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Is There Clinically Relevant Plasma Interference with ELISA Detection of APS Antibodies? Reproducibility of Real-World Paired Serum and Plasma