

**Antiphospholipid Antibody Recovery in Serum and Citrated  
Plasma by 4 different Assays.  
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**ABSTRACT**

The presence of high serum levels of antiphospholipid antibodies are strongly associated with the antiphospholipid syndrome (APS) clinically characterized by thrombosis. The serologic diagnosis of APS requires a positive result of a coagulation assay for lupus anticoagulant activity or ELISA. Therefore, these assays are performed in immunology and coagulation laboratories on both serum and plasma samples. To study the recovery of 4 different antiphospholipid assays on serum vs. plasma, 36 paired serum and 3.2% sodium citrated plasma samples were collected from both normal and disease state patients. All samples were tested in duplicate for IgG and IgM anticardiolipin (aCL), antiphosphatidylserine (aPS), anti-beta 2 glycoprotein I (aB2GPI), and antiprothrombin (aPT) antibodies using commercially available test kits (REAADS) following the manufacturers recommended procedure. (Table). The mean serum and plasma values for each assay were statistically similar (single factor ANOVA,  $p > 0.05$ ). Positive and negative interpretation was identical between serum and plasma for all of the assays and antibody isotypes, except for 5 determinations that were borderline values. When comparing the individual values of the paired samples, the correlation coefficient ( $r$ ) was  $> 0.960$ . In summary, the overall reactivity of the serum and plasma samples was comparable for all of the assays. These results indicate that testing a patient's serum or plasma specimen will produce similar values in any of the test kits studied. Results from either serum or plasma would be valuable in the serologic diagnosis of APS (and evaluation of thrombosis) in the clinical laboratory.

**INTRODUCTION**

The presence of high serum levels of antiphospholipid antibodies is strongly associated with the antiphospholipid syndrome (APS), clinically characterized by recurrent arterial or venous thrombosis, thrombocytopenia and fetal loss. The current serologic diagnostic criteria for APS includes a positive result in coagulation assays for lupus anticoagulant (LA) activity and/or a positive ELISA result for antiphospholipid antibodies (commonly for IgG or IgM anticardiolipin antibodies). Due to the association of these antibodies with thrombosis, antiphospholipid assays are frequently performed in immunology and coagulation laboratories on both serum and/or plasma samples. In addition, due to the heterogeneous nature of antiphospholipid antibodies, their serologic evaluation in the clinical laboratory may require several assays.

**OBJECTIVE**

- To evaluate and compare the recovery of serum and plasma samples using four different antiphospholipid ELISA assays (aCL, aPS, aB2GPI, and aPT) on matched serum and 3.2% sodium citrate plasma samples from both healthy donors and disease state populations.

**MATERIALS AND METHODS**

36 matched serum and 3.2% sodium citrate plasma samples were collected from healthy donors and disease state populations. All samples were tested in duplicate for IgG and IgM aCL, aPS, aB2GPI, and aPT antibodies using REAADS ELISA test kits, following the manufacturer's recommended procedures:

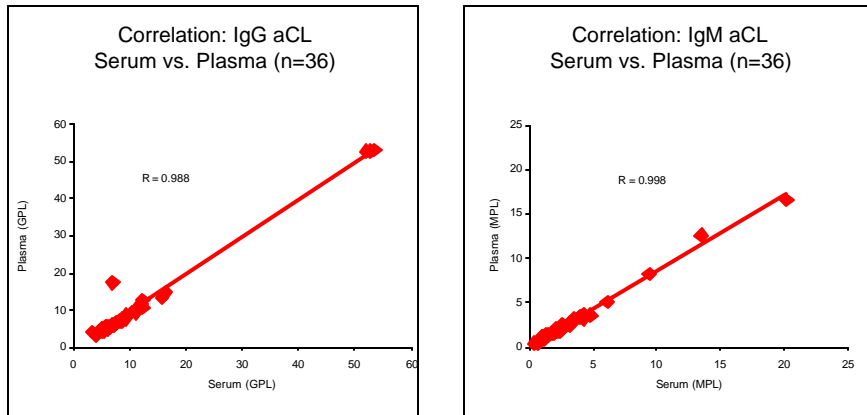
**anti-Cardiolipin ELISA:** Purified bovine cardiolipin was coated onto 96-microwell plates, blocked and stabilized. 100uL of diluted patient serum (1:50) in sample diluent containing bovine B2GPI was incubated in coated microwells for 15 minutes at room temperature. After washing, 100uL of HRP conjugated anti-human antibody heavy chain specific for IgG or IgM was added for another 15 minute incubation, followed by TMB substrate. The reaction was stopped with 0.36N H2SO4 and absorbance read at 450/650 nm.

