label Rx Usage: NONE
LA-APL: Testing Guidelines Update

• **Overview**
  - Synoptic review: LA, APL Antibodies, Antiphospholipid Syndrome
    - Terminology, Pathophysiology, Clinical Significance/Implications
  - Comparative review of selected features of newer (and older) LA-APL testing guidelines

• **Objectives**
  - Compare recent LA-APL testing guidelines
  - Recall cardinal criteria for LA diagnosis
  - Describe screen, mix, confirmatory, and exclusionary LA testing
  - Discuss specific LA testing recommendations
  - Summarize APL Antibody serologic testing recommendations
# LA & APL Antibodies and Syndrome

## TERMINOLOGY

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<th>Term</th>
<th>Name</th>
<th>Definition</th>
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<td>Antiphospholipid antibodies</td>
<td>Antibodies to phospholipid and protein cofactor neo-antigens (β₂-GPI, prothrombin, etc.)</td>
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<td>LA</td>
<td>Lupus anticoagulant</td>
<td>APL antibodies, detected &amp; confirmed by phospholipid-dependent clot-based functional testing &amp; qualitative interpretation</td>
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<td>ACL</td>
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<td>APL antibodies (IgG, IgM) to cardiolipin (+β₂-GPI), detected and quantitated by immunoassay (ELISA)</td>
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<td>APS</td>
<td>Antiphospholipid syndrome</td>
<td>APL antibodies (persistent) and thrombosis or recurrent fetal loss (not otherwise explained)</td>
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LA & APL Antibodies / Syndrome

SIGNIFICANCE

• Thrombosis risk
  • LA > ACL IgG > ACL IgM*
  • LA + IgG ACL (β2-GPI) >> LA alone** (double positive)
  • LA + ACL + β2-GPI >> single/double*** (triple positive)

• Pregnancy loss or complications
  • LA > (or =?) ACL/ β2-GPI IgG
  • LA + ACL &/or β2-GPI IgG >> ACL/β2-GPI alone****

• Bleeding risk
  • LA / hypoprothrombinemia (or F X def.)
  • Thrombocytopenia (AITP)
  • Anticoagulation -related bleeding

• Asymptomatic

LA & APL Antibodies / Syndrome

Pathophysiology (Multiple mechanisms suggested / identified)

- Antibody inhibition of natural anticoagulant pathways (PL-dependent assembly):
  - Proteins C &/or S (APC inactivation of Factors Va & VIIIa)
  - Tissue factor pathway inhibitor (TFPI-TF-VIIa -> Xa inhibition)
  - Protein Z - ZPI complex (Factor Xa inhibition)

- Down-regulation of physiological B₂-GPI functions

- Disruption of protective Annexin V binding to anionic phospholipids (Rand JH. *Thromb Res* 2004; deLaat B. *Blood* 2007)

- Dysregulation of Fibrinolysis (inhibition)

- Platelet activation; enhanced thrombin generation

- Endothelial cell activation or injury
Beta$_2$-Glycoprotein I (β$_2$-GPI)

- Principal antigen for APL Abs (& LA activity)
- Abundant plasma protein (~4 µMol/L; ~150-200 mg/L)
- Domain V binds PL; APL Abs target domain I
- Function(s) include anticoagulant activities:
  - inhibiting VWF binding to platelet GP Ib-IX complex
  - enhancing TFPI down-regulation of factor VIIa (TF-TFPI)
  - enhancing APC-PS down-regulation of factor Va
  - inhibiting factor XI activation by thrombin
  - promoting fibrinolysis
- APL antibodies may impair 1 or more functions
- APL Abs with β$_2$-GPI specificity may confer greater thrombosis risk than do other APL Abs
- β$_2$-GPI deficiency: no thrombosis (humans); mice can have provoked fetal loss (homozygous deletion)

Miyakis *Thromb Res* 2004 (114:335); Giannakopoulos *Blood* 2007 (109:422)
Lupus Anticoagulant (LA) 
SUMMARY

• Lupus anticoagulants (LA) are “anti-phospholipid” antibodies (IgG, IgM isotypes)

• Directed against antigens comprised of a combination of anionic (negatively charged) phospholipids and a protein cofactor (eg, prothrombin or β₂-glycoprotein I [β₂-GPI])

• Inhibit in vitro phospholipid-dependent clotting time tests (eg, APTT, DRVVT, KCT, DPT, PCT [PRP recalcification])

• “LA” is a:
  • Misnomer: most patients do not have SLE
  • Paradox: procoagulant, not anticoagulant
  • Riddle: prothrombotic mechanisms not clear
  • Epiphenomenon?

