

## Overview

### Useful For

Testing for phospholipid antibodies is indicated in the following clinical situations:

- 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 an unexplained cutaneous circulatory disturbance, eg, livido reticularis or pyoderma gangrenosum
- Presence of a systemic rheumatic disease especially lupus erythematosus
- Unexplained thrombocytopenia or hemolytic anemia
- Possible nonbacterial, thrombotic endocarditis

### Profile Information

Test Id	Reporting Name	Available Separately	Always Performed
MCLIP	Phospholipid Ab IgM, S	Yes	Yes
GCLIP	Phospholipid Ab IgG, S	Yes	Yes

### Method Name

Enzyme-Linked Immunosorbent Assay (ELISA)

### NY State Available

Yes

## Specimen

### Specimen Type

Serum

### Specimen Required

**Container/Tube:**

**Preferred:** Serum gel

**Acceptable:** Red top

**Specimen Volume:** 0.5 mL

### Forms

If not ordering electronically, complete, print, and send 1 of the following forms with the specimen:

-[General Request](#) (T239)

[-Coagulation Test Request \(T753\)](#)

[-Renal Diagnostics Test Request \(T830\)](#)

## 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

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 and most immunoassays for phospholipid antibodies employ a composite substrate consisting of cardiolipin plus beta-2 glycoprotein 1(beta-2 GP1beta-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. Not all

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phospholipid antibodies possess lupus anticoagulant activity.<sup>(3)</sup> 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.

Phospholipid antibodies occur in patients with a variety of clinical signs and symptoms notably thrombosis (arterial or venous) pregnancy morbidity (unexplained fetal death, premature birth, severe preeclampsia, or placental insufficiency) unexplained cutaneous circulation disturbances (livido reticularis or pyoderma gangrenosum) thrombocytopenia or hemolytic anemia and nonbacterial thrombotic endocarditis. Phospholipid antibodies and lupus anticoagulants are found with increased frequency in patients with systemic rheumatic diseases especially lupus erythematosus. The term antiphospholipid syndrome (APS) or Hughes syndrome is used to describe the triad of thrombosis, recurrent fetal loss and thrombocytopenia accompanied by phospholipid antibodies or a lupus anticoagulant. The diagnosis of APS requires 1 or more of the above mentioned clinical findings plus positive test results for phospholipid antibodies (> or =40 GPL or MPL) or positive tests for a lupus anticoagulant on more than 1 occasion separated by at least 6 weeks.<sup>(4)</sup>

**Reference Values**

<15.0 MPL or GPL (negative)

15.0-39.9 MPL or GPL (weakly positive)

40.0-79.9 MPL or GPL (positive)

> or =80.0 MPL or GPL (strongly positive)

MPL refers to IgM Phospholipid Units. One MPL unit is 1 microgram of IgM antibody.

GPL refers to IgG Phospholipid Units. One GPL unit is 1 microgram of IgG antibody.

Reference values apply to all ages.

**Interpretation**

Positive and strongly positive results for phospholipid antibodies (> or =40 GPL and/or MPL) are a diagnostic criterion for antiphospholipid syndrome (APS). Lesser levels of phospholipid antibodies and antibodies of the IgA isotype may occur in patients with clinical signs of APS but the results are not considered diagnostic.

Detection of phospholipid antibodies is not affected by anticoagulant treatment.

**Cautions**

The immunoassay for phospholipid 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).

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.<sup>(5,6)</sup>

**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, Vermynen 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. Wilson WW, Gharavi AE, Koike T, et al: International consensus statement on preliminary classification criteria for definite antiphospholipid syndrome. Arthritis Rheum 1999;42(7):1309-1311

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 IgM or IgG conjugate is added to each well. A second incubation allows the enzyme-labeled antihuman IgM or IgG to bind to any patient antibodies that have become attached to the microwells. After washing away any unbound enzyme-labeled antihuman IgM or IgG, 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 or IgG anticardiolipin units (MPL and GPL). (Package inserts: QUANTA Lite ACA IgM III September 2015 Revision 21 and QUANTA Lite ACA IgG III Inova Diagnostics February 2015 Revision 22)

### PDF Report

No

### Day(s) Performed

Monday through Saturday

### Report Available

Same day/1 to 2 days

### Specimen Retention Time

See Individual Mayo Test IDs.

### Performing Laboratory Location

Rochester

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**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 x 2

**LOINC® Information**

Test ID	Test Order Name	Order LOINC® Value
CLPMG	Phospholip Ab (Cardiolip) IgM/IgG	24319-6

Result ID	Test Result Name	Result LOINC® Value
GCLIP	Phospholipid Ab IgG, S	3181-5
MCLIP	Phospholipid Ab IgM, S	3182-3