



## EVALUATION OF THE RELATIVE SENSITIVITY AND ACCURACY OF 4 ANTIPHOSPHOLIPID ANTIBODY ASSAYS

Due in part to the heterogeneous nature of antiphospholipid antibodies, the majority of antiphospholipid syndrome (APS) patients as well as those individuals at risk for developing thrombosis, present with more than one antiphospholipid antibody in different combinations. For this reason, a single assay performed in the clinical laboratory may not be sufficient to establish the diagnosis of APS or to properly assess the risk of thrombosis. In addition, elevated serum levels of one type of antiphospholipid antibody may indicate the presence of medical conditions unrelated to APS or thrombosis. For example, cofactor-independent anti-cardiolipin (aCL) and anti-phosphatidylserine (aPS) antibodies may occasionally be elevated in healthy individuals and are frequently found in patients with infectious diseases (i.e. syphilis, viral infections, etc.). These antibodies are not necessarily associated with thrombosis. Because of the low specificity (frequent false positives for thrombotic risk), aCL and aPS have been recommended for screening purposes. In contrast, antibodies to the cofactors B2GPI (anti-B2GPI) or prothrombin (aPT) are almost exclusively seen in APS patients and are strongly associated with thrombosis. This information has been incorporated into a practical algorithm developed by Corgenix to assist the clinical laboratory in the serologic evaluation of antiphospholipid antibodies.

Corgenix recently evaluated the clinical performance of four REAADS antiphospholipid assays with a large population of healthy and disease state samples. This evaluation studied the relative sensitivity and accuracy (2 x 2 analysis) of each antiphospholipid antibody against the "APL status" of the sample. A positive APL

status most likely indicates the presence of antiphospholipid antibodies clinically relevant for thrombosis or APS. The following serologic criteria were used to classify the samples as APL negative or positive: *Negative APL status:* 1) aCL, aPS and anti-B2GPI (anti-cofactor) tests negative; or 2) aCL and/or aPS positive but anti-B2GPI negative. *Positive APL status:* 1) aCL and/or aPS and anti-B2GPI (anti-cofactor) positive; or 2) positive only for anti-B2GPI, with moderate to high titer (>50 units). The study included serum samples from 40 healthy controls and 87 diseased states (40 autoimmune, 36 SLE, and 11 APS patients). In addition, plasma samples from 100 healthy controls, 81 LA positive and 56 LA controls (abnormal coagulation times but negative by accepted criteria for LA) were tested. The LA positive plasma samples were obtained from 2 independent institutions (group #1 and group #2). Because the LA activity was determined independently by each institution, the results are presented separately. All serum and plasma samples were tested with REAADS antiphospholipid ELISAs for IgG aCL, aPS, anti-B2GPI and aPT antibodies.

As expected, the prevalence of each IgG antiphospholipid antibody and APL status classification varied between the populations studied (controls versus disease states). Interestingly, only 64% of the APS patients had a positive APL status. This may be explained by the fact that the criteria for determining "APL status" is based only on serologic information, while the diagnosis of APS also includes clinical manifestations. A summary of the relative sensitivity (% RS) and accuracy (% acc) of each assay against the APL status of the diseased samples is shown below:

