



## Anti-Phospholipid Antibody Testing: A Current Review

Anti-cardiolipin (aCL) antibody testing has been utilized by physicians for over a decade to assess the risk of thrombotic episodes. Historically, the VDRL test, which uses an antigenic lipid mixture containing cardiolipin, was the first assay to detect anti-phospholipid (aPL) antibodies. Some patients with autoimmune disease result in false positive VDRL. ELISA methods were developed using cardiolipin to specifically detect and measure aCL antibodies. The Anti-Phospholipid Syndrome (APS) is now recognized as a separate clinical entity characterized by the production of autoantibodies against negatively charged phospholipids in relationship with specific binding proteins, and clinically associated with the following:

- arterial or venous thrombosis
- thrombocytopenia
- recurrent fetal loss

The presence of high level aPL antibodies has also been well documented in stroke patients, and is utilized by neurologists to predict risk of reoccurrence<sup>1</sup>.

As a result of research in this field, new information is available to give us a better understanding of the mechanisms involved. aPL antibodies are now recognized as a group of heterogeneous autoantibodies with reactivity against various negatively charged phospholipids, including cardiolipin and phosphatidylserine. Also, there is increased interest in  $\beta$ 2-Glycoprotein I (B2GPI) because of its requirement *in-vitro* as a serum cofactor in solid phase assays. The interaction between B2GPI and the phospholipid appears to create new binding sites on the B2GPI molecule for aPL antibodies.

Despite lacking a physiological role in coagulation, aCL remains the most commonly requested aPL test in the clinical laboratory. However, phosphatidylserine is more physiologically relevant than cardiolipin in coagulation, and demonstrates unique structural properties:

- *In-vivo*, cardiolipin's location is restricted to the inner mitochondrial membrane, isolated from the immune and coagulation systems.

- Phosphatidylserine, phosphatidylethanolamine, and phosphatidylcholine are primary components of the plasma membrane.
- Phosphatidylserine is almost exclusively located on the inside surface of the membrane at rest, and is externalized during cell activation.
- Phosphatidylserine promotes the anticoagulant protein C pathway that provides feedback inhibition of thrombin formation<sup>2</sup>.
- Phosphatidylserine has been shown to possess intercellular membrane fusion capabilities on the placental membrane<sup>3</sup>.

Most commercial aPL methods utilize bovine serum as the source of cofactor (B2GPI). It is provided either on the coating surface, in the samples diluent, or both. Even though placing the cofactor directly on the coating surface allows binding of cofactor dependent aPL antibodies, it also introduces additional proteins to which other irrelevant serum antibodies can bind (i.e. rheumatoid factor). This could contribute to lot-to-lot variation with aCL methods utilizing bovine serum on the plate surface. REAADS aCL and anti-phosphatidylserine (aPS) kits contain bovine B2GPI in the sample diluent only. B2GPI in the sample diluent binds to the phospholipid on the plate during incubation with the patient sample, favoring the detection of clinically relevant aPL antibodies. Both REAADS aCL and aPS assays demonstrate excellent lot-to-lot consistency.

Since the actual binding site for clinically relevant antibodies is believed to be located on the cofactor, much attention has shifted to the development of anti-B2GPI assays. Even though testing directly for anti-B2GPI has demonstrated increased specificity for some clinically relevant aPL antibodies, other important antibodies may be missed. Continued testing for antibodies in the presence of phospholipids (aPS and aCL) is still necessary. Research suggests that the binding site generated on the B2GPI molecule when bound to cardiolipin is different from that generated with phosphatidylserine<sup>4</sup>. It has not been

determined whether the binding site on the B2GPI molecule, when bound to a plastic surface, is similar to those formed when bound to cardiolipin, phosphatidylserine, or if in fact, is completely different.

REAADS has analyzed the clinical performance of its aPS, aCL, and anti-B2GPI ELISA assays on several healthy and autoimmune populations, including selected SLE patients with clinical manifestations of APS. These results demonstrate that patients positive for all three assays had the best correlation with thrombosis<sup>5</sup>. We have also shown that aPS antibodies are more closely related to cofactor dependency than aCL<sup>6</sup>. These findings indicate that testing for aPS and anti-B2GPI provides additional information along with aCL. Also, testing either for aPS plus anti-B2GPI, or aPS alone, may be more clinically relevant than testing for aCL only. There is no evidence at this time to support replacing aCL or aPS testing with anti-B2GPI only<sup>7,8</sup>.

#### Feature article references:

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

## REAADER PRODUCT FEATURE

### **REAADS Anti-Phosphatidylserine IgG/IgM ELISA Test Kit**

For *in vitro* Diagnostic Use

Assay format -	96-well microtiter plate (8 x 12 strips) with breakaway wells
Antigen substrate -	Brain phosphatidylserine
Conjugate -	Horseradish peroxidase (HRP) / goat anti-human IgG/IgM
Chromogenic substrate -	TMB (single component)
Sample dilution -	1:50
Incubations	
Sample -	15 min @ room temperature
Conjugate -	15 min @ room temperature
Substrate -	10 min @ room temperature
Stopping solution -	2.5 N Sulfuric acid
Wavelength -	450 nm
Clinical specificity -	IgG: 96%; IgM: 96%
Clinical sensitivity -	unselected SLE: IgG 32%; IgM 7.5%
	SLE with thrombosis: IgG 54%; IgM 15%

## REAADER ANNOUNCEMENTS

**NOW AVAILABLE:** Three new antigenic coagulation ELISA test kits:

#### Catalog #

034-001	von Willebrand Factor Antigen
035-001	Protein C Antigen
036-001	Protein S Antigen (Free and Total)

All products are now FDA cleared. These rapid and convenient assays provide highly accurate and precise quantitative results in human plasma. Discounted evaluation kits are available. Please contact your REAADS sales representative or distributor for details.

**aPS CPT Code:** The American Medical Association has assigned and published a new, distinct CPT code for the anti-Phosphatidylserine (aPS) assay. The aPS test is classified under Immunology, with the code #86148.

**Anti-B2GPI ELISA:** Evaluation kits for investigational use will be available soon. Contact REAADS customer service department for further information.

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