

RELATIVE TO COFACTOR DEPENDENCY, ANTI-PHOSPHATIDYLSERINE (aPS) ANTIBODIES ARE DISTINCT AND MORE CLINICALLY RELEVANT THAN ANTI-CARDIOLIPIN (aCL) ANTIBODIES

The immunology and clinical relevance of antiphospholipid antibodies have been the subject of an intense multi-disciplinary research effort. Significant progress has been made, and we now have a better understanding of their possible pathogenic role (i.e. their relationship to arterial or venous thrombosis in patients with primary or secondary antiphospholipid syndrome). As discussed in previous issues of **THE READER**, antiphospholipid antibodies are a heterogeneous group of autoantibodies commonly detected either by coagulation assays or ELISA. Cardiolipin is the most common antigen used in current ELISA systems for the detection of antiphospholipid antibodies, however, cardiolipin is not found in cell membranes nor does it participate in the coagulation cascade. Because phosphatidylserine is both present in cell membranes and participates in the coagulation cascade, it is considered more physiologic or relevant and should be the antigen of choice for routine use in antiphospholipid testing. Comparative studies on the prevalence of aCL and aPS antibodies in certain patient populations may not show significant differences, suggesting that the measurement of one antibody (either aCL or aPS) would be sufficient. In addition to prevalence studies, we have been exploring better methods to demonstrate that aCL and aPS are two distinct populations of antibodies, and that aPS antibodies are more clinically relevant than aCL antibodies.

"Autoimmune" antiphospholipid antibodies may require the presence of a serum cofactor (i.e. B2GPI) for optimal binding to the antigen in ELISA. (Both READS aCL and aPS test kits provide cofactor in the sample diluent). This enhanced binding has been referred to as "cofactor effect" and has been proposed to be an important distinguishing feature of antiphospholipid antibodies in patients with autoimmune diseases (associated with thrombosis) from those found in some patients with infectious diseases (i.e. syphilis). Furthermore, the requirement of a serum cofactor in the antigen-antibody reaction has been used to develop hypotheses to explain the immunogenicity and the generation of thrombosis in patients with high serum levels of antiphospholipid antibodies. In addition, we have previously demonstrated

that all 3 isotypes (IgG, IgM, and IgA) of both aCL and aPS antibodies may show B2GPI cofactor effect. This study was recently expanded by using a larger number of sera from selected patients with various autoimmune diseases (i.e. SLE) and syphilis. All of these serum samples were antiphospholipid positive over a wide range of reactivity. The B2GPI cofactor effect for IgG aPS and aCL antibodies was measured and compared.

In our study, 18% of the antiphospholipid positive serum samples showed cofactor effect; if syphilis samples are excluded from this group, the prevalence increases to 24%. As expected, none of the syphilis samples that were aCL positive reacted for aPS antibodies and none showed B2GPI cofactor effect. In this study, most of the samples with cofactor effect were from SLE patients. An unexpected finding was that only one sample showed cofactor effect for both IgG aPS and aCL antibodies. The rest showed a strong cofactor effect for aPS antibodies, and weak or no effect for aCL antibodies.

Our results with the syphilis serum samples (aCL positive, aPS negative and no cofactor effect) are in agreement with the published literature. These antibodies may be directed only to the phospholipid cardiolipin. The measurement of aPS antibodies may eliminate or decrease false positive results seen in patients with infections during routine antiphospholipid testing. With respect to the autoimmune disease samples, our results suggest the following: not all "autoimmune" antiphospholipid positive samples will show B2GPI cofactor effect (24% did in our study). However, if a strong cofactor effect is present, it is most likely associated with SLE. In addition, strong B2GPI cofactor effect was seen more frequently with IgG aPS than with aCL antibodies. One sample may have both aCL and aPS antibodies but cofactor effect may be seen only for aPS antibodies. These results strongly support the concept that aCL and aPS are two distinct populations of autoantibodies with different physiologic roles, aPS being more clinically relevant for thrombosis than aCL antibodies. The association between cofactor dependency of IgG aPS antibodies and clinical manifestations of the antiphospholipid syndrome (i.e. thrombosis) will need to be further explored.

Q. Do you recommend screening patient samples for anticardiolipin (aCL) antibodies using polyvalent conjugates?

A. REAADS does not recommend polyvalent aCL screening, nor is their use supported in the literature. Most experts in the field use isotype specific conjugates in their laboratories to routinely test patients for cardiolipin antibodies.

Two problems with the use of polyvalent conjugates have been observed and described in the literature. The first is that some samples test negative using the polyvalent aCL conjugates while testing positive with IgG, IgM, or IgA specific conjugates. In our experience, up to 20% of samples that test positive with specific conjugates are negative with polyvalent conjugates. These findings have been corroborated by several independent groups.

The second problem is that specific isotypes are associated with certain clinical events. Elevated serum levels of IgG and IgA anticardiolipin antibodies are strongly correlated with thrombosis and thrombocytopenia, while IgM in the absence of IgG or IgA is less likely to be associated with thrombosis. For these reasons, REAADS recommends against the use of polyvalent conjugates for "screening" samples during routine anticardiolipin determinations.

Q. "Cofactor" is becoming more important in anti-cardiolipin testing. What is cofactor and how does it relate to the REAADS Anti-Cardiolipin Test Kit?

A. The presence of a specific serum protein is required for optimal binding between anti-cardiolipin antibodies (aCL) and cardiolipin in ELISA. This protein has been referred to as "cofactor" and identified as Beta 2 glycoprotein I (apolipoprotein H). The exact role of the cofactor has not been fully resolved. It is believed that the aCL antibodies recognize a complex of cardiolipin and cofactor or specific cofactor epitopes that are presented when cofactor is bound to cardiolipin.

REAADS aCL kit uses bovine serum in the sample diluent which provides the "cofactor" necessary to facilitate binding of aCL as reported.

Independent studies comparing REAADS Anti-Cardiolipin Test Kit to another test kit which uses a cofactor-cardiolipin complex as an antigen demonstrate that both kits correlate closely with positive vs. negative results on SLE patient samples:

Comparison of REAADS Anti-Cardiolipin Assay with Cofactor-CI Assay		
Serum	REAADS	Cofactor-CI
SLE + thrombosis	9 + 10 -	9 + 10 -
SLE - controls	0 + 10 -	1 + 9 -

REAADS anti-dsDNA ELISA Test Kit
For *in vitro* Diagnostic Use

Assay format -	96-well micro plate (8 x 12)
Shelf life -	One year
Antigen substrate -	dsDNA (from calf thymus)
Conjugate -	horseradish peroxidase goat α 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 -	98%
Clinical sensitivity -	65%(expected 50-75% SLE)

The REAADS anti-dsDNA ELISA kit is specially treated so that virtually no ssDNA is present. Results are reported in AU/mL, traceable to a CDC reference preparation.

READER ANNOUNCEMENTS

- **AACC Annual Meeting** in Chicago IL, July 28 - August 1, 1996. REAADS Medical Products invites you to visit us at booth #1339-40 during the Clinical Laboratory Exposition at the McCormick Convention Center.
- **Clinical Laboratory Management Association (CLMA) Annual Conference**, August 22-25, 1996, in Denver, CO. REAADS Medical Products will sponsor booth #1200-02 where we look forward to meeting with you.
- **REAADS** is moving to a new and larger facility within the same building complex in early July. Our new mailing address will be:

12061 Tejon Street
Westminster, CO 80234

Our phone and fax numbers will remain the same. We look forward to the expansion, and will provide uninterrupted service to our customers during the relocation process.

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