



## Determination, Prevalence and Clinical Significance of anti-Phosphatidylserine Antibodies

The determination of antibodies to phosphatidylserine in human serum is an important aid for assessing the risk of thrombosis in individuals with systemic lupus erythematosus (SLE), lupus-like disorders, and the antiphospholipid syndrome (APS). Unlike anti-Cardiolipin (aCL), an assay for anti-Phosphatidylserine (aPS) antibodies uses a specific antigen that participates in coagulation. When aPS antibodies are injected into a murine model, an experimental antiphospholipid syndrome is induced, confirming their pathogenic role (1). These antibodies can interfere with normal hemostatic mechanisms in humans, and require cofactor (as do autoimmune aCL antibodies) for optimal binding in ELISA test systems (2).

There are currently no recognized aPS antibody standards available. However, aCL IgG/IgM calibrators available from the Antiphospholipid Standardization Laboratory have been described to bind other negatively charged phospholipid antigens in a roughly equivalent manner. Hence, the GPL and MPL designation used to describe the cardiolipin binding activity of these calibrators can also be applied to phosphatidylserine binding activity. To distinguish them from aCL units, aPS antibody activity is reported in GPS and MPS units (IgG or IgM PhosphatidylSerine). These calibrators were used to assign values to various sera. From these, a serum panel has been developed which includes multiple individual samples to represent the heterogeneity of antiphospholipid antibodies in assay calibration and to ensure lot to lot consistency.

The clinical specificity of the aPS assay has been compared to the specificity of the aCL assay. When sera from multiple healthy blood donor populations were tested for IgG aPS antibodies, antibody levels above the established cutoff of 16 GPS were demonstrated in 4% of the samples, for a specificity of 96%. The specificity of the aPS assay for IgM antibodies was also 96%, with a cutoff of 22 MPS when the same samples were tested. The aCL assay is reported to have 97% specificity for IgG antibodies and 96% specificity for IgM antibodies for a given normal population.

Some interesting differences were noted when the clinical sensitivity of the aCL and aPS assays was compared by testing serum from an unselected SLE

population. When 53 SLE samples were analyzed over three lots of aCL and aPS kits, 32.1% were positive for IgG aPS, compared to 33.9% for IgG aCL. With IgM, 7.5% of the samples were positive for aPS antibodies, while 12.6% were positive for aCL. The detection of more clinically relevant antibodies with the aPS assay may explain the lower sensitivity of aPS in this population, especially for IgM. Additional differences in sensitivity were seen in the following 2 x 2 analysis derived from the mean sample values for this population:

Unselected SLE Population:

	IgG aPS +	IgG aPS -
IgG aCL +	12	6
IgG aCL -	5	30

	IgM aPS +	IgM aPS -
IgM aCL +	3	3
IgM aCL -	1	46

Although the correlation of aPS and aCL assay results was good, the value of testing for both aPS and aCL antibodies is evident from the samples testing positive by only one of the assays. Also, a recent study found a higher correlation with thrombosis in patients testing positive for both aCL and aPS antibodies (3).

The clinical sensitivity of the assay for thrombosis and thrombocytopenia was determined by comparing aPS test results from two groups of selected SLE patients: Group 1) with a clinical history of thrombosis and/or thrombocytopenia; and Group 2) with no history of thrombosis or thrombocytopenia. The samples were assayed in duplicate with three lots of aPS kits. The results are summarized below:

Group 1: SLE w/ thrombosis &/or thrombocytopenia

	IgG aPS	IgM aPS
Average Value	27.1 GPS	13.6 MPS
% Positive	54%	15%

Group 2: SLE w/out thrombosis or thrombocytopenia

	IgG aPS	IgM aPS
Average Value	7.2 GPS	6.6 MPS
% Positive	0%	10%

The prevalence and mean levels of IgG aPS antibodies were significantly higher in SLE patients with a history of thrombosis or thrombocytopenia, than in the group with no clinical history of thrombosis or thrombocytopenia ( $p = 0.002$ ). The difference in the mean IgM aPS antibody levels for the two groups was not statistically significant ( $p > 0.05$ ). It can be concluded that increased IgG aPS antibody levels correlate with the clinical manifestations of APS. This is further supported in a recently published study which compared aPS levels and the obstetric history of selected female SLE patients either with or without a clinical history of thrombosis and/or thrombocytopenia (4). High serum aPS levels and an increased prevalence of positive IgG aPS results correlated with a higher rate of fetal abortions in the group with clinical manifestations of APS.

