



CLINICAL SIGNIFICANCE OF IgG AND IgM AUTOANTIBODIES THAT TARGET THE COMPLEX OF PHOSPHATIDYLSERINE AND PROTHROMBIN (PS/PT)

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ABSTRACT

Objective: To demonstrate performance characteristics and clinical utility of an ELISA assay for detecting autoantibodies that react with a complex of phosphatidylserine and prothrombin (PS/PT).

Method: We tested 71 patients with anti-phospholipid syndrome (APS), 24 known Lupus Anticoagulant (LAC) positives, 247 random normals and 52 disease controls for IgG and IgM antibodies to PS/PT. These results were used to calculate performance characteristics and the new assays were compared to traditional anti- β_2 GPI and LAC assays. Matched serum and citrated plasma samples were tested to see if sample matrix had any influence on results.

Results: All LAC positive patients were found to be strongly positive for either IgG, IgM PS/PT antibodies or both. Most patients with APS were found to be PS/PT positive. Forty eight of the 71 APS patients (67.6%) were PS/PT positive and many of these individuals were found to be negative using more traditional assays such as anti- β_2 GPI and LAC.

Only 7 of 247 normals and 1 of the 52 disease controls were found to be positive for either IgG or IgM PS/PT antibodies for a combined specificity of 97.3% (8/299).

The assays were found to have high inter and intra run precision. Equivalent results were obtained with either serum or citrated plasma.

Conclusion: IgG and IgM autoantibodies that react with a physiologic complex of phosphatidylserine and prothrombin are sensitive markers for anti-phospholipid syndrome. These antibodies are present in APS patients that are negative by methods currently in use. The tests exhibit high specificity and reproducibility and can be run with serum or plasma specimens.

BACKGROUND

Antiphospholipid antibodies represent a large, heterogeneous group of immunoglobulins of considerable clinical importance due to their association with arterial and/or venous thrombosis, recurrent pregnancy loss, neurological disorders, pulmonary hypertension and thrombocytopenia. Clinical laboratories routinely use the anticardiolipin antibody ELISA and the lupus anticoagulant (LAC) clotting assay for aiding in diagnosis of antiphospholipid syndrome (APS). More and more laboratories are now including tests for detecting antibodies directed against phospholipid binding proteins, the best studied of which is β_2 GPI.

Prothrombin (factor II) is another phospholipid binding protein with procoagulant activity. A number of groups have definitively shown that antibodies targeting the complex of phosphatidylserine (PS) and prothrombin (PT) have significant clinical relevance due to their strong correlations with clinical features of APS and with the presence of LAC.¹⁻⁴ It was also shown that it is antibody to the PS/PT complex rather than antibodies that target prothrombin alone that correlate

with LAC and APS. (3,4) The PS/PT antibodies provide useful sensitivity for APS and have high specificity. Their inclusion into the laboratory criteria for classification of APS has been proposed⁴.

ASSAY CHARACTERISTICS

Antigen on solid phase is a layer of phosphatidylserine and human prothrombin coated in the presence of Ca ++.

Does not detect β_2 GPI reactive antibodies

- Sapporo monoclonals do not react
- Strong positive β_2 GPI do not react

Performance goals

- Detect many ACA and β_2 GPI negative APS patients
- Close approximation of LAC
- Replace ACA and/or β_2 GPI?

SPECIFIC PERFORMANCE CHARACTERISTICS

The performance of the QUANTA Lite™ PS/PT IgG and IgM kits was evaluated in one external and one internal study. Combined results are tabulated below (Table 1).

	No. Samples	PS/PT IgG Pos	PS/PT IgM Pos	IgG and/or IgM
Normals	247	3 (1.2%)	4 (1.6%)	7 (2.8%)
Lupus Anticoagulant Positive (LAC)	24	21 (87.5%)	19 (79.2%)	24 (100%)
Anti-Phospholipid Syndrome (APS)	71	33 (46.5%)	34 (47.9%)	48 (67.6%)
Rheumatoid Arthritis	6	0	0	0
Crohn's	2	0	0	0
Ulcerative Colitis	2	0	0	0
Celiac	5	0	0	0
LAC negative	8	0	1 (12.5%)	1 (12.5%)
Infectious disease (CMV, Toxo, rubella, HSV HBV HCV)	14	0	0	0
Syphilis	12	0	0	0
Actin Antibody Positive	1	0	0	0
H. Pylori	2	0	0	0

Performance of PS/PT IgG & IgM

The combined use of IgG and IgM PS/PT detected all 24 known lupus anticoagulant positives and 67.6% of the APS patients. Only 7 of 247 normals and 1 of another 52 disease controls tested positive for a combined specificity of 97.3% (8/299).

