



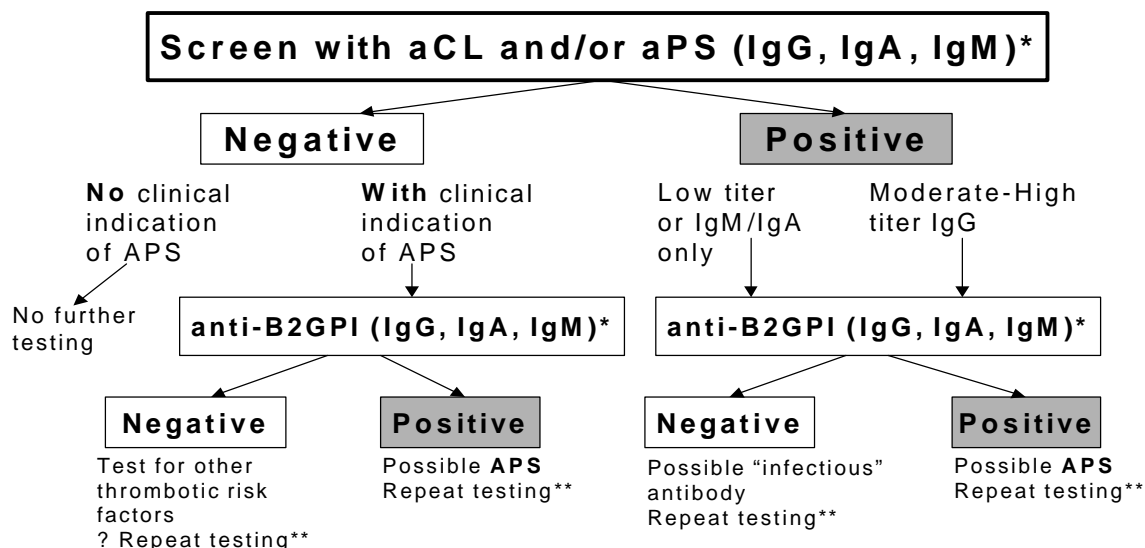
### A PRACTICAL ALGORITHM FOR THE LABORATORY EVALUATION OF ANTIPHOSPHOLIPID ANTIBODIES

The antiphospholipid syndrome (APS) is currently recognized as a common risk factor for arterial or venous thromboembolic disease. The presence of high serum levels of antiphospholipid antibodies in patients with APS has been strongly associated with thrombosis, and experimental evidence suggests that these antibodies play an important pathogenic role in this disease. Antiphospholipid antibodies are a very heterogeneous group of autoantibodies (IgG, IgM, IgA) initially thought to be specific only to anionic phospholipids. It is now well recognized that many clinically relevant antiphospholipid antibodies are directed to phospholipid/protein complexes, and furthermore, to proteins (cofactors) in the absence of phospholipids. In addition to various immunologic targets, laboratory methodologies used to detect these antibodies i.e. coagulation assays for Lupus Anticoagulants (LA), and ELISAs for anti-cardiolipin (aCL), anti-phosphatidylserine (aPS), and anti-Beta2 glycoprotein I (aB2GPI) antibodies, also demonstrate the heterogeneity of antiphospholipid antibodies. However, recent studies on the immunologic nature of some of these antibodies have provided a better

understanding of their clinical relevance and possible mechanisms of action in thrombosis (APS).

In the past few years, the evaluation of antiphospholipid antibodies has been surrounded by confusion in many clinical laboratories, and with the anticipated introduction of new technologies and serologic markers, it is likely that some confusion will continue. To assist the clinical laboratory in its routine evaluation of this heterogeneous group of antibodies, Corgenix has reviewed both in-house and current published research in this area to develop a practical algorithm for the evaluation of antiphospholipid antibodies. This algorithm takes into consideration the antibody titer and isotype as well as the patient's clinical information. This should facilitate the decision making process for subsequent testing and the final interpretation of the results. Laboratory results should always be interpreted in the context of clinical findings. LA has not been included in this algorithm, however, it must be kept in mind that the presence of LA has been shown to be more specific for thrombosis than antiphospholipid antibodies detected by ELISA and

### ANTIPHOSPHOLIPID ANTIBODY SCREENING PROCEDURE



\* Screen for each antibody isotype. Polyvalent screen not recommended  
 \*\*Repeat testing in 8-10 weeks for seroconversion or antibody persistence

## THE READER RESPONSE

**Q.** Our laboratory is evaluating methods for measuring Total and Free Protein S (PS) Antigen. We have noticed that Free PS values, expressed as a percentage (%), are higher in some samples than Total PS values, also reported in %. How can Free PS values be higher than Total PS, when normally only 40% of PS circulates in the free form?

**A.** Patient Total and Free PS antigen levels are compared to levels seen in a normal population. The % of Total PS in a patient sample is compared to the average Total PS level in a normal population, which, by definition, is 100%. Likewise, the % of Free PS in a patient sample is relative only to Free PS levels in a normal population (100%). Even though both levels are expressed as a % of normal, the measurements (Total and Free) are independent of each other.

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**(cont. from page 1)**

adds valuable information to the serologic diagnosis of APS.

ELISAs for aCL and aPS antibodies may detect different populations of antiphospholipid antibodies, including some not associated with thrombosis (APS), i.e. "infectious" antiphospholipid antibodies. These assays may also detect antibodies to bovine proteins present in blocking reagents or sample diluent. Thus, these assays may be used to screen for a wide range of antiphospholipid antibodies. A negative screening result in the absence of clinical findings of thrombosis (APS) would not require further testing. However, a negative screen with clinical findings suggestive of thrombosis would require additional testing with a more specific assay such as an anti-cofactor ELISA. Antibodies to the cofactor B2GPI have shown to be more specific for thrombosis than aCL or aPS. If anti-B2GPI ELISA is negative, testing for other thrombotic risk markers is recommended (Protein C, Protein S, antithrombin, APC Resistance, etc.), as well as repeat testing for seroconversion. An anti-B2GPI ELISA positive result would strongly point towards APS. Repeat testing is also recommended as the serologic diagnostic criteria requires the demonstration of persistently high serum levels of antiphospholipid antibodies.

A positive aCL and/or aPS ELISA would also require follow up testing for anti-B2GPI antibodies. A negative anti-B2GPI result may suggest the presence of "infectious" antiphospholipid antibodies which are usually present in low titers and transient. Repeat testing would help to clarify the significance of this antibody. A positive anti-B2GPI result would strongly suggest APS and/or increased risk of thrombosis.

## REAADER PRODUCT FEATURE

### **REAADS Anti-Beta 2 Glycoprotein I Semi-Quantitative Test Kits**

For *In Vitro* Diagnostic Use

Assay format -	96-well microtiter plate (8 x 12 strips) with breakaway wells
Antigen substrate -	Purified Beta 2 Glycoprotein I (human)
Conjugate -	Horseradish peroxidase (HRP) goat anti-human IgG, IgM, or IgA
Chromogenic substrate -	TMB (single component)
Stopping solution -	0.36 N Sulfuric acid
Sample -	Human serum, 1:50 dilution
Incubations	
Sample -	15 min @ room temperature
Conjugate -	15 min @ room temperature
Substrate -	10 min @ room temperature
Wavelength -	450 nm
Clinical specificity -	IgG 100%; IgM 93%; IgA 95%
Clinical sensitivity -	autoimmune population: IgG 28%; IgM 23%; IgA 27%
Product number -	037-001 IgG anti-B2GPI 038-001 IgM anti-B2GPI 039-001 IgA anti-B2GPI

## REAADER ANNOUNCEMENTS

- **NEW Reference Plasma Assay Sheet:** Corgenix is now including an assay sheet for the Reference Plasma in all new product lots of REAADS Hemostasis Antigen ELISAs. The levels for REAADS von Willebrand Factor Antigen, Protein C Antigen, Total Protein S Antigen and Free Protein S Antigen (Monoclonal and PEG) have been assigned against the ISTH/SSC Secondary Coagulation Standard (calibrated against WHO Standards).

- **Luis R. Lopez, M.D., CEO and Chairman of Corgenix, Inc.** has been invited by the American Society of Clinical Pathologists (ASCP) to present a teleconference on antiphospholipid antibodies in the Spring, 2000. Registration is currently open for the session entitled, "Laboratory Evaluation of the Antiphospholipid Syndrome" scheduled for May 17, 2000. Please contact ASCP or Corgenix for additional information.

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