

THE INCREASING IMPORTANCE OF ANTI-PHOSPHATIDYLSERINE ANTIBODIES

Antiphospholipid (aPL) antibodies are defined as a family of immunoglobulins that bind to several negatively charged phospholipids, including phosphatidylserine, cardiolipin (diphosphatidylglycerol), phosphatidic acid, phosphatidylinositol, and others. Elevated serum levels of aPL antibodies have been associated with increased risk for recurrent arterial and venous thrombotic events, thrombocytopenia and fetal loss. These are now recognized as the main clinical manifestations of the Antiphospholipid Syndrome (APS). Historically, the VDRL test, which uses a lipid mixture containing cardiolipin, was the first assay to detect a subgroup of aPL antibodies in patients with syphilis and autoimmune disease. aPL antibodies are now classified according to the method used to detect them. Antibodies detected by solid-phase ELISA using cardiolipin as the antigen, are referred to as anticardiolipin (aCL) antibodies. Coagulation assays, such as activated Partial Thromboplastin Time (aPTT), and dilute Russell Viper Venom Time (dRVVT) detect some aPL antibodies which are referred to as lupus anticoagulants. Because aPL antibodies represent a very heterogeneous group, it has been recommended that both types of assays, ELISA and coagulation, be performed when evaluating for APS.

Recently it has been described that some aPL antibodies interact with complexes formed by anionic phospholipids and plasma protein cofactors such as β_2 glycoprotein I (β_2 GPI). In addition, autoimmune aPL antibodies can be distinguished from non-autoimmune by their higher β_2 GPI requirement. *In vivo*, this interaction appears to play an important role in the generation of thrombosis, hence the nature of the phospholipid is important to fully understand the mechanism of action of antiphospholipid antibodies.

Cardiolipin is currently the phospholipid most commonly used to test for aPL 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 β_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.

Although the heterogeneity of aPL antibodies complicates the laboratory diagnosis of APS, one approach is to perform more specific tests. It has recently been proposed that laboratories include aPS antibody determinations by ELISA with their routine 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 β_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.

THE READER RESPONSE

Q. What is the clinical significance of aCL antibody levels around the cutoff?

A. Results from aCL testing should be used to assess the risk of developing coagulation complications such as thrombosis, thrombocytopenia, or fetal loss associated with Antiphospholipid Syndrome (APS). This risk increases with higher aCL values. Low positive aCL values can be found in a variety of patient populations, such as infectious disease, early stages of APS, and even healthy individuals. The exact clinical significance or prognostic value of low positives is unknown. These patients should be retested in 2-3 months to monitor disease activity and/or change in risk status. Patients with high serum levels of aCL (>40GPL, >50MPL, >60APL) are at increased risk of coagulation complications, and should be handled appropriately. Patients with aCL values around the cutoff are at a lower risk than those with higher values; these levels should not be dismissed as insignificant until further evaluation is performed.

Q. Does READS ANA ELISA detect autoantibodies to centromere?

A. No. The READS ANA ELISA contains specifically selected purified nuclear antigens that include dsDNA, Sm, RNP, SSA, and histones. These antigens are the most prevalent in SLE and in other rheumatic diseases. Antibodies to centromere are associated with CREST Syndrome, a rare subclass of scleroderma. CREST stands for: **C**alcinosis, **R**aynaud's phenomenon, **E**sophageal motility abnormalities, **S**clerodactyly, and **T**elangiectasia. These are all symptoms and manifestations that can be detected by the physician. If CREST Syndrome is suspected and the result of the READS ANA is negative, the patient should be specifically tested for antibodies to centromere either by IFA or ELISA.

READER ANNOUNCEMENTS

The following abstract has been accepted for presentation during the poster session of the 1995 ASCP/CAP Spring National Meeting in Orlando, Florida, April 24-26 (Poster # 102):

Enhanced Clinical Correlation of Antinuclear Antibody Determination by ELISA. Ken J Dier B.S., I(ASCP), Luis R Lopez, M.D.

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READS Medical Products, Inc.
12001 Tejon Street, Suite 120
Westminster, Colorado 80234, USA
Phone: (303) 457-4345
Toll Free: 1-800-729-5661



READER PRODUCT FEATURE

READS ANA ELISA

Antinuclear antibody (ANA) determination by IFA has long been the gold standard in the clinical laboratory, but consider bringing your laboratory to the next level of technology. Supplement the information available from standard IFA methodology with additional clinically significant information by ELISA. The READS ANA ELISA uses selected purified nuclear antigens in a 96 well microplate format, which results in better specificity and increased confirmation rate of positive specimens for specific autoantibodies. This assay can be used in sequence with IFA to help physicians determine appropriate follow-up testing on their patients. Best of all this can be accomplished with no increase in overall cost or turn around time. For more information on how your laboratory can benefit from the use of the READS ANA ELISA, contact technical service (1-800-729-5661).

READS ANA ELISA Test Kit For *in vitro* Diagnostic Use

| | |
|-------------------------|---|
| Assay format - | 96-micro well plate (8 X 12) |
| Shelf life - | One year from manufacture |
| Antigen substrate - | dsDNA, Sm, RNP, SSA, and histone |
| Conjugate - | horseradish peroxidase (HRP) goat α human IgG/IgM |
| Chromogenic substrate - | TMB |
| Sample dilution - | 1:50 |
| Incubations | |
| samples - | 15 minutes @ room temp. |
| conjugate - | 15 minutes @ room temp. |
| substrate - | 10 minutes @ room temp. |
| Stopping solution - | 2.5 N sulfuric acid |
| Wavelength - | 450 nm |

Feature Article References:

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