



## Complexities of Anticardiolipin Assay Standardization

Recent research in the field of antiphospholipid antibodies has provided significant new information which may help unravel some of the complexities of anticardiolipin (aCL) assay standardization. These findings suggest that while standardization of aCL assays has long been desired and actively pursued, measures currently in use may have been scientifically premature, and may even have contributed to the confusion surrounding antiphospholipid antibody testing. In the light of these recent developments we are beginning to appreciate the complexity of the standardization issue.

With the discovery of antibodies to phospholipids in patient sera, important clinical differences were soon recognized: patients with syphilis or other infectious diseases in whom antiphospholipid antibodies were detected showed no evidence of coagulation disorders; in autoimmune patients, antiphospholipid antibodies were frequently associated with venous and arterial thrombosis, thrombocytopenia, and recurrent fetal loss (antiphospholipid syndrome). Current research on the nature and role of cofactors such as beta 2 glycoprotein I has increased our understanding of the interactions between phospholipids and antiphospholipid antibodies. It has been found that antibodies occurring with syphilis and other infectious diseases do not require a cofactor to react with cardiolipin, whereas those classified as autoimmune are more dependent on the presence of cofactor for optimal binding. The anticoagulant properties of beta 2 glycoprotein I suggest a possible mechanism for the development of thrombosis in patients with antiphospholipid syndrome. This association is strengthened by the finding that other proteins involved in the coagulation cascade, including Protein C, Protein S, and Prothrombin, also act as cofactors in forming autoimmune complexes with phospholipids.

In addition to the heterogeneity of antiphospholipid antibodies, other factors may influence the reactivity of antiphospholipid antibodies *in vitro*. These include antibody isotype, avidity, crossreactivity, and the conformation of the phospholipid on ELISA microtiter plates. The recent discovery of antibodies to cofactor along with antiphospholipid antibodies in some autoimmune patient sera has further complicated efforts to standardize aCL assays.

Understanding the relationship between cofactor and antiphospholipid antibodies should also help explain why the design of test systems to detect these antibodies greatly influences reactivity. Our experience indicates that different antibodies may be favored by different aCL assays, depending on where the cofactor is incorporated in the kit. Assays which include cofactor in plate coating detect both cofactor dependent and independent aCL antibodies as well as anti-cofactor antibodies, producing a maximum assay response. When cofactor is included only in the sample diluent, cofactor dependent antibodies are favored and anti-cofactor antibodies may be neutralized, resulting in a relatively smaller measurable response. Other assays which include cofactor in plate coating and sample diluent may favor both cofactor dependent and independent aCL antibodies, while possibly neutralizing anti-cofactor antibodies to produce an intermediate assay response.

Thus, the magnitude of the antibody response in aCL assays may depend on many factors, including the nature of the antibodies present in the test serum and the reference preparations used to standardize the assay, as well as the design and formulation of different components in the assay system. Better standardization of aCL assays may remain elusive until it is possible to identify and characterize the antibodies in the sera used as standards, and/or until more specific assays are available. Our research has shown that cofactor independent antiphospholipid antibodies, such as those associated with syphilis, do not cross-react with phosphatidylserine coated to microtiter plates, even in the presence of cofactor. This suggests that assays measuring antiphosphatidylserine antibodies may be more selective for phospholipid antibodies associated with autoimmune disease.

With our current level of understanding, consistency within an aCL test system may be a more important consideration than how well unit values compare with another method. A consistent product is assured at READS by the extensive quality control procedures performed during kit production. Clinical relevance of aCL test results may be the best index of assay performance until more progress is made toward assay standardization

## READER PRODUCT FEATURE

### READS IgA anticardiolipin ELISA Test Kit

For *in vitro* Diagnostic Use

|                         |   |
|-------------------------|---|
| Assay format -          | 96-well microtiter plate (8 x 12)                 |
| Shelf life -            | One year  |
| Antigen substrate -     | cardiolipin (diphosphatidyl glycerol)             |
| Conjugate -             | horseradish peroxidase<br>goat $\alpha$ human IgA |
| 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 -  | 95%   |
| Clinical sensitivity -  | 26% in SLE population; 79% in SLE with thrombosis |

**Q.** Our laboratory is evaluating anticardiolipin kits to begin testing in house. As our volume of testing is expected to be fairly small, we would like to know if microtiter plates with break-away wells are available in READS aCL IgG/IgM kits?

**A.** Currently, microtiter plates in the break-away format are not available in our kits, although we are working to make this change in our manufacturing process. In response to the continuing cost constraints in today's laboratories, we feel this change may help our customers control reagent costs and operate more efficiently. The wells in these new plates snap apart and can be used individually, allowing customers to use the exact number of wells they need for their run. This should help minimize wasted materials, as entire strips must be run with the plates currently used. READS plans to incorporate the new plates in our aCL IgG/IgM kits later this year, and as soon as possible thereafter in our other products. The new plates are compatible with spectrophotometers currently used with READS kits.

**Q.** We have a few microtiter strips left from the plate in our IgA aCL kit, but have run out of IgA conjugate. Since both assays use plates coated with cardiolipin, can I use these strips with my IgG/IgM kit?

**A.** No, we do not recommend using components from an IgA kit with an IgG/IgM aCL kit, nor should components from one kit lot be used with another lot of the same kit type. The reagents (including plates) for each kit are carefully tested for optimum performance only with the other specific components of that kit lot. We can not predict how the substitution of components from another kit lot will affect the reactivity of a kit. The extensive quality control testing performed at READS assures that the particular combination of lot specific components used in each kit will produce results that are standardized and consistent.

---

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

---

## READER ANNOUNCEMENTS

**Revised Instructions for Use:** The latest revision of our *Instructions for Use*, in booklet format, will soon appear in READS ELISA kits. A separate sheet detailing the changes in the instructions will be included in the first few kit lots with the new inserts. One of the most significant changes can be found in the IgA assay insert. We have revised the washing procedure for **READS IgA aCL** assays to include **five** washes with PBS per wash cycle, rather than four. The concentration of HRP-conjugated antibody used in IgA conjugates is stronger than with IgG or IgM conjugates, requiring a more vigorous wash procedure with IgA assays to minimize non-specific binding and keep reagent blanks in the acceptable range. Please notify your technologists, and change the procedure in your laboratory to include an additional wash with each cycle for optimum results.

**ASCP/CAP Spring Meeting** in Orlando, Florida, April 24-26, 1995: Ken J Dier B.S., I(ASCP), will be presenting his abstract, "Enhanced Clinical Correlation of Antinuclear Antibody Determination by ELISA," at the poster session.

Published by  
READS Medical Products, Inc.  
12001 Tejon Street, Suite 120  
Westminster, Colorado 80234  
Phone: 1-800-729-5661  
Outside the US: (303)457-4345

