MAYO CLINIC LABORATORIES

Test Definition: ACLIP

Phospholipid (Cardiolipin) Antibodies, IgA, Serum

Overview

Useful For

May be of diagnostic significance for patients at risk for antiphospholipid syndrome or systemic lupus erythematosus who test negative for criterial antiphospholipid antibodies

Method Name

Enzyme-Linked Immunosorbent Assay (ELISA)

NY State Available

Yes

Specimen

Specimen Type

Serum

Specimen Required

Collection Container/Tube:

Preferred: Serum gel

Acceptable: Red top

Submission Container/Tube: Plastic vial

Specimen Volume: 0.5 mL

Collection Instructions: Centrifuge and aliquot serum into a plastic vial.

Specimen Minimum Volume

0.4 mL

Reject Due To

Gross hemolysis	Reject
Gross lipemia	Reject
Gross icterus	OK

Specimen Stability Information

Specimen Type	Temperature	Time	Special Container
Serum	Refrigerated (preferred)	21 days	
	Frozen	21 days	

Clinical & Interpretive

Clinical Information

Antiphospholipid syndrome (APS) is a systemic autoimmune disease characterized by thrombosis and/or specific pregnancy-related death. Based on the 2006 revised Sapporo consensus classification criteria, the laboratory requirements for diagnosing APS include the presence of at least one of the following: lupus anticoagulant (LAC), anticardiolipin (aCL) and anti-beta2 glycoprotein 1 (anti-B2GP1) IgG or IgM antibodies.(1) To avoid overdiagnosis, and to exclude patients with transient antiphospholipid (aPL) levels, the APS guidance also recommends confirmation of any positive result at least twelve weeks after the initial evaluation. Of note, aPL antibodies also occur in patients with autoimmune diseases with significant prevalence in systemic lupus erythematosus (SLE) as well as other clinical manifestations (eg, heart valve disease, livedo reticularis, thrombocytopenia, nephropathy and neurological) often associated with APS.(1-3) Thus, in addition to the 2006 APS guidance, the 2012 derivation and validation of the Systemic Lupus International Collaborating Clinics (SLICC) classification criteria for SLE recommends testing for the criteria aPL antibody tests as well as aCL IgA and anti-B2GP1 IgA.(2)

Unlike LAC, which is evaluated using functional assays, aCL and anti-B2GP1 IgG and IgM antibodies are measured with diverse solid-phase immunoassays (SPA).(4) For aCL IgG and IgM determinations, the APS classification guidance recommends antibody cut-off values greater than 40 IgG phospholipid (GPL) or IgM phospholipid (MPL) units (units traceable to the Harris standards for aCL antibody assays) or more than the 99th percentile for the testing laboratory's population for positivity. It also advocates for the use of values greater than the 99th percentile for the laboratory's population in the establishment of reference intervals for anti-B2GPI IgG and IgM antibody tests. The use of cutoff values greater than 40 GPL or MPL units to define positivity is not be applicable to all aCL antibody immunoassays, as the threshold used to distinguish moderate-to-high positive from low positive results are test dependent.(4-6) In addition, the cutoff used at the 99th percentile of a laboratory's testing population may not be consistent with kits from the same manufacturer or 40 GPL units, in the case of aCL antibodies.(4-7)

Early observations that aCL antibody determinations made in the presence of B2GPI were more specific for APS led to the recommendation of B2GPI-dependent cardiolipin enzyme-linked immunosorbent assay (ELISA) for APS evaluation.(1,8) Cardiolipin is a negatively charged phospholipid (PL) capable of binding diverse proteins, of which B2GP1 is one of the best characterized in APS. B2GP1 is a 326-amino acid protein that contains five repetitive structures or "sushi domains," termed domain 1 through to 5, for a combined molecular weight of 54?kDa for the protein.(6). Anti-B2GP1 antibodies associated with thromboembolic events target domain 1 of the molecule and are responsible for LAC (functional, phospholipid-dependent prolongation of the clotting time) and aCL antibody positivity.(7) Compared to LAC and anti-B2GPI IgG antibodies, aCL IgG antibodies are less specific but sensitive for the diagnosis of APS. Of the aCL IgG and IgM, the IgG and not IgM confers higher diagnostic relevance and risk for definite APS.(1,6,7)

