

UNDERSTANDING ANTICARDIOLIPIN ANTIBODY TESTING

In the April 1995 issue of THE READER, possible explanations for the discrepancies encountered by several laboratories when comparing anticardiolipin (aCL) antibody results were briefly discussed. It is now recognized that quantitative measurement of aCL antibodies 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 with results between laboratories, the increasing number of in-house and commercial kits available for aCL testing, coupled with the increasing popularity of the aCL assay 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*, 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 are worth reviewing.

Nine commercial aCL kits were used to test simultaneously a group of 90 selected serum samples including the standards provided by the Antiphospholipid Standardization Laboratory (University of Louisville, Kentucky, USA). The rate of agreement between laboratories participating in the study was good with 96% for IgG and 97% for IgM aCL. However, the positivity rate was different among the kits with ranges from 31% to 60% for IgG and from 6% to 50% for IgM aCL. Agreement between kits was only 59% for IgG and 51% for IgM aCL. Even though all kits used GPL and MPL units to express the results, the differences may have resulted from the way the cut-offs were

calculated which varied among kits used in the study. Furthermore, the antiphospholipid standards were tested on all kits and the measured versus assigned values compared. The slopes of the regression lines obtained differed significantly between kits and these results were interpreted as an indication that the use of reference standards does not improve the standardization of the assay system. Due to the inherent differences of the kits and the heterogeneity of these antibodies, it is unlikely that the use of a single serum or even a set of calibrators would achieve the standardization of aCL testing, the authors concluded.

Cofactor requirement for anticardiolipin antibody binding was also analyzed as a determinant for the differences observed between kits. All kits provided a source of cofactor. Despite noticeable differences in the content of B2GPI, this could not be related directly to the differences observed nor used to determine the ability of any kit to preferentially bind autoimmune (cofactor-dependent) versus non-autoimmune (cofactor-independent) aCL antibodies in this study. Whether cofactor was provided on the wells or in the sample diluent may contribute to some of the differences. This is important to point out due to the coexistence of cofactor-dependent and independent aCL antibodies in some serum samples. Moreover, antibodies to B2GPI which also may coexist in some samples, were detected similarly in most kits. However, samples without antibodies to B2GPI showed variable reactivity between kits. This is relevant as it may be interpreted that certain kits detect more "true" antiphospholipid (aCL) antibodies than others.

READS aCL kit demonstrated positive rates for IgG and IgM aCL antibodies in the upper end of the range (48% and 39% respectively), B2GPI was measured only in the sample diluent buffer as expected, and it showed one of the highest detection rates of aCL antibodies in samples that

lacked antibodies to B2GPI. Based on these results, it can be concluded that most of the antibodies detected by the READS aCL kit are "true" antiphospholipid (aCL) antibodies. Because B2GPI is present in the sample diluent, the detection of autoimmune aCL antibodies may be favored. Interestingly, these results were obtained in spite of a relatively low slope obtained by the READS aCL kit against the University of Louisville standards in this particular study. This might have suggested low positive rates with the READS aCL kit, however, the study showed just the opposite.

In spite of expressing results in common GPL or MPL units, significant variability in the results was observed between kits. READS QC/QA procedures uses a panel of different serum samples with values over the entire reported range to account for the heterogeneity of aCL antibodies. This procedure allows for better lot to lot consistency of values over time. The use of any established and well characterized commercial kit should ensure some degree of standardization for that laboratory. This study clearly showed that GPL or MPL value comparison between the diverse technologies used by commercial kits should not be used as the sole criteria even when using the available standards. Standardization of aCL antibody testing will need to be reassessed when more information on the nature of the antigen-antibody reaction is obtained in this complicated system.

Feature article references:

Reber G., Arvieux J., Comby E. *et al.*
Multicenter Evaluation Of Nine Commercial Kits for the Quantitation of anticardiolipin Antibodies.
Thrombosis and Haemostasis 73:444-452 (1995)

Ward A.M., White P.A.E.
Measuring Cardiolipin Antibodies
British Medical Journal 310:1472-1473 (1995)

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

READS Autoantibody Identification System
For In-Vitro Diagnostic Use

Individual antigen-coated well packs are tested with a common Reagent Pack. Well Packs & Reagent Packs are ordered separately. Well packs contain coated microwells, positive control, and calibrator for either 32 or 96 determinations.

All assays utilize a common procedure, which offers additional convenience allowing flexibility when setting up different combinations of specific autoantibody tests.

Sample dilution - 1:100
Incubations
Sample - 30 min @ room temp.
Conjugate - 30 min @ room temp.
Substrate - 30 min @ room temp.

Anti-SSA well pack 32 well: Order # 041-001
96 well: Order # 041-002
Anti-SSB well pack: 32 well: Order # 042-001
96 well: Order # 042-002
Anti-Sm well pack 32 well: Order # 043-001
96 well: Order # 043-002
Anti-Sm/RNP well pack 32 well: Order # 044-001
96 well: Order # 044-002
Anti-Jo-1 well pack 32 well: Order # 045-001
96 well: Order # 045-002
Anti-Scl-70 well pack 32 well: Order # 046-001
96 well: Order # 046-002

Reagent pack: Order # 040-001
(sample diluent, conjugate, substrate, stop solution, and wash buffer to run 192 wells)

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