



ANTIPHOSPHOLIPID ANTIBODY TESTING: STANDARDIZATION AND CLINICAL INTERPRETATION OF RESULTS

The field of antiphospholipid testing has experienced rapid growth within the last decade, however, in spite of the progress made to date, there remains considerable confusion in interpreting results among laboratories and clinicians. The published literature on antiphospholipid antibodies has been reviewed to understand the reasons for this confusion and to suggest certain guidelines for the clinical interpretation of laboratory results.

Antiphospholipid antibodies are now recognized to be a group of heterogeneous autoantibodies with reactivity against various negatively-charged phospholipids, i.e. cardiolipin, phosphatidylserine. These autoantibodies can be detected either by solid-phase ELISA, where the antigen most commonly used is the phospholipid cardiolipin (anticardiolipin antibodies), and/or by coagulation assays from which the name lupus anticoagulant was derived. Lupus anticoagulant is today considered a true misnomer because the majority of patients with these antibodies do not have systemic lupus erythematosus (SLE).

The clinical importance of antiphospholipid antibodies resides in their strong association with recurrent venous and arterial thrombosis, thrombocytopenia and spontaneous fetal abortion. These are the main clinical manifestations of the antiphospholipid syndrome. Furthermore, certain clinical and serologic criteria have been proposed to aid in the detection of, or to diagnose antiphospholipid syndrome. From the serologic point of view, a high positive IgG anticardiolipin test and/or a positive lupus anticoagulant test result will confirm the diagnosis of antiphospholipid syndrome provided the patient also presents clinical manifestations. IgM anticardiolipin alone may not be used to support the diagnosis of antiphospholipid syndrome. The IgA isotype, however, appears to be as important as the IgG in the diagnosis of antiphospholipid syndrome. It is important to note that many individuals with a positive serologic test do not have clinical manifestations. The routine determination of antiphospholipid antibodies in these patients should be considered for its predictive value. The intended use of antiphospholipid antibody determinations is to assess the risk for thrombosis in patients with SLE or related diseases.

Standardization of anticardiolipin testing has encountered difficulties as a result of the complexity of the antiphospholipid system and its relationship with

thrombosis. Several technologies in current use may provide results including cut-off values which are apparently different from each other.

We now recognize that measurement of aCL antibodies in the laboratory is complicated by the heterogeneity of these antibodies as well as the differential requirement of a serum cofactor for optimal antibody binding. The lack of correlation of results between laboratories, the increasing number of in-house and commercial aCL assays, coupled with the growing popularity of the aCL test has prompted several groups to re-evaluate the current status of aCL standardization. The results of a European study, recently published in *Thrombosis and Haemostasis* (73:444-452, 1995) may enhance our understanding of aCL testing. The working group on Methodologies in Hemostasis of Switzerland carried out a multicentric comparative study to estimate the degree of standardization among several commercial aCL kits. This study concluded that aCL testing standardization has not been achieved and that it is debatable whether standardization of this heterogeneous system is possible. Some of the results and suggestions presented by the authors of this multicentric study were discussed in more detail in the August 1995 (Vol 5, No 4) issue of **THE READER**.

Until there is a better understanding of the immunology of antiphospholipid antibodies, the cardiolipin cofactor and the pathogenic role of both in thrombosis, standardization will continue to present some challenges which complicate the clinical interpretation of results by physicians. Because of these issues, it has been recommended that aCL results by ELISA be considered semiquantitative. To assist in the interpretation of results in relation to their association with clinical manifestations, results may be reported as ranges (negative, low +, moderate +, and high +) according to the degree of positivity. As with any laboratory result, the diagnosis and therapeutic intervention must be considered in the context of clinical manifestations presented by the patient.

(cont. on page 2)

(From page 1)

READS has done extensive testing and research in the field of antiphospholipid antibodies. This experience has resulted in the development of an anticardiolipin assay which leads the industry in providing accurate, reproducible results. READS has analyzed the clinical performance of its ELISA anticardiolipin test on several healthy and diseased populations including SLE patients with and without a history of thrombosis. Based on this analysis, the following table contains our recommended ranges. This may be useful when interpreting results for diagnostic and therapeutic purposes.

DEGREE OF POSITIVITY

	GPL RANGE
NORMAL	< 23
LOW +	23 - 40
MODERATE +	40 - 100
HIGH +	> 100
	MPL RANGE
NORMAL	< 11
LOW +	11 - 20
MODERATE +	20 - 50
HIGH +	> 50
	APL RANGE
NORMAL	<22
LOW +	22 - 35
MODERATE +	35 - 60
HIGH +	> 60

It is important to point out that up to 5-7% of healthy individuals may present a positive anticardiolipin test result. Commonly, their levels are considered as low positives and a high degree of variability between test results is frequently seen. The clinical significance of low positives and their variability are not well understood. In general, treatment is not recommended in these individuals and long term follow up would be advisable.

On the other hand, patients with autoimmune disease or with obvious manifestations of thrombosis in association with antiphospholipid autoantibodies will usually show positive results in the medium to high ranges, and treatment should be considered. High positive results in the absence of clinical manifestations indicate increased risk for a thrombotic event, however, the type and schedule of treatment for these patients remains controversial.

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

READER PRODUCT FEATURE

READS Anti-Phosphatidylserine ELISA Test
For *in vitro* Diagnostic use

Assay format -	96-well microtiter plate (8 x 12)
Antigen substrate -	Bovine brain phosphatidylserine
Conjugate -	Horseradish peroxidase (HRP) 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 -	IgG: 96%; IgM: 96%
Clinical sensitivity -	SLE: IgG: 30%; IgM: 7.5% SLE with thrombosis: IgG: 54%; IgM: 15%

READER ANNOUNCEMENTS

- READS Anti-Phosphatidylserine (aPS) IgG/IgM ELISA Test kit received FDA clearance and will be launched soon. This is the first commercial aPS kit available in the US for *in vitro* diagnostic use.
- READS is pleased to announce the recent appointment of Ann L. Steinbarger as Vice President, Sales of READS Medical Products.

Published by
READS Medical Products, Inc.
12001 Tejon Street, Suite 120
Westminster, Colorado 80234
Phone: (800)729-5661
Outside the US: (303)457-4345