Thrombosis and obstetric complications are common clinical events in the general population and are not unique to APS; therefore, the presence of aPL antibodies is an absolute requirement for the diagnosis of definite APS.(1,6,7) Furthermore, aPL antibodies are heterogeneous with overlapping tendencies; the lack of aPL test harmonization or standardization requires the use of all three tests for optimal APS diagnosis.(1,4) The aPL antibodies were traditionally determined using classic ELISA, with more diverse methods recently developed and adapted for clinical testing. Recognizing the analytical and diagnostic challenges associated with aPL antibody testing, initiatives to support assay

harmonization and utilization, including the development of calibrators, test development, and validation efforts as well as pre-analytical, analytical, and post-analytical measures have been published. (4-8) Based on these and other published studies, the interpretation and relevance of aPL antibody tests are dependent on factors such as the type of aPL (LAC, aCL or anti-B2GPI), the source of cardiolipin and/or B2GPI, aPL antibody class (IgG, IgM, or IgA) and level as well as whether antibody positivity is single, double, or triple.(1,4-8)

In conclusion, although the APS classification criteria were not established for routine clinical use, in the absence of formal diagnostic guidelines, these have widely been adopted to diagnose or assess risk for APS and the need for treatment or prophylaxis. Therefore, in clinical practice, if suspicion for disease is high but criteria aPL antibody tests are inconclusive or negative, deviation from the APS diagnostic criteria may be justified. This may include testing for non-criteria aPL antibody tests such the aCL IgA and anti-B2GPI IgA recommended in 2012 SLICC guidance for SLE and/or evaluation of anti-phosphatidylserine/prothrombin (aPS/PT) IgG and IgM autoantibodies amongst others.(2,6,9,10)

Reference Values

APL refers to IgA phospholipid units. One APL unit is 1 microgram of IgA antibody.

Negative: <15.0 APL
Weakly positive: 15.0-39.9 APL
Positive: 40.0-79.9 APL
Strongly positive: > or =80.0 APL

Reference values apply to all ages.

Interpretation

The presence of anticardiolipin (aCL) IgA antibodies (greater than 15 IgA phospholipid units [APL]) may be associated with a diagnosis of antiphospholipid syndrome (APS) and/or systemic lupus erythematosus (SLE). In the absence "criteria" aPL antibodies for APS and diagnostic tests for SLE, isolated aCL IgA must be interpreted with a high degree of caution.

Documentation of persistence aCL IgA as is the case for criteria aCL IgG and IgM antibodies would be consistent with best clinical practice.

Detection of anticardiolipin antibodies using the method is not affected by anticoagulant treatment.

Cautions

Immunoassays for the detection of certain antibodies including anticardiolipin (aCL) may not completely distinguish between autoantibodies specific for antiphospholipid syndrome (APS) and those antibodies produced in response to infectious agents with or without thrombosis. Since these antibodies may be transiently produced, documentation of persistence, as outlined for aCL IgG and IgM antibodies in the 2006 revised Sapporo guidance for definite APS, is required (see Clinical Information).

Comparative studies and interlaboratory proficiency surveys indicate that results of phospholipid antibody tests can be

highly variable, and results obtained with different commercial immunoassays may yield substantially different results.(4-7)