**anti-Phosphatidylserine ELISA:** Purified bovine phosphatidylserine was coated onto 96-microwell plates, blocked and stabilized. 100uL of diluted patient serum (1:50) in sample diluent containing bovine B2GPI was incubated in coated microwells for 15 minutes at room temperature. After washing, 100uL of HRP conjugated anti-human antibody heavy chain specific for IgG or IgM was added for another 15 minute incubation, followed by TMB substrate. The reaction was stopped with 0.36N H2SO4 and absorbance read at 450/650 nm.

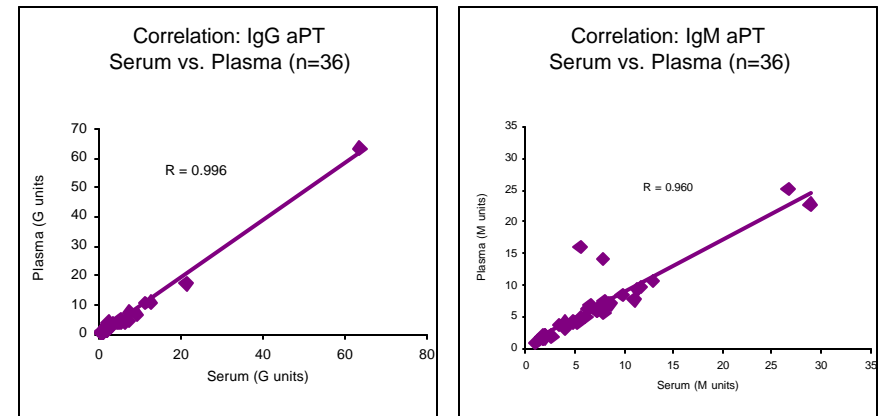
**anti-Beta 2 Glycoprotein I ELISA:** Purified human B2GPI (purity  $> 95\%$  SDS-PAGE) was coated onto 96 well micro-plates, blocked, and stabilized in the absence of exogenous B2GPI. 100uL of diluted patient serum (1:50) in sample diluent containing no B2GPI was incubated in coated microwells for 15 minutes at room temperature. After washing, 100uL of HRP conjugated anti-human antibody heavy chain specific for IgG or IgM was added for another 15 minute incubation, followed by TMB substrate. The reaction was stopped with 0.36N H2SO4 and optical density read at 450/650 nm.

**Anti-Prothrombin ELISA:** Purified human prothrombin was coated onto 96 well micro-plates, blocked and stabilized. 100uL of diluted patient serum (1:51) in sample diluent was incubated in coated microwells for 15 minutes at room temperature. After washing, 100uL of HRP conjugated anti-human antibody heavy chain specific for IgG or IgM was added for another 15 minute incubation, followed by TMB substrate. The reaction was stopped with 0.36N H2SO4 and optical density read at 450/650 nm.

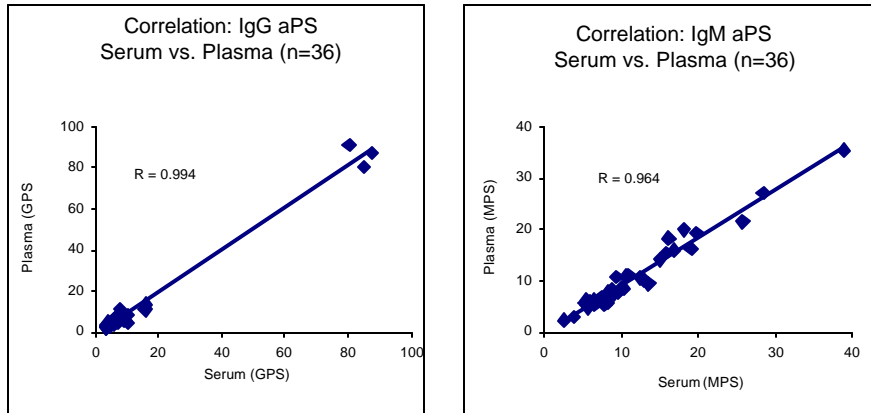
**Graph #1 Correlation of 36 Matched Serum vs. Plasma for aCL antibodies**