DA Triplett & JT Brandt 1988 Hematol Pathol 2(3):121-43
Antiphospholipid Antibodies (APL) CLASSIFICATION

• Autoantibodies (persistent)
  • Primary APL/APS (no other autoimmune Dz)
  • Secondary APL/APS (eg, SLE or other AI Dz)
  • “Catastrophic APS” (acute multifocal thrombi)

• Alloantibodies (transient)
  • Infections
  • Inflammation

• Drug-induced APL Antibodies (& DI-SLE)
  • Procainamide, Quinidine, Quinine
  • Phenothiazines
  • Hydralazine
  • Antibiotics (quinolones, others)
  • Other Rxs
Antiphospholipid (APL) Antibodies
CLASSIFICATION

• Lupus Anticoagulants (LA)
  • Identified by functional coagulation testing
  • Qualitative, interpretive

• Anticardiolipin Antibodies (ACL)
  • Identified by immunoassays (eg, ELISA)
  • IgG, IgM isotyping & quantitative titering
  • Supplemental immunoassays for antibodies to β₂-GPI component

• LA & ACL/β₂-GPI testing must both be performed to identify or exclude APL antibodies
  • LA +, ACL/β₂-GPI +
  • LA -, ACL/β₂-GPI +
  • LA +, ACL/β₂-GPI -
  • LA -, ACL/β₂-GPI -
When should one test for antiphospholipid antibodies?
Suspicion of APS (&/or unexplained prolonged APTT)

- **Clinical Criteria**
  - Vascular Thrombosis (Arterial, Venous) &/or
  - Pregnancy Morbidity
    - ≥ 1 fetal death ≥ 10 weeks
    - ≥ 3 consecutive spontaneous abortions <10 weeks
    - ≥ 1 premature birth ≤ 34 weeks
    - Placental insufficiency, Severe pre-eclampsia

- **Laboratory Criteria** (persistent ≥ 12 wks)
  - ACL / β2-GPI Abs (IgG, IgM) mod.- high titer &/or
  - Lupus anticoagulant (by ISTH criteria)

- 1 clinical + 1 lab criterion fulfills classification definition

*Clinical events not otherwise readily explained

APS Non-Criteria Clinical Manifestations

• Cardiac valve lesions (vegetations, thickening)
• Thrombocytopenia (immune-mediated: AITP)
• Livedo reticularis & other skin manifestations
• Non-thrombotic neurologic manifestations, including cognitive dysfunction, chorea
• Small artery vasculopathy/vasculitis (APL-associated nephropathy, etc.)
• Thrombotic microangiopathic syndromes (TTP, HUS, HELLP, etc.)
Antiphospholipid Syndrome (APS)
Non-Criteria Laboratory Findings

- Low titer IgG or IgM ACL (<40 GPL or MPL)
- Low titer anti-β₂GPI antibodies (no standards)
- IgA ACL or β₂-GPI antibodies
- Anti-Prothrombin (F II) antibodies
- Anti-Phosphatidylserine (PS) antibodies
- Anti-Phosphatidylethanolamine (PE) antibodies
- Anti-Annexin A5 antibodies
- Transient LA &/or ACL/β₂-GPI antibodies
LA & APL Antibodies / Syndrome
Diagnostic Guidelines - Publications

**LA testing guidelines (criteria, recommendations)**
- **CAP (2002):** Triplett DA. Arch Pathol Lab Med 126:1424
- **CLSI/NCCLS (4-2014):** H-60A (www.clsi.org)

**APS classification criteria**
- **Wilson WA et al. (1999):** Arthritis Rheum 42:1309
- **Miyakis S et al. (2006):** J Thromb Haemost 4:295

- All of the above are primarily based on expert opinions together with results of limited studies; evidence-based recommendations are lacking (as are standards).
Lupus Anticoagulant (LA)
ISTH Diagnostic Criteria (1995 & 2009) - Summarized

