Test Definition: MCLIP

Phospholipid (Cardiolipin) Antibodies, IgM, Serum

Overview

Useful For

The following clinical situations, when used in conjunction with other criterial antiphospholipid antibody tests:
-Unexplained arterial or venous thrombosis
-A history of pregnancy morbidity defined as 1 or more unexplained deaths of a morphologically normal fetus beyond the 10th week of gestation, 1 or more premature births before 34 weeks of gestation caused by severe preeclampsia or placental insufficiency, or 3 or more unexplained, consecutive spontaneous abortions before the 10th week of gestation with no identifiable maternal hormonal or anatomic, or maternal or paternal chromosomal causes
-Presence of a systemic autoimmune rheumatic disease, especially systemic lupus erythematosus
-Unexplained thrombocytopenia
-Presence of an unexplained cutaneous manifestations varying from livedo reticularis to cutaneous necrosis, such as leg ulcers
-Possible nonbacterial, thrombotic endocarditis

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	Reject

hemolysis	
Gross lipemia	Reject
Gross icterus	OK
Heat-treated specimen	Reject

Specimen Stability Information

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

Clinical & Interpretive

Clinical Information

Antiphospholipid syndrome (APS) has traditionally been described as a systemic autoimmune disease characterized by thrombosis and/or specific pregnancy-related morbidities associated with persistent documentation of "criterial" antiphospholipid antibody (aPL) tests.(1,2) Based on the 2006 revised Sapporo consensus classification, the "criterial" aPL antibody tests include lupus anticoagulant (LAC) and IgG/IgM antibodies to the cardiolipin (aCL) and beta2-glyocoprotein I (anti-B2GPI) with all tests carrying equal diagnostic significance for disease.(1) In 2023, the American College of Rheumatology (ACR)/ European League Against Rheumatism (EULAR) published new classification criteria for APS that includes an entry criterion of at least one positive aPL antibody test within 3 years of identification of an aPL-associated clinical criterion, followed by additive weighted criteria (score range 1-7 points each) clustered into 6 clinical domains (macrovascular venous thromboembolism, macrovascular arterial thrombosis, microvascular, obstetric, cardiac valve, and hematologic) and 2 laboratory domains (LAC functional coagulation assays, and solid-phase enzyme-linked immunosorbent assays (ELISA) for IgG/IgM aCL and/or IgG/IgM anti-B2GPI).(3)

Unlike LAC, which is evaluated using functional assays, diverse solid-phase immunoassays (SPA) such as ELISA, multiplex bead assay, chemiluminescent immunoassay, and fluorescence enzyme immunoassay are used in the clinical laboratories for the detection and measurement of aCL and anti-B2GPI IgA, IgG, and IgM antibodies.(4,5) 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.(1) 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.(5-7) 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.(2,5-7)

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 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 5, for a combined molecular weight of 54 kDa for the protein.(8) 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.(2) 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.(1,3,6,7)

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.(1,2,6) 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.(1,3) 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 preanalytical, analytical, and postanalytical measures have been published. (2,4,5,7) 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.(1,7)

The 2023 ACR/EULAR classification criteria for APS are meant for clinical studies and may not be appropriate for routine patient evaluation and management. 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 noncriteria aPL antibody tests such the aCL IgA, anti-B2GPI IgA and anti-phosphatidylserine/prothrombin complex IgG and IgM antibodies.(2,5,9,10) However, there is no formal guidance for the measurement and interpretation of these noncriteria aPL antibodies in patients with APS or SLE.

Reference Values

MPL refers to IgM phospholipid units. One MPL unit is 1 microgram of IgM antibody.

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

Reference values apply to all ages.

Interpretation

Moderate-to-strong positive results for anticardiolipin (aCL) IgM antibodies (> or =40 IgM phospholipid [MPL] units) are a diagnostic criterion for antiphospholipid syndrome (APS). Compared to aCL IgG, isolated and low levels aCL IgM antibodies have a very low risk for APS and should be interpreted with a high degree of suspicion.

Documentation of persistent aCL IgM antibodies is a requirement for the diagnosis of definite APS. Antibodies must be detected on 2 or more occasions at least 12 weeks apart to fulfill the laboratory diagnostic criteria for APS.

Detection of anticardiolipin antibodies using the enzyme-linked immunosorbent assay or other solid-phase immunoassays is not affected by anticoagulant treatment.

Cautions

Immunoassays for the detection of antiphospholipid antibodies including anticardiolipin may not completely distinguish between autoantibodies specific for antiphospholipid syndrome 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 in the 2006 revised Sapporo guidance for the criteria antibodies, would constitute best practice (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 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;4(2): 295-306
2. Pengo V, Bison E, Denas G, Jose SP, Zoppellaro G, Banzato A. Laboratory diagnostics of antiphospholipid syndrome. Semin Thromb Hemost. 2018;44(5):439-44
3. Barbhaiya M, Zuily S, Naden R, et al. The 2023 ACR/EULAR antiphospholipid syndrome classification criteria. Arthritis Rheumatol. 2023;75(10):1687-1702
4. Tebo AE. Laboratory evaluation of antiphospholipid syndrome: An update on autoantibody testing. Clin Lab Med. 2019;39(4):553-65
5. Devreese KMJ. Solid phase assays for antiphospholipid antibodies. Semin Thromb Hemost. 2022 Sep;48(6):661-671. doi:10.1055/s-0042-1744364
6. 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;6(10):1693-6
7. 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;64(1):1-10
8. Matsuura E, Igarashi Y, Fujimoto M, et al. Heterogeneity of anticardiolipin antibodies defined by the anticardiolipin cofactor. J Immunol. 1992;148(12):3885-91.
9. Cousins L, Pericleous C, Khamashta M, et al. Antibodies to domain I of ß-2-glycoprotein I and IgA antiphospholipid antibodies in patients with ‘seronegative’ antiphospholipid syndrome. Ann Rheum Dis. 2015;74(01):317-9
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;70(4):627-34

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 IgM conjugate is added to each well. A second incubation allows the enzyme-labeled antihuman IgM to bind to any patient antibodies that have become attached to the microwells. After washing away any unbound enzyme-labeled antihuman IgM, 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 IgM anticardiolipin units.(Package inserts: QUANTA Lite ACA IgM III. Inova Diagnostics; Version 23, 08/2020)

PDF Report
No

Day(s) Performed
Monday through Saturday

Report Available
1 to 3 days

Specimen Retention Time
14 days

Performing Laboratory Location
Mayo Clinic Laboratories - Rochester Superior Drive

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 account representative. 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

MCLIP	Phospholipid Ab IgM, S	3182-3

Result ID	Test Result Name	Result LOINC® Value
MCLIP	Phospholipid Ab IgM, S	3182-3