



## THE FIFTH INTERNATIONAL SYMPOSIUM ON ANTIPHOSPHOLIPID ANTIBODIES

Sponsored by the University of Texas Health Sciences Center at San Antonio, The Fifth International Symposium on Antiphospholipid Antibodies attracted over 300 scientists from various regions of the world. During 3 days (September 9-12, 1992), the results of recent research on antiphospholipid antibodies were presented and discussed by the attendees. In addition, this symposium offered optional lupus anticoagulant and anticardiolipin workshops. The following is a brief review of the most significant topics included in the program.

The association between antiphospholipid antibodies and clinical manifestations of the antiphospholipid syndrome (i.e. thrombosis) has been well established. However, the exact nature of the phospholipid antigen and how it induces antibody formation *in vivo* remained controversial. The nature, specificity and molecular biology of the antiphospholipid immune response were discussed by renowned researchers. In a mouse model, only the hexagonal form of phosphatidylethanolamine and the mixture of  $\beta_2$  glycoprotein I with cardiolipin induced the production of antibodies that had both lupus anticoagulant and anticardiolipin reactivities. These results suggest that structural changes of phospholipids such as those produced by inflammatory stimuli may induce the formation of these antibodies. In the case of cardiolipin, its interaction with  $\beta_2$  glycoprotein I appears to be important to induce antibody formation. This provides strong evidence that under certain conditions phospholipids can be immunogenic and autoantibodies may be produced.

The cardiolipin cofactor ( $\beta_2$  glycoprotein I) generated considerable discussion. Although some discrepant results were presented, the consensus is that  $\beta_2$  glycoprotein I is not only required in solid phase assays (i.e. ELISA's) to measure anticardiolipin antibodies but also may have an important role in the pathogenesis of thrombosis *in vivo* as further explained below.

It was found that certain antiphospholipid antibodies have both anticardiolipin and lupus anticoagulant activities. Other antibodies showed only anticardiolipin or only lupus anticoagulant activity. These results supported the concept that antiphospholipid antibodies are heterogeneous in nature. Furthermore, data was presented to show that lupus anticoagulants may also bind phospholipids complexed with prothrombin, whereas anticardiolipin antibodies bind phospholipids complexed with  $\beta_2$  glycoprotein I. Both prothrombin and  $\beta_2$  glycoprotein I have high affinity for membrane phospholipids "activated" by inflammation. These results offer an explanation for the *in vitro* prolonged coagulation test while *in vivo* these antibodies produce the opposite effect (thrombosis).

The above results support recent claims that both anticardiolipin and lupus anticoagulant assays should be performed to accurately determine the presence of antiphospholipid antibodies. In addition, based on reviews presented by guest speakers, the need to routinely measure all 3 antibody isotypes (IgG, IgM and IgA) was emphasized. Presentations on serologic testing for anticardiolipin antibodies also generated several controversial opinions mostly due to the existing information and experience related to the anticardiolipin standardization process.

Finally, additional data was presented on the prevalence of antiphospholipid antibodies in patients with neurological and cardiac conditions as well as in adverse pregnancy outcomes. These results along with discussions on therapeutic considerations for these patients, indicate that progress is being made in understanding the significance of antiphospholipid antibodies. This significance is reflected in the continual growth of clinical applications of antiphospholipid antibody determinations.

## THE READER RESPONSE

**Q.** Sometimes there are droplets of moisture on the bottom of the wells of the READER microtiter plate. What is the cause of this and will it affect the performance of the assay?

**A.** The droplets are a normal byproduct of the coating process of the microtiter plates and do not interfere with the assay performance.

**Q.** I have noticed that the reagent blank for the READER IgA anticardiolipin test kit is higher than the reagent blank for the IgG/IgM anticardiolipin test kit. Why does this occur and how can the reagent blank be decreased?

**A.** The higher reagent blank of the IgA kit is due to a difference in the strength of the HRP attached to the IgA conjugate compared to the IgG and IgM conjugates. The conjugates are optimized differently due to various assay characteristics. To decrease the reagent blank of the IgA assay, increase the washing of the IgA plate, either add an additional wash or wash the plates more vigorously. The antigens and antigen/antibody complexes will not be affected by the increased washing.

**Q.** Because both come in red solutions, occasionally I have mispipetted the IgM conjugate into wells which required the IgG conjugate, is there some way to distinguish the 2 different conjugates?

**A.** In the near future the IgG anticardiolipin conjugate will be colored blue to distinguish it from the red IgM conjugate. The availability of the blue conjugate will be announced in the READER EVENTS when the conjugate is available.

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### FEATURE ARTICLE REFERENCES:

For more information on papers presented at the Symposium, contact READER Medical Products, Inc.

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## READER PRODUCT FEATURE

**READER Anti-dsDNA Test Kits - For the semi-quantitation of IgG/IgM anti-dsDNA antibodies to aid in the diagnosis and management of systemic lupus erythematosus (SLE).**

Incubation Times: (at room temp.)	Sample 15 min. Conjugate 15 min. Substrate 10 min.
Sample Dilution:	1:50
Conjugate:	HRP
Substrate:	TMB
Standardization:	Center of Disease Control Reference preparation
Reproducibility: Clinical SLE	CV's < 10%
Specificity: Clinical SLE	98%
Sensitivity:	65%
Shelf Life:	One year
Kit Size:	96-well order #D022-01 288-well order #D022-03

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**The READER ANA Test Kit - For the detection and semi-quantitation of IgG/IgM Antinuclear Antibodies in human serum. The ANA test kit protocol and kit configuration are identical to other READER test kits.**

Antigen Substrate:	Purified nuclear antigens (mixture)
Incubation Times: (at room temp.)	Sample 15 min. Conjugate 15 min. Substrate 10 min.
Sample Dilution:	1:50
Conjugate:	HRP
Substrate:	TMB
Stopping Solution:	2.5 N sulfuric acid
Clinical SLE	
Specificity:	95%
Clinical SLE	
Sensitivity:	96%
Reproducibility:	CV's < 15%
Kit Size:	96-well order #D027-01