MAYO CLINIC LABORATORIES

Test Definition: ACLIP

Phospholipid Ab IgA, S

Overview

Useful For

Evaluation of patients with suspected antiphospholipid syndrome by identification of phospholipid IgA antibodies

Method Name

Enzyme-LinkedImmunosorbentAssay(ELISA)

NY State Available

Yes

Specimen

Specimen Type

Serum

Specimen Required

Container/Tube:

Preferred: Serum gel

Acceptable: Red top

Specimen Volume: 0.5 mL

Reject Due To

Gross hemolysis	Reject
Gross lipemia	Reject
Gross icterus	OK

Specimen Minimum Volume

0.4 mL

Specimen Stability Information

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

Clinical & Interpretive

Clinical Information

The plasma membranes of mammalian cells are formed from phospholipids. Anionic phospholipids (eg, phosphatidylserine) are found on the cytoplasmic surface and neutral phospholipids (eg, phosphatidylcholine) predominate on the external surface. Membrane phospholipids participate in several important cellular functions including exchanging metabolites across membranes, transferring molecular signals and serving as a platform for the

assembly of protein-lipid complexes.(1) Cellular activation is often accompanied by the translocation of anionic phospholipids to the external membrane surface. For example, during platelet-mediated blood coagulation, phosphatidylserine is translocated from the inner platelet membrane and provides a surface for the assembly of the prothrombinase enzyme complex that catalyzes the formation of thrombin.

Complexes of negatively charged (anionic) phospholipids and endogenous plasma proteins provide epitopes recognized by natural autoantibodies.(2) Plasma from normal individuals contains low concentrations of natural IgG autoantibodies of moderate affinity. Pathologic levels of autoantibodies reflect loss of tolerance and increased production of antibodies. These autoantibodies are called phospholipid or cardiolipin antibodies when they are detected by immunoassays that employ anionic phospholipids as substrates. The most commonly used phospholipid substrate is cardiolipin. The term phospholipid antibody is actually a misnomer. The autoantibodies react with epitopes of protein molecules that associate noncovalently with reagent phospholipids. The best characterized phospholipid-binding protein is beta 2-glycoprotein 1 (beta-2 GP1) and most immunoassays for phospholipid antibodies employ a composite substrate consisting of cardiolipin plus beta-2 GP1. Beta-2 GP1 is a 326-amino acid polypeptide that contains 5 homologous domains of approximately 60 amino acids each. Most phospholipid antibodies bind to an epitope associated with domain 1 near the N-terminus. Autoantibodies can also be detected by the use of functional, phospholipid-dependent coagulation assays. Phospholipid antibodies detected by functional assays are often called lupus anticoagulants because they produce prolongation of phospholipid-dependent clotting in vitro and are found in some patients with systemic lupus erythematosus. Not all phospholipid antibodies possess lupus anticoagulant activity.(3) Only those phospholipid antibodies that are capable of cross-linking beta-2 GP1 molecules can interact efficiently with phospholipid surfaces in functional coagulation assays. It is hypothesized that complexes formed in vivo between bivalent, natural autoantibodies and beta-2 GP1 bind to translocated, anionic phospholipid on activated platelets at sites of endothelial injury. This binding is believed to promote further platelet activation that may lead to thrombosis.

Antiphospholipid syndrome (APS) is an autoimmune disorder characterized by thromboses, complications of pregnancy, and certain laboratory abnormalities. The diagnosis of APS requires at least 1 clinical criteria and 1 laboratory criteria be met.(4) The clinical criteria include vascular thrombosis (arterial or venous in any organ or tissue) and pregnancy morbidity (unexplained fetal death, premature birth, severe preeclampsia, or placental insufficiency). Other clinical manifestations, including heart valve disease, livedo reticularis, thrombocytopenia, nephropathy and neurological symptoms, are often associated with APS but are not included in the diagnostic criteria. The laboratory criteria for diagnosis of APS are presence of lupus anticoagulant, presence of IgG and/or IgM anticardiolipin antibody (>40 GPL, >40 MPL, or >99th percentile), and/or presence of IgG and/or IgM anti-beta-2 GP1 antibody (>99th percentile). All antibodies must be demonstrated on 2 or more occasions separated by at least 12 weeks. Anticardiolipin and anti-beta-2 GP1 antibodies of the IgA isotype are not part of the laboratory criteria for APS due to lack of specificity.(4)

Reference Values

<15.0 APL (negative)
15.0-39.9 APL (weakly positive)
40.0-79.9 APL (positive)
> or =80.0 APL (strongly positive)
APL refers to IgA Phospholipid Units. One APL unit is 1 microgram of IgA antibody.
Reference values apply to all ages.

Interpretation

APL, GPL and MPL units refer to arbitrary units. The abbreviation APL denotes the result is from the IgA isotype, the abbreviation GPL denotes the result is from the IgG isotype and the abbreviation MPL denotes the result is from the IgM isotype. The letters "PL" denote specificity for phospholipid antigens. Positive and strongly-positive results for IgG and IgM phospholipid (cardiolipin) antibodies (>40 GPL and/or >40 MPL) are diagnostic criteria for antiphospholipid

syndrome (APS). Lesser levels of IgG and IgM phospholipid (cardiolipin) antibodies and antibodies of the IgA isotype (APL) may occur in patients with clinical signs of APS but the results are not considered diagnostic. Phospholipid (cardiolipin) antibodies must be detected on 2 or more occasions at least 12 weeks apart to fulfill the laboratory diagnostic criteria for APS.
IgA phospholipid (cardiolipin) antibody results greater than 15 APL with negative IgG and IgM phospholipid (cardiolipin) antibody results are not diagnostic for APS.
Detection of phospholipid (cardiolipin) antibodies is not affected by anticoagulant treatment.

Cautions

The immunoassay for phospholipid (cardiolipin) antibodies does not distinguish between autoantibodies and antibodies produced in response to infectious agents or as epiphenomena following thrombosis. For this reason, a single positive test result is not sufficient to meet accepted serologic criteria for the diagnosis of antiphospholipid syndrome (APS) (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.(5,6)

Clinical Reference

1. Bevers EM, Comfurius P, Dekkers DW, et al: Lipid translocation across the plasma membrane of mammalian cells. Biochim Biophys Acta 1999;1439:317-330
2. Arnout J, Vermylen J: Current status and implications of autoimmune antiphospholipid antibodies in relation to thrombotic disease. J Thromb Haemost 2003;1:931-942
3. Proven A, Bartlett RP, Moder KG, et al: Clinical importance of positive test results for lupus anticoagulant and anticardiolipin antibodies. Mayo Clin Proc 2004;79:467-475
4. 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: 295-306
5. Fontaine MJ, Jacob GL, Nichols WL, et al: Comparative evaluation of three assays for anti-cardiolipin antibodies. J Autoimmun 2000;15(2):A56
6. Favaloro EJ, Wong RC, Silvertrini R, et al: A multilaboratory peer assessment quality assurance program-based evaluation of anticardiolipin antibody, and beta 2 glycoprotein 1 antibody testing. Semin Thromb Hemost 2005;31(1):73-84

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, November 2014 Revision 20)

PDF Report

No

Specimen Retention Time

14 days

Performing Laboratory Location

Rochester

Fees & Codes

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