



EVALUATION OF ANTIPHOSPHOLIPID ANTIBODY RECOVERY OF MATCHED SERUM AND CITRATED PLASMA SAMPLES ON FOUR ANTIPHOSPHOLIPID ASSAYS

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. To evaluate the recovery of serum and plasma samples on 4 different antiphospholipid ELISA assays, 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 anti-cardiolipin (aCL), anti-phosphatidylserine (aPS), anti-beta 2 glycoprotein I (aB2GPI), and anti-prothrombin

(aPT) antibodies using READS test kits, following the recommended procedures. The values of each matched serum and citrated plasma sample were calculated against the kit calibrator and expressed in the respective kit units. Results are summarized in the table below.

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 and citrated plasma specimen produced similar values. Results from either serum or 3.2% citrated plasma samples would be valuable in the serologic diagnosis of APS (and evaluation of thrombosis) in the clinical laboratory.

READS assay (units)	Serum Mean (1SD)	Citrated Plasma Mean (1SD)	Correlation Coefficient (r value)
aCL IgG (GPL)	11.6 (13.0)	11.2 (13.1)	0.988
aCL IgM (MPL)	3.5 (4.2)	3.0 (3.5)	0.998
aPS IgG (GPS)	13.1 (22.0)	12.4 (22.8)	0.994
aPS IgM (MPS)	13.6 (8.5)	12.4 (8.1)	0.996
aB2GPI IgG (G units)	11.6 (33.6)	10.8 (33.8)	0.996
aB2GPI IgM (M units)	8.2 (13.1)	6.9 (12.1)	0.985
aPT IgG (G units)	5.4 (10.8)	4.8 (10.7)	0.996
aPT IgM (M units)	7.4 (5.9)	6.5 (5.2)	0.960

THE READER RESPONSE

Q. Our coagulation laboratory is running the **REAADS** Anti-Cardiolipin (aCL), Anti-Phosphatidylserine (aPS), and Anti-Beta-2 Glycoprotein I (aB2GPI) IgG, IgM, and IgA ELISAs, along with lupus anticoagulant testing as an antiphospholipid antibody panel. Since citrated plasma is required as the sample matrix for lupus anticoagulant testing, can the same sample be used in the REAADS antiphospholipid ELISAs?

A. Your question is very timely, since **Corgenix** has just completed a study comparing the recovery of serum and 3.2% citrated plasma with the **REAADS** ELISA antiphospholipid kits (see feature article). In our study, serum and plasma results were shown to be equivalent with both normal and disease state patient samples. We are currently compiling the data to support the use of 3.2% citrated plasma or serum as the sample matrix in the REAADS aPS and aB2GPI assays, for submission to the FDA. (The sample matrix for the REAADS aCL IgG/IgM and IgA assays currently includes plasma, in addition to serum). As soon as the change is cleared by the FDA, we will modify the sample matrix section of the aPS and aB2GPI product inserts to include 3.2% citrated plasma. This change will be announced in a future edition of *THE READER*. We will also notify our customers when the change is implemented. In the meantime, contact Technical Support at **Corgenix** for additional information or a copy of our data.

NCCLS UPDATE

NCCLS recently announced that the *I/LA22-P Detection of Anticardiolipin Antibodies by ELISA: Proposed Guideline* project has been cancelled and NCCLS has discontinued distribution of the related document. As many of you are aware, NCCLS drafted the proposed guideline to "provide guidance for performing an ELISA for the detection of anticardiolipin (aCL)" antibodies. Unfortunately, some of the recommendations included in the document were controversial and not scientifically justified. After reviewing the responses submitted during the comment period, the Subcommittee on Anti-Phospholipid Antibodies was apparently unable to reach a consensus and the project was discontinued. The opinions of all who participated in the NCCLS consensus process were valuable to the efforts to improve anti-cardiolipin testing.

READER 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
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

READER ANNOUNCEMENTS

- A new, unique **CPT Code for Anti-Beta 2 Glycoprotein I, #86146**, established by the AMA, is in effect, as of January 2001. The AMA now recognizes three distinct markers for the measurement of antibodies related to the Antiphospholipid Syndrome: Anti-Cardiolipin (**86147**), Anti-Phosphatidylserine (**86148**), and Anti-Beta-2 Glycoprotein I (**86146**).
- The 3rd **Annual Symposium on Hemostasis and Thrombosis** for 2001, organized by the Midwest Hemostasis and Thrombosis Laboratories, will be held in Indianapolis, Indiana, on May 10-11. The *Diagnosis and Management of the Thrombophilic Patient* is the focus of this year's meeting, which includes a wet workshop in addition to the scientific symposium. For additional information, call (888) 272-2624, or log on to the sponsor's website at www.midwestcoag.com.

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