1. **Prolongation** of at least 1 phospholipid-dependent clotting time assay

2. **Inhibition** shown by mixing patient and normal pooled plasma

3. **Phospholipid-dependent** inhibition demonstrated

4. Evaluate for other coagulopathies that may give similar results and/or confound LA diagnosis
   - Anticoagulants - warfarin, heparin, DOACs (DTIs, anti-Xa)
   - Factor inhibitor or deficiency
   - PT & TT screening and reflexive specific factor assays may be useful

Lupus Anticoagulant (LA)

**Other ISTH Diagnostic Recommendations**

- Testing for LA should be limited to patients who have a significant probability of antiphospholipid syndrome (APS), or who have unexplained APTT prolongation.

- Two (or more?) tests, based on different principles (eg, APTT, DRVVT) should be used to screen for LA.

- Results should be reported as ratios of patient to normal pooled plasma - for screen, mix and confirm.

- An interpretive report with quantitative results and explanation should be provided.

- LA positivity should be retested @ ≥12 weeks.

- Solid phase assays for APL antibodies (eg, ACL Abs &/or β₂-GPI Abs) should not be considered as confirmatory for LA (but should be performed).

Clinical Laboratory Standards Institute (CLSI)
H60-A: LA Testing Guideline

Timeline

- January 2010: Call for subcommittee member nominations
  24 members from 7 countries representing academia, reference &
  hospital laboratories, EQA programs, industry, and government
  Includes ISTH-SSC guideline authors: Exner (1991), Brandt (1995), de
  Groot and Ortel (2009); and BCSH author: Moore (2012)


Goals

• Continue to build upon previous global initiatives and also harmonize with
  and add clarity to current guidelines
• Present information in a succinct, practical, and easy to understand format
CLSI H60-A LA Testing Guideline

- **Document Development Committee Members**
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CLSI H60-A LA Testing Guideline

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(October 2010 - Atlanta attendees)

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Not Shown: Moore, Depasse, Exner, Favaloro, Hollensead, Meijer, Moffat, Ortel, Sanfelippo, Scott-Grillo, Stang, Thiagarajan, Van Cott
In order to make a diagnosis of LA, a sample should identify with the following:

A. PROCUREMENT: adherence to standardized protocols for collection and processing of blood to be used for testing

B. SCREENING: prolongation of at least one of two different phospholipid-dependent clotting assays based on different principles and coagulation pathways

C. CONFIRMATION: evidence that prolongation of the screening test(s) demonstrates phospholipid dependence by using a similar second test(s) using altered concentrations and/or composition of phospholipids

D. MIXING: if mixing assays are performed, evidence of inhibitory activity shown by the effect of patient plasma on an equal volume of normal pooled plasma

E. EXCLUSION: distinguish LA from other causes of prolonged clotting times that may mask, mimic, or coexist with LA, such as anticoagulant therapies or other coagulopathies

F. REPORTING & INTERPRETATION: numerical results of all testing should be reported, and interpretive comments that address and integrate these results should be provided
CLSI H60 Recommendations (selected) for LA Diagnosis

A. PROCUREMENT: 1. Testing should preferably be performed in the absence of anticoagulant therapy

B. SCREENING: 1. Two tests (LA-responsive), representing different principles/pathways
   2. APTT & dRVVT recommended as preferred minimal screening
   3. Other tests for LA referenced in this document may supplement the preferred minimal screening test
   4. Where test design permits, results should be calculated using the mean of the reference range and reported as a normalized ratio
   5. Routine tests (PT, APTT & TT) as indicated may help characterize anticoagulant effects or sample suitability for LA testing / interpreting

C. CONFIRMATION:
   1. Use same assay principle as the abnormal screening test (eg, dRVVT)
   2. For paired tests, results should be ... reported as a normalized screen to confirm ratio
   3. Solid phase immunoassays for antibodies to phospholipid (eg, aCL, aβ2-GPI) should not be considered as LA confirmatory procedures
**CLSI H60 Recommendations (selected) for LA Diagnosis**

**D. MIXING:**

3. **The dilution effect of a 1:1 mixing test may mask LA inhibitory activity.** Other mix ratios (e.g., 4:1 patient:pool) may be used if validated.