Clinical Reference

1. Miyakis S, Lockshin MD, Atsumi T, et al: International consensus statement on an update of the classification criteria for definite antiphospholipid syndrome (APS). J Thromb Haemost. 2006 Feb;4(2): 295-306
2. Petri M, Orbai AM, Alarcon GS, et al: Derivation and validation of the Systemic Lupus International Collaborating Clinics classification criteria for systemic lupus erythematosus. Arthritis Rheum. 2012 Aug;64(8):2677-86
3. Sciascia S, Amigo MC, Roccatello D, Khamashta M: Diagnosing antiphospholipid syndrome: 'extra-criteria' manifestations and technical advances. Nat Rev Rheumatol. 2017 Sep;13(9):548-560
4. Devreese KMJ. Solid phase assays for antiphospholipid antibodies. Semin Thromb Hemost. 2022 Sep;48(6):661-671. doi: 10.1055/s-0042-1744364
5. Ruffatti A, Olivieri S, Tonello M, et al: Influence of different IgG anticardiolipin antibody cut-off values on antiphospholipid syndrome classification. J Thromb Haemost. 2008 Oct;6(10):1693-1696
6. Lakos G, Favaloro EJ, Harris EN, et al: International consensus guidelines on anticardiolipin and anti-beta 2-glycoprotein I testing: report from the 13th International Congress on antiphospholipid antibodies. Arthritis Rheum. 2012 Jan;64(1):1-10
7. Pengo V, Bison E, Denas G, Jose SP, Zoppellaro G, Banzato A. Laboratory diagnostics of antiphospholipid syndrome. Semin Thromb Hemost. 2018 Jul;44(5):439-444
8. Matsuura E, Igarashi Y, Fujimoto M, et al: Heterogeneity of anticardiolipin antibodies defined by the anticardiolipin cofactor. J Immunol. 1992 Jun 15;148(12):3885-91
9. Abisror N, Nguyen Y, Marozio L, et al. Obstetrical outcome and treatments in seronegative primary APS: data from European retrospective study. RMD Open. 2020 Aug;6(2):0. doi: 10.1136/rmdopen-2020-001340
10. Nakamura H, Oku K, Amengual O, et al: First-line, non-criterial antiphospholipid antibody testing for the diagnosis of antiphospholipid syndrome in clinical practice: A combination of anti-beta2 -glycoprotein I domain I and anti-phosphatidylserine/prothrombin complex antibodies tests. Arthritis Care Res (Hoboken). 2018 Apr;70(4):627-634

Performance

Method Description

Purified cardiolipin antigen is bound to the wells of a polystyrene microwell plate under conditions that will preserve the antigen in its native state. Prediluted controls and diluted patient sera are added to separate wells, allowing any cardiolipin antibodies present to bind to the immobilized antigen. Unbound sample is washed away, and an enzyme-labeled antihuman IgA conjugate is added to each well. A second incubation allows the enzyme-labeled antihuman IgA to bind to any patient antibodies that have become attached to the microwells. After washing away any unbound enzyme-labeled antihuman IgA, the remaining enzyme activity is measured by adding a chromogenic substrate and measuring the intensity of the color that develops. After stopping the enzymatic production of colored product, the presence or absence of cardiolipin antibody is determined by comparing the sample optical density with that of a 5-point calibration curve. Results are reported out semiquantitatively in standard IgA anticardiolipin units (APL).(Package insert: QUANTA Lite ACA IgA III. Inova Diagnostics; Version 22, 08/2020)

PDF Report

No

Day(s) Performed
Monday, Wednesday, Friday

Report Available
3 to 5 days

Specimen Retention Time
14 days

Performing Laboratory Location
Rochester

Fees & Codes

Fees
- Authorized users can sign in to Test Prices for detailed fee information.
- Clients without access to Test Prices can contact Customer Service 24 hours a day, seven days a week.
- Prospective clients should contact their Regional Manager. For assistance, contact Customer Service.

Test Classification
This test has been cleared, approved, or is exempt by the US Food and Drug Administration and is used per manufacturer's instructions. Performance characteristics were verified by Mayo Clinic in a manner consistent with CLIA requirements.

CPT Code Information
86147

LOINC® Information

Test ID	Test Order Name	Order LOINC® Value
ACLIP	Phospholipid Ab IgA, S	5076-5

Result ID	Test Result Name	Result LOINC® Value
ACLIP	Phospholipid Ab IgA, S	5076-5