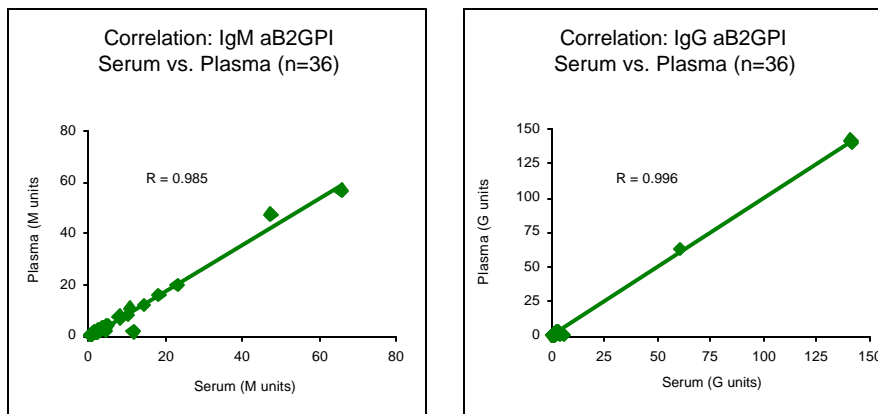
**Graph #4: Correlation of 36 Matched Serum vs. Plasma for aPT antibodies**



**Graph #1 Correlation of 36 Matched Serum vs. Plasma for aPS antibodies**



**Graph #1 Correlation of 36 Matched Serum vs. Plasma for aB2GPI antibodies**



## SUMMARY

The values of each matched serum and citrated plasma sample were calculated against the kit calibrator and expressed in the respective kit units. Results (mean + 1SD) are summarized in the table below.

Assay	Serum Mean (1SD)	Plasma Mean (1SD)	p-value (ANOVA single factor)	Correlation Coefficient (r value)
aCL IgG (GPL)	<b>11.6</b> (13.0)	<b>11.2</b> (13.1)	0.891	0.988
aCL IgM (MPL)	<b>3.5</b> (4.2)	<b>3.0</b> (3.5)	0.586	0.998
aPS IgG (GPS)	<b>13.1</b> (22.0)	<b>12.4</b> (22.8)	0.893	0.994
aPS IgM (MPS)	<b>13.6</b> (8.5)	<b>12.4</b> (8.1)	0.547	0.964
aB2GPI IgG (G units)	<b>11.6</b> (33.6)	<b>10.8</b> (33.8)	0.982	0.996
aB2GPI IgM (M units)	<b>8.2</b> (13.1)	<b>6.9</b> (12.1)	0.662	0.985
aPT IgG (G units)	<b>5.4</b> (10.8)	<b>4.8</b> (10.7)	0.834	0.996
aPT IgM (M units)	<b>7.4</b> (5.9)	<b>6.5</b> (5.2)	0.687	0.960

## CONCLUSIONS

- The mean serum and citrated plasma values for each assay were statistically similar (single factor ANOVA,  $p > 0.05$ ).
- The positive and negative interpretation of the results was identical between serum and citrated plasmas for all of the assays and antibody isotypes (IgG, IgM), except for 5 determinations in which the results were classified as borderline (near the cut-off value of the assay).
- When comparing the individual values of the matched samples, the correlation coefficient (r) was  $> 0.960$  for all assays.
- These results indicate that individual values as well as the overall reactivity of matched serum and citrated plasma samples were comparable for all assays performed. With any of the test kits studied, testing a patient's serum or citrated plasma specimen should produce similar values.