4. Mixing test inhibition is assessed by either comparison of normalized ratios to cut-off values specific for each screen or confirm mixing test or calculating an Index of Circulating Anticoagulant (ICA).

5. **Incubated mixing tests are not recommended for routine LA testing, but should be performed when indicated** (e.g., when a specific factor inhibitor is suspected).

**E. EXCLUSION:**

1. LA should be distinguished from anticoagulant therapies and/or other coagulation disorders which may interfere with LA testing / interpreting.

2. Factor assays should be performed whenever there is suspicion of a specific factor deficiency or inhibitor, using 3 or more dilutions...
CLSI H60 Recommendations (selected) for LA Diagnosis

F. REPORTING & INTERPRETATION:

1. Numerical results of all testing should be reported with reference interval or cut-off values.

2. Interpretive comments that address and integrate all test results (LA panel) should be provided.

3. The interpretive report should indicate whether LA is detected, not detected, or indeterminant.

4. Solid phase assays for PL-dependent antibodies (eg, aCL and/or αβ₂-GPI) are recommended as part of an evaluation for antiphospholipid syndrome (APS).

5. If LA is present, the test panel should be repeated at or beyond 12 weeks to determine persistence of LA as part of the evaluation for LA.
CLSI H60 **Recommendations** (other) for LA Diagnosis

**Establishment of Reference Intervals & Cut-off Values**

1. Follow guidelines in CLSI C28-A3

2. +/- 2 SD RI recommended rather than +/- 3SD (requires ≥40 normals rather than ≥120 normals)

3. Although 2SD is less stringent, for LA testing it is the composite LA testing (Screen + Confirm + Mix) results that collectively increase specificity of LA diagnosis

**Patient Selection**

**Timing of LA Testing**

**Alternative LA Testing (supplementing DRVVT, APTT)**

DPT, KCT, PNP, Others - permitted if validated by laboratory
CLSI H60-A: LA Guidelines
Summary of Other Features

• 15+ Sections -- including:
  • Foreword: Synopsis of Diagnostic Criteria and Testing Recommendations
  • Historical Perspective; Principles of LA Assays; Test Validation
  • Definitions and Terminology; Quality Control (Internal, External)
  • Pre-examination Issues (Patients, Specimens, Screening PT+APTT+TT)
  • Examination/Testing Details: Screen; Confirm; Mix; Exclusion
  • Post-examination Issues: Interpretation and Reporting; Examples
  • Harmonization with Other Guidelines

• 6 Laboratory Diagnostic Criteria

• 24 Testing Recommendations, relative to Criteria

• 116 Pages; ≥331 References; 8 Appendices; ≥12 Tables; ≥11 Figures, including Algorithms
LA Testing Guidelines Review
Selected Comparisons: ISTH-SSC 2009 vs CLSI H60-A*

**ISTH SSC (2009)**

- Cutoffs (Ref. Interval): 99% (nonparametric)
- dRVVT first, then APTT
- APTT activator: Silica only, not Ellagic acid
- Tests not recommended: dPT, KCT, PNP, etc.
- Algorithm order: Screen, Mix, Confirmatory
- Normalized ratio reporting: relative to mean normal pool

**CLSI H60-A (2014)**

- Cutoffs (Ref. Interval): +2SD (parametric) for each test
- Both dRVVT & APTT screen
- APTT activator: no restriction (Silica, Ellagic acid, Kaolin)
- Does not restrict supplemental tests, if validated by lab
- Algorithm order: Screen, Confirmatory, Mix
- Normalized ratio reporting: relative to mean ref. interval

*Table 3, Section 15: Harmonization - excerpts
LA Testing Guidelines Update

Summary

• The 4 Cardinal Criteria for LA assessment and diagnosis - Screen, Confirm, Mix, Exclusion - are paramount for guiding accurate testing and interpretive reporting - as reconfirmed in recently updated LA testing guidelines:
  • ISTH-SSC (2009)
  • BCSH (2012)
  • CLSI H60-A (2014)

• Adherence to specific recommendations, insofar as feasible and practical, contributes importantly to the diagnostic specificity of LA testing.