Performance of PS/PT IgG and IgM kits with 20 Positive LAC Samples and 4 Borderline Positives (Table 2)

LAC POSITIVE PS/PT Units (pos>30)			LAC BORDERLINE PS/PT Units (pos>30)		
Sample	IgG	IgM	Sample	IgG	IgM
1	141	81.2	1	22.1	132.6
2	146	61.6	2	75.2	7.8
3	139	60.2	3	217.5	21.6
4	151	67.6	4	278.5	35.6
5	144	54.4			
6	112	39.7			
7	213	11.5			
8	217	10.4			
9	21.2	98.7			
10	125.6	51.6			
11	51.3	406			
12	224	16.6			
13	97	138			
14	157	132			
15	152	65.3			
16	173	305			
17	147	253			
18	136	63.7			
19	15.5	114			
20	88.3	109			

Relative Performance to β_2 GPI IgG

		IgG PS/PT		<ul style="list-style-type: none"> • Relative Sensitivity = 79.2% • Relative Specificity = 87.9% • Relative Agreement = 85.6%
		+	-	
IgG β_2 GPI	+	38	10**	
		-	16*	116

* 1 of the 16 was LAC positive and the other 15 were APS patients

** All 10 were from the APS group

Relative Performance to β_2 GPI IgM

		IgM PS/PT		<ul style="list-style-type: none"> • Relative Sensitivity = 80% • Relative Specificity = 82.8% • Relative Agreement = 82.2%
		+	-	
IgG β_2 GPI	+	28	7†	
		-	25‡	120

† 1 of these was a normal and 6 were from the APS group

‡ 22 were from the APS group and 3 were LAC positives

RELATIVE PERFORMANCE

The relative agreement for both IgG and IgM PS/PT kits with respect to the β_2 GPI kits is good at 85.6% and 82.2%. Most of the discrepant results are due to the higher sensitivity of the IgG and IgM PS/PT kits for both the LAC positives and especially the APS patients.

The PS/PT kits displayed excellent specificity. The PS/PT IgG plus IgM kits detected all LAC positive samples and most of the APS patients. In fact, the sensitivity of the PS/PT kits for APS exceeded that of the predicate β_2 GPI assays.

It was noticed that the vast majority of APS patients were positive for PS/PT and/or β_2 GPI .

Precision and Reproducibility

Six samples were tested as six replicates on the PS/PT IgG ELISA on six different days. Total n= 36. The unit value results are summarized below (Table 5).

Table 5						
IgG unit value	139.3	79.3	36.8	22.2	8.3	53.8
Intra-assay Sdev	6.8	4.9	2.5	1.7	0.8	3.4
%CV	4.9	6.2	6.7	7.8	9.9	6.4
Inter-assay Sdev	4.2	3.3	1.6	1.2	0.4	2.3
%CV	3.0	4.2	4.5	5.3	5.2	4.2

Another six samples were tested as six replicates on the PS/PT IgM ELISA on the same day. Total n= 36. The unit value results are summarized below (Table 6).

Table 6						
IgG unit value	153.2	75.8	36.0	18.2	9.8	58.2
Intra-assay Sdev	5.9	3.3	1.7	0.7	0.6	2.6
%CV	3.8	4.4	4.6	3.8	6.3	4.5
Inter-assay Sdev	4.3	2.4	1.1	0.5	0.4	2.1
%CV	2.8	3.2	3.0	2.6	3.8	3.6

PS/PT Serum vs Plasma

Table 7				
Sample	PS/PT Units (pos>30)		PS/PT Units (pos>30)	
	plasma	serum	plasma	serum
1	134.1	129.4	52.5	49.3
2	149.3	148.5	61.1	61.2
3	135.7	125.7	53.3	53.3
4	26.2	26	103.1	113
5	251.9	239.9	9.6	9.7
6	245.6	236	9.1	8.3
7	2.4	2.5	8.7	8
8	15.7	14.2	22.1	15.5
9	2.8	2.4	7.1	5.4
10	5.5	5.9	4.9	4.4
11	2.4	2.3	7.3	5.8
12	3.2	3.7	6.7	5.4
coeff	0.99972		0.996411	
intercept	0.20278		2.734478	
slope	1.043495		0.921563	

CONCLUSIONS

- **PS/PT IgG and IgM ELISA detects a majority of LAC positives.**
- **Appears to be very specific.**
- **Detects most APS patients including many that are ACA, β_2 GPI , LAC negative.**
- **Provides an efficient solution for APS testing.**

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