In conclusion, the anti-Phosphatidylserine (aPS) assay, when run in concert with aCL and Lupus Anticoagulant assays, will provide a more complete profile for establishing a diagnosis of APS and for assessing the risk of thrombotic complications in SLE patients.

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#### Feature article references:

1. Blank M, Tincani A, Shoenfeld Y. Induction of Experimental Antiphospholipid Syndrome in Naive Mice with Purified IgG Antiphosphatidylserine Antibodies. *Journal of Rheumatology*, 1994; 21:1.
2. Keedy K, Santos M, Lopez L. Antiphosphatidylserine Antibodies Require  $\beta_2$ Glycoprotein I as Cofactor in ELISA. *Lupus*, Vol. 3: 327, 1994.
3. Barna L, Triplett D, Foster W, Gaddis M. A Study of Relationships Among Antiphosphatidylserine and Anticardiolipin Antibodies, the Lupus Anticoagulant and Clinical Complications. *Lupus*, Vol.3: 357, 1994.
4. Keedy K, Santos M, Lopez L. Prevalence and Clinical Significance of Antiphosphatidylserine Antibodies. *Arthritis and Rheumatism*, Vol. 37: 1384, Sept. 1994.
5. McNeil H, Chesterman C, Krillis S. Immunology and Clinical Importance of Antiphospholipid Antibodies. *Advances in Immunology*, 1991; 49:193-280.
6. Branch D, Rote N, Dostal D, Scott J. Association of Lupus Anticoagulant with Antibody Against Phosphatidylserine. *Clinical Immunology and Immunopathology*, 1987; 42:63-75.
7. Schick P, Kurica K, Chacko G. Location of Phosphatidyl-ethanolamine and Phosphatidylserine in the Human Platelet Plasma Membrane. *Journal of Clinical Investigation*, 1976; 57:1221-1226.

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**Additional questions or requests for references regarding the information or opinions presented in this newsletter, current applications and clinical significance of antiphospholipid antibodies or autoantibodies detected by ELISA may be directed to our customer service / technical support staff at READS Medical Products, Inc.**

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## READER PRODUCT FEATURE

### READS anti-Phosphatidylserine ELISA Test

For investigational use only

Assay format -	96-well microtiter plate (8 x 12)
Antigen substrate -	Bovine brain phosphatidylserine
Conjugate -	Horseradish peroxidase (HRP) goat $\alpha$ -human IgG;IgM
Chromogenic substrate -	TMB
Sample dilution -	1:50
Incubations	
Sample -	15 min @ room temp.
Conjugate -	15 min @ room temp.
Substrate -	10 min @ room temp.
Stopping solution -	2.5 N sulfuric acid
Wavelength -	450 nm
Clinical specificity -	IgG: 96%; IgM: 96%
Clinical sensitivity -	SLE: IgG: 30%; IgM: 7.5%
	SLE with thrombosis: IgG: 54%; IgM: 15%

### READER ANNOUNCEMENTS

**AACC Annual Meeting** in Anaheim, CA, July 16-20, 1995. READS Medical Products will sponsor booth #803 in Hall C during the Clinical Laboratory Exposition at the Anaheim Convention Center. We invite you to stop by to see us. Technical and sales representatives will be available to answer your questions, and to discuss current and new products that we are about to introduce. The following abstracts will be presented at Session 2, from 2:00pm - 4:30pm, Tuesday, July 17:

**Clinical Utility of Serum Hyaluronic Acid Levels in Liver Disease.** Santos M, Kondo T, Wieczorek A, Collier D, Lopez L. Abstract #171.

**Laboratory Management of Antinuclear Antibody Testing.** Dier K, Lopez L. Abstract #255.

**Performance of a New ELISA Assay for Protein C, Total Protein S, and Free Protein S.** Butler M, Santos M, Lopez L. Abstract #304.

Session 2, 2:00pm - 4:30pm, Wednesday, July 19:

**Protein C, Protein S, and Free Protein S Levels in Patients with Liver Disease Measured by ELISA.** Butler M, Santos M, Collier D, Lopez L. Abstract #606.

**Clinical Laboratory Management Association (CLMA) Annual Conference**, August 27-30, in Minneapolis, MN. Please visit READS Medical Products at booth #347, where technical and sales representatives look forward to meeting with you.

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