

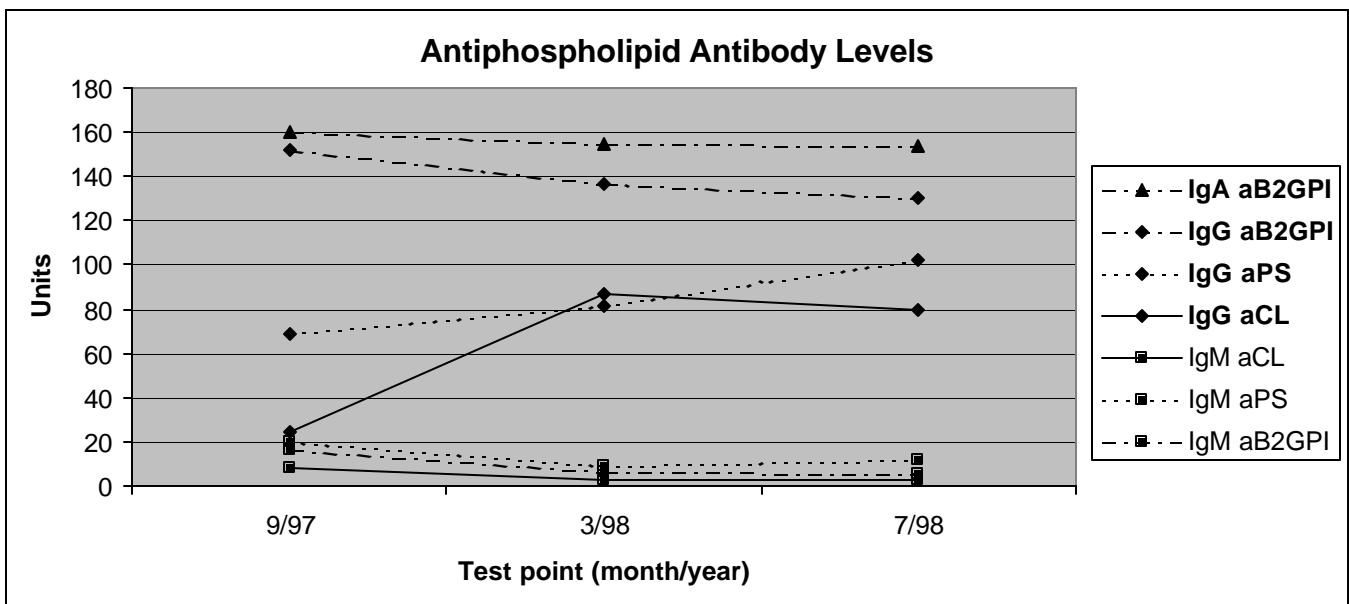


PRACTICAL APPLICATION OF SUGGESTED ANTIPHOSPHOLIPID TESTING ALGORITHM IN aCL NEGATIVE, aPS POSITIVE ANTIPHOSPHOLIPID SYNDROME PATIENT

Antiphospholipid antibodies are a heterogeneous group of autoantibodies with reactivity toward: 1) negatively-charged phospholipids (eg. cardiolipin, phosphatidylserine); 2) phospholipid-protein complexes; and 3) certain plasma proteins (cofactors) in the absence of phospholipids. Elevated levels of these antibodies are serologic markers for the diagnosis of the antiphospholipid syndrome (APS), clinically characterized by recurrent arterial or venous thrombosis, thrombocytopenia and/or fetal abortion. The anticardiolipin (aCL) ELISA, the test most commonly used for the diagnosis of APS, includes bovine serum in the test system as source of cofactor (B2GPI). This test may detect both "infectious" as well as cofactor-dependent or "autoimmune" antibodies. The anti-phosphatidylserine (aPS) ELISA uses a more physiologically relevant phospholipid antigen in addition to bovine serum as a source of cofactor. As with the aCL ELISA, the aPS assay may also detect both types of antibodies. It is widely accepted that the antigen for "autoimmune" antiphospholipid antibodies is located on the protein (cofactor) molecule, which may lead one to assume that the same "autoimmune" antibody would react in the aCL and aPS assays as they both contain cofactor. Accordingly, it has been suggested that aPS are the same as aCL antibodies and do not add to the diagnosis of APS. More recently it has been shown that "autoimmune" antibodies also

react in ELISA tests using purified cofactor (B2GPI) as the antigen, in the absence of phospholipids. Anti-B2GPI assays have been described as more specific for thrombosis (and APS) than aCL or aPS ELISAs.

