



## THE SEROLOGIC EVALUATION OF THROMBOSIS: COMPARATIVE PERFORMANCE OF FOUR ANTIPHOSPHOLIPID ANTIBODY ASSAYS

Antiphospholipid antibodies are a heterogeneous group of autoantibodies associated with the antiphospholipid syndrome (APS), which is clinically characterized by recurrent arterial or venous thrombosis. In addition, APS is the most common acquired risk factor for the development of deep vein thrombosis. High serum levels of anticardiolipin (aCL) antibodies measured by ELISA are accepted serologic criteria for the diagnosis of APS. In the last decade, it has become apparent that many assays for aCL antibodies show low sensitivity or specificity for thrombosis (and APS). More recently, new assays for various antiphospholipid antibodies (i.e. to phosphatidylserine [aPS] or to protein cofactors such as beta-2 glycoprotein 1 [anti-B2GPI], prothrombin [aPT], etc.) have been developed with enhanced sensitivity and specificity for thrombosis. Several recent reports suggest that anti-B2GPI antibodies may be more specific for thrombosis (and APS) than aCL antibodies. Based on this information and several clinical studies, Corgenix proposed an algorithm for the laboratory evaluation of antiphospholipid antibodies (*THE READER Vol 9, No 5, October 1999*). This algorithm suggests the use of aCL and/or aPS assays as screening tools, followed by a more specific assay (anti-B2GPI) if indicated by serologic results or clinical findings.

To assist the clinical laboratory in becoming familiar with the performance of these assays (including the newer anti-Prothombin) and the use of the algorithm, Corgenix studied the sensitivity and specificity for thrombosis of four antiphospholipid antibodies in a group of 24 plasma samples. The samples were obtained from patients with abnormal coagulation times who were referred to a coagulation laboratory for evaluation of lupus anticoagulant (LA) activity. IgG, IgM and IgA antibodies to cardiolipin (aCL), phosphatidylserine (aPS), beta 2 glycoprotein I (anti-B2GPI) and prothrombin (aPT) were measured by ELISA kits. Patients' clinical records were reviewed for clinical manifestations of thrombosis (APS) and/or autoimmune disease (i.e. SLE).

While all 24 plasma samples were classified as LA positive by accepted classification criteria, 5 (21%)

showed no clinical manifestations of thrombosis, APS or autoimmune disease. The majority (79%) had a diagnosis of APS or SLE and/or had a history of thrombosis in their clinical records. Four of the 5 patients without APS were negative for antiphospholipid antibodies; the other patient tested positive for 3 antibodies. Fifteen of the 24 patients were positive for anti-B2GPI antibodies, for a prevalence of 62%. The prevalence of each antibody (aCL, aPS, and aPT) was 46% in this population. When reviewing the pattern of reactivity of each patient, 3 patients (17.6%) reacted to one antibody, 2 patients (11.8%) to two antibodies, while the majority (70.6%) reacted to 3 or more antibodies in various combinations, as shown in the bar graph on page 2.

A 2x2 analysis of positive or negative results for each antibody versus the presence or absence of clinical manifestations of thrombosis (APS) showed that anti-B2GPI antibodies had the best sensitivity (68%), followed by aPS (58%), and both aCL and aPT antibodies with 52%. The best specificity for thrombosis (APS) was observed with aPS antibodies (100%), followed by aCL and aPT (80%), and anti-B2GPI antibodies (60%). When the results of all four antiphospholipid antibody assays were combined, the sensitivity for APS was 80% with a specificity of 60%, giving an overall accuracy of antiphospholipid antibodies of 75%. These results are summarized in the table on the following page.

These results not only confirmed the association of antiphospholipid antibodies with thrombosis (APS) but showed that the majority of patients developed more than one antiphospholipid antibody in different combinations. This suggests that measuring only one antibody may result in a missed diagnosis in some patients. For example, 5 patients with APS (21%) in this population tested negative for aCL and positive for other antibodies. In this study, the antiphospholipid antibody with the best specificity was aPS (100%) while anti-B2GPI showed the best sensitivity (68%), suggesting that aPS followed by anti-B2GPI may be the best assay combination for the laboratory screening of antiphospholipid antibodies.