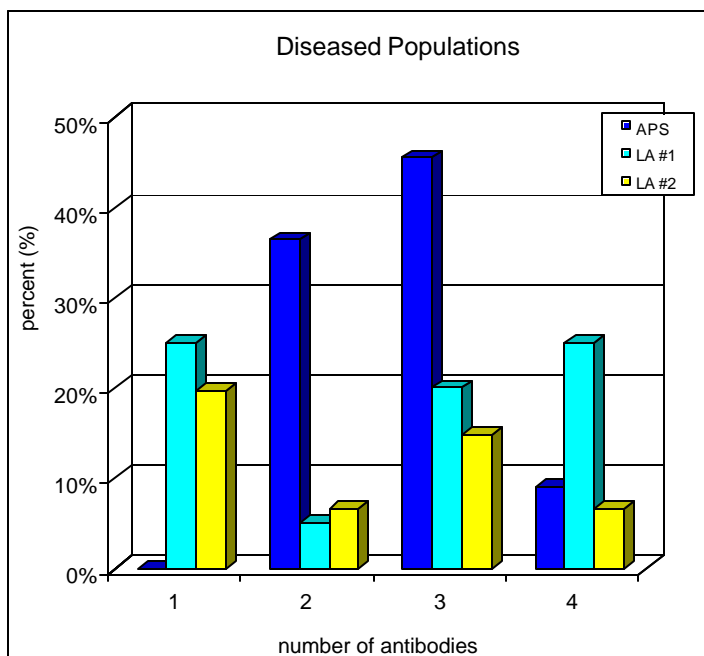
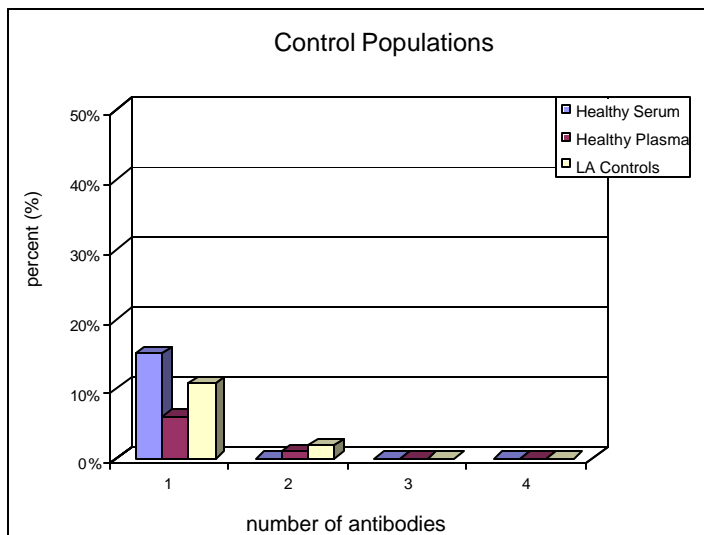
Test Sample	IgG aCL		IgG aPS		IgG anti-B2GPI		IgG aPT		APL Status % +
	% RS	% acc	% RS	% acc	% RS	% acc	% RS	% acc	
Autoimmune	95	72	95	77	100	92	53	72	47
SLE	71	83	86	91	100	94	14	69	19
APS	71	54	100	72	100	100	28	54	64
LA+ (grp 1)	64	75	50	65	100	100	57	65	70
LA+ (grp 2)	84	83	92	92	100	93	33	77	20
<b>mean</b>	<b>77</b>	<b>73</b>	<b>85</b>	<b>79</b>	<b>100</b>	<b>96</b>	<b>37</b>	<b>67</b>	

(cont. from pg. 1)

The high performance of the IgG anti-B2GPI assay (100% relative sensitivity, and 96% mean accuracy when compared to the APL status) was somewhat biased due to the prominent role of this assay in the defined serologic criteria for the APL status. The second best relative sensitivity (mean 85%, and 79% accuracy) was seen with the IgG aPS assay. IgG aCL, the most commonly used assay, ranked third (mean relative sensitivity 77%, and 73% accuracy). The mean relative sensitivity and % accuracy of the aPT assay (RS 37%, accuracy 67%) may be a reflection of the lower prevalence of antibodies to prothrombin compared to aB2GPI, as reported in the literature. Similar relative sensitivity and accuracy results were obtained in a previous study when a different group of LA positive plasma samples was evaluated using clinical criteria, i.e. history of thrombosis (*THE READER*, Vol. 11, # 2, April 2001). The results from the current study confirm the previous conclusion that

aPS and anti-B2GPI may be the best assay combination for the laboratory evaluation of antiphospholipid antibodies.

The pattern of reactivity (number of antibodies present in each sample) was also evaluated. The following graphs demonstrate that individuals in the control group frequently present only one antibody. In contrast, most of the patients in the diseased group present with 2 or more antibodies. This distribution was also seen in the previous study of LA positive samples with thrombosis (mentioned above), and confirms that most APS (autoimmune) patients present several antiphospholipid antibodies. For this reason, one assay may not be sufficient to establish the diagnosis of APS or to properly assess the risk of thrombosis. In addition, this information should be considered by clinical laboratories when selecting assays for the serologic evaluation of antiphospholipid antibodies and when interpreting results.



## READER PRODUCT FEATURE

### READS 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
Sample matrix -	Human serum
Sample dilution -	1:51
Antigen -	Purified human Beta 2 Glycoprotein I
Conjugate -	Horseradish peroxidase (HRP) / goat anti-human IgG, IgM, or IgA
Chromogenic substrate -	TMB (single component)
Stopping solution -	0.36 N Sulfuric acid
Assay 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 aB2GPI Test Kit 038-001 IgM aB2GPI Test Kit 039-001 IgA aB2GPI Test Kit

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