

## LABORATORY DETERMINATION OF ANTI-PHOSPHATIDYLSERINE ANTIBODIES

Cardiolipin is currently the phospholipid most commonly used to test for antiphospholipid antibodies. Phosphatidylserine, unlike cardiolipin, is found in the membranes of platelets and endothelial cells, and participates in the coagulation cascade. Phosphatidylserine comprises about 10-15% of the total phospholipids in plasma membranes, and is normally located in the interior of the lipid bilayer. Upon cell activation, phosphatidylserine is redistributed to the external surface where it may bind  $\beta_2$ GPI or other serum cofactor, leading to clot formation. For this reason, phosphatidylserine is a more physiologically relevant anionic phospholipid than cardiolipin, which has not been shown to be involved in coagulation. The determination of antibodies to phosphatidylserine (aPS) should provide more clinically relevant results in the diagnosis of antiphospholipid syndrome (APS).

Although the heterogeneity of antiphospholipid antibodies complicates the laboratory diagnosis of APS, one approach is to perform several more specific tests. It has recently been proposed that laboratories include aPS antibody determinations by ELISA with their routine anticardiolipin (aCL) and lupus anticoagulant panels. Patients with elevated levels of antibodies to both cardiolipin and phosphatidylserine are more likely to have clinical complications than those positive to only one phospholipid. In addition, we have shown that binding of aPS antibodies also requires the presence of  $\beta_2$ GPI as cofactor. Anticardiolipin antibodies associated with syphilis do not bind to phosphatidylserine coated plates even in the presence of cofactor, indicating that "infectious" aCL antibodies do not cross react with phosphatidylserine. We have also shown that aPS antibodies are prevalent in autoimmune populations and correlate with clinical manifestations of APS. All of these findings support the increased importance of aPS antibody determinations in the clinical laboratory.

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 READS 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, an increased risk of thrombosis has been shown in patients testing positive for both aCL and aPS antibodies.

The clinical sensitivity of the aPS assay for thrombosis and thrombocytopenia was determined by comparing 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 either ( $p = 0.002$ ). 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. 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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**Additional questions or requests for references regarding the information or opinions presented in this newsletter, current applications and clinical significance of antiphospholipid or autoimmune antibodies 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

<b>READS Anti-Phosphatidylserine ELISA Test Kit</b>	
For <i>in vitro</i> Diagnostic use	
Assay format -	96-well microtiter plate (8 x 12)
Antigen substrate -	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: 32%; IgM: 7.5%
	SLE with thrombosis: IgG: 54%; IgM: 15%

## READER ANNOUNCEMENTS

- READS Anti-Phosphatidylserine (aPS) IgG/IgM ELISA, the first commercial aPS assay to receive FDA clearance, is now available for *in vitro* diagnostic use. Contact your READS sales representative or our customer service department to order an evaluation kit.
- READS is pleased to announce the opening of our new European sales/marketing office:  
READS Bio-Medical Products (UK) Ltd.  
The Hunstman, Great North Road, Connington  
Peterborough, Cambridgeshire, PE7 3QU  
United Kingdom  
Phone: (01487) 930121  
Fax: (01487) 831097
- READS kits with the new packaging are already appearing on the shelves of some laboratories. In addition to our attractive new, recyclable boxes, the kits include additional volumes of kit sera, stopping solution, and sample diluent. Microtiter plates with breakaway wells, and liquid PBS concentrate will also be incorporated with the new packaging. These changes will not affect kit performance and are provided for the convenience of our customers at no additional cost.

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