• There is need for yet further improvement in LA testing and guidelines!
Immunoassays for Antibodies to β₂-GPI
With or without added cardiolipin (CLIP)

• Anticardiolipin (ACL) ELISAs
  • Cardiolipin + β₂-GPI (bovine or human) -->
    β₂-GPI neo-antigenic epitopes (domain I)
  • IgG & IgM calibrator standards (GPL, MPL units)
    • weak = ~15-40; positive = >40-80; strong = >80
    • Poor inter-assay correlation for different kits

• Direct β₂-GPI ELISAs (no added CLIP)
  • β₂-GPI on plate -> neo-antigenic epitopes
  • No currently available calibrator standards
  • “Positive” = >99th percentile of normals (>3 SD)
  • Poor inter-assay correlation for different kits
Immunoassays for APL antibodies
Testing Features & Recommendations

- **ACL ELISA (IgG & IgM isotypes)**
  - Somewhat more sensitive vs. direct $\beta_2$-GPI assay
  - Sufficient screening for many patients?

- **$\beta_2$-GPI (direct) ELISA (IgG & IgM isotypes)**
  - Somewhat more specific vs. ACL assay
  - Reflexive testing of ACL-positive results?
  - Supplemental testing if strong APS suspicion?

- **Not Recommended**:
  - IgA isotype testing
  - Antibodies to Prothrombin, Annexin A5, etc.
  - Antibodies to other PLs: PS, PE, Cardiolipin alone

Lakos G et al. (2010 International APL Congress) *Arth Rheum* 2012;64:1-10
Devreese KMJ et al. (ISTH-SSC Guidelines) *JTH* 2014;12:792-5
Lupus Anticoagulant Testing
WHAT TESTS ARE AVAILABLE?

- APTT  (activated partial thromboplastin time)
- DRVVT  (dilute Russell’s viper venom time)
- DPT  (dilute prothombin time)
- Staclot-LA  (APTT + hexag. phase phospholipid)
- KCT/SCT  (kaolin or silica clot time)
- PCT  (plasma clot time)
- Textarin-Ecarin Time  (venoms)
Mayo LA (& APL) Test Algorithm

- **Activated Partial Thromboplastin Time (APTT)**
  - Is result within normal range?
    - Yes, stop
    - No
      - APTT Mixing Test (1:1 mix with normal plasma)
        - Does result correct sufficiently?
          - Yes, stop
          - No
            - Platelet Neutralization Procedure (PNP)

- **Thrombin Time (TT)**
  - Is TT normal?
    - Yes, stop
    - No
      - Reptilase Time (RT)

- **Prothrombin Time (PT)**
  - Is PT normal?
    - Yes, stop
    - No
      - PT Mixing Test

- **Dilute Russell Viper Venom Time (DRVVT)**
  - Is result within normal range?
    - Yes, stop
    - No
      - DRVVT Mixing Test (1:1 mix with normal plasma)
        - Does result correct sufficiently?
          - Yes, stop
          - No
            - DRVVT Confirmatory Testing

- **Review all test results.**
  - Is further clarification needed?
    - Yes: perform 1 or more as needed
    - No
      - Interpretation of all results is performed

- **Factor inhibitor screen & titer**
- **Factor assays**
- **Staclot LA and/or DPT**
- **Clinical information review**

- **Repeat testing in ≥12 weeks if positive for LA and/or APL antibodies, if indicated**

- **Review ACL and/or β2-GP-I test results**

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LA & APL Antibodies / Syndrome
Summary & Recommendations

• LA &/or ACL positivity are relatively frequent
  • Test for both LA and IgG & IgM ACL (+/- supplemental β2-GPI Abs)
  • Important to evaluate persistence of positivity (>3 mo.)
  • Numerous pre-analytical, analytical and post-analytical variables (patient, sample, AC Rx, interpretation and reporting, etc.)
  • Know your laboratory’s quality --> select a good one
• Not all LA or ACL are pro-thrombotic; no predictive tests yet
• Rarely, bleeding can occur with LA (II or X deficiency, AITP)

• APL Syndrome (APS) - diagnosis and management challenging
  • Thrombotic events &/or fetal losses, otherwise unexplained
  • Persistently significantly positive LA &/or ACL (β2-GPI)
  • Anticoagulation management can be difficult (& Rx impacts Dx)
  • Heparin (inhibited APTT); Warfarin (inhibited PT/INR); DOACs (false positive)
Questions & Discussion