**REAADER ANNOUNCEMENTS**

• Coming soon: **REAADS Anti-Phosphatidylserine (aPS) IgG/IgM Test Kit** will be available soon in a convenient three plate format. The 3-plate kit (product number 030-002) will include proportionally larger serum and wash buffer volumes to accommodate automated testing. The new format will minimize shipping costs and storage space requirements for large volume customers.

• **The XVIIIth Congress of The International Society on Thrombosis and Haemostasis (ISTH)** will convene in Paris, France on July 6-12, 2001 at the Palais des Congrès. An impressive Scientific Program with state-of-the-art lectures and a wide array of Symposia covering the most important advances in the field will be offered at the Congress, which is held every two years. **Corgenix** will participate in the Congress, with the presentation of two abstracts at the meeting, the publication of a third in the official Congress CD-ROM of abstracts published by Schattauer, and an exhibition booth. We invite you to visit our booth during the exhibition, or meet with us during the Poster session on Wednesday, July 11 at the Hall Havane. Contact Corgenix for additional information or visit the Congress website at: [www.isth2001.com](http://www.isth2001.com). Make your plans now to attend. The deadline for registration by mail is June 1, 2001. The following Corgenix abstracts will be presented at the meeting:

**Ten Month Follow up of Various Antiphospholipid Antibody Levels in a Patient with Antiphospholipid Syndrome**, Dier K, Taylor D, Fink CA, Lopez L. Abstract #5761.

**Comparative Sensitivity and Specificity of Various Antiphospholipid Antibodies for Thrombosis in Samples with Lupus Anticoagulant**, Lopez L, Dier K, Taylor D, Olsen L, Fink C, Levy V, Adcock D. Abstract #6005.

Selected for publication:

**Antiphospholipid Antibodies in Angioimmunoblastic Lymphadenopathy**, Taylor D, Cary E, Woodard J, Fink CA, Lopez L. Abstract #5760.

• **Reminder: The Third Annual Symposium on Hemostasis and Thrombosis** and a Coagulation Wet Workshop, sponsored by the Midwest Hemostasis and Thrombosis Laboratories under the direction of Dr. Douglas A. Triplett, will be held in Indianapolis, Indiana, on May 10-11, 2001. This year's Symposium will focus on the evaluation and management of the thrombotic patient, and will provide a comprehensive review of pathophysiology, laboratory testing, genetics and treatment modalities. The registration deadline is April 15, 2001. For additional information, call (765) 747-8445 or log on to the sponsor's website at [www.midwestcoag.com](http://www.midwestcoag.com).

**REAADER PRODUCT FEATURE**

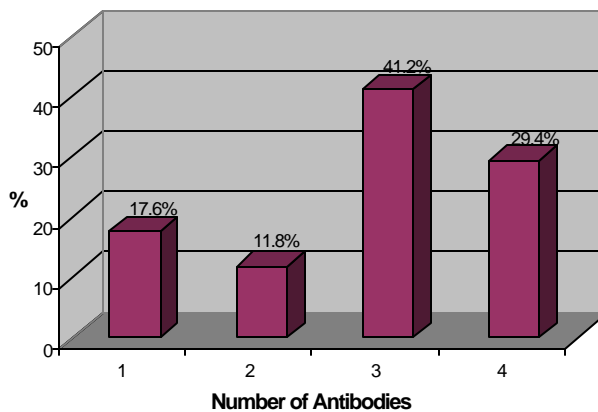
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Sample -	15 min @ room temperature
Conjugate -	15min @ room temperature
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Clinical Sensitivity -	SLE with thrombosis: IgG 75%, IgM 16%, IgA 40%; Primary APS: IgG 84%, IgM 60%, IgA 36%
Product numbers -	030-001 aPS IgG/IgM 10206 aPS IgA

Cont. from pg. 1:

**Patterns of Reactivity**



**Clinical Performance of APA Assays**

	aCL	aPS	aB2GPI	aPT	All assays combined
Sensitivity (%)	52	58	68	52	80
Specificity (%)	80	100	60	80	60
Accuracy (%)	58	67	67	58	75

**Published by:  
Corgenix, Inc.**

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