In a previous issue of THE READER (Vol 6, #3, June 1996), Corgenix presented the results from an internal study that suggested that aCL and aPS antibodies were distinct, relative to cofactor dependency. More recently, Corgenix proposed an algorithm for the serologic evaluation of antiphospholipid antibodies (THE READER Vol 9, #5, October 1999), derived from a large in-house study of several hundred patients and a review of the literature. Corgenix has been following the case of an APS patient with an unusual serologic presentation over a period of time. This case illustrates the points discussed above. A 31 year-old female patient was hospitalized for renal failure. She was previously diagnosed with SLE and lupus nephritis, and had suffered 2 strokes several years before. On exam, skin changes suggestive of livedo reticularis and ulcers on both ankles were recorded. SLE serology (ANA and anti-dsDNA) was negative at this time. Her coagulation profile was interpreted as positive for Lupus Anticoagulant, with elevated D-dimer levels. No evidence of clinical thrombosis was found. These findings prompted an aCL antibody evaluation which was reported as

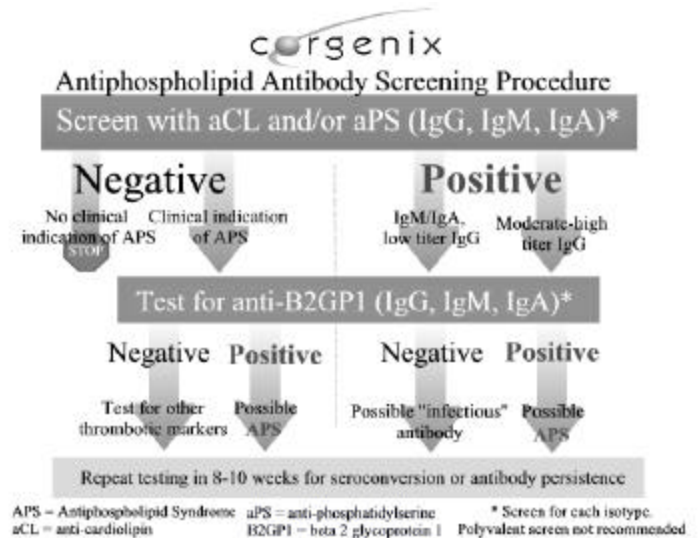


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negative/borderline for IgG aCL (24 GPL with a cut-off of 23 GPL), negative for IgM and IgA aCL. Because the clinical picture was suggestive of APS, the sample was also tested for aPS antibodies. IgG aPS was strongly positive (69 GPS with a cut-off of 16 GPS), with IgM aPS negative. This aCL negative, aPS positive patient was followed for 10 months, as shown in the graph on the previous page.

During the course of testing, IgG aCL levels increased with time from negative/borderline (24 GPL) to strongly positive (87 GPL) and remained high several months after diagnosis. IgM aCL remained negative throughout the follow-up period. IgG aPS levels gradually increased from the initial 69 GPS positive level to 102 GPS, while IgM aPS remained negative. The samples were later tested for anti-B2GPI antibodies, when the anti-B2GPI assay became available. The patient was strongly positive for both IgG and IgA anti-B2GPI from the outset.

This case illustrates the following points: 1) if the serologic diagnosis had been based only on aCL results, the diagnosis of APS would have been missed or delayed for 6 months when the patient seroconverted to a strong aCL positive. 2) Because IgG aPS was strongly positive when IgG aCL was low, it can be concluded that these antibodies are not only different, but behaved differently over time. In the proposed algorithm, it is suggested to use both aCL and aPS assays to screen for antiphospholipid antibodies, to minimize the number of patients missed. 3) Interestingly, IgG and IgA anti-B2GPI antibodies were strongly positive from the beginning, indicating that one test (i.e. aCL) may not be enough. Following the algorithm would have lead to an earlier diagnosis of APS and treatment in this patient. While the case presented is only one patient, the serologic follow-up suggests that the diagnosis of APS can be missed or delayed in some patients if the diagnosis is based on only one test (i.e. aCL) at a single testing point in time.



Proposed antiphospholipid algorithm READER PRODUCT FEATURE

REAADS Anti-Phosphatidylserine IgG/IgM ELISA Test Kit

For *In Vitro* Diagnostic Use

Assay format -	96-well microtiter plate (8 x 12 strips) with breakaway wells
Sample matrix -	Human serum
Sample dilution -	1:51
Antigen substrate -	Phosphatidylserine
Conjugate -	Horseradish peroxidase (HRP) conjugated anti-human IgG/M
Chromogenic substrate -	TMB (single component)
Stopping solution -	0.36 N Sulfuric acid
Assay incubations	
Sample -	15 min @ room temperature
Conjugate -	15min @ room temperature
Substrate -	10 min @ room temperature
Wavelength -	450 nm
Clinical specificity -	IgG 96%; IgM 96%

Clinical Sensitivity -	SLE with thrombosis: IgG 75%, IgM 16%; Primary APS: IgG 84%, IgM 60%
Product number -	030-001 aPS IgG/IgM

HAPPY HOLIDAYS!

*Best wishes to you and your families this Holiday Season from all of us at **Corgenix**! We appreciate your business and look forward to your continued support in the NEW YEAR.*

Corgenix will be operating on a limited schedule during the holidays, from 12:00 noon on Thursday, December 21 until 8:00 am on Tuesday, January 2, 2001. Please check your inventory and plan ahead to assure an adequate supply of product during the holidays. All standing orders due by the end of the month will be shipped during the week of December 18, to arrive before the holiday break. For technical assistance or an emergency delivery, please leave a message at (800) 729-5661 or (303) 457-4345, and a Customer Service Representative will return your call.

NEW Anti-Beta 2 Glycoprotein CPT Code: 86146

Through the efforts of **Corgenix**, the AMA established and recently published a unique CPT code for the anti-B2GPI assay (**86146**). This assay complements the Anti-Cardiolipin assay (**86147**) and the Anti-Phosphatidylserine assay (**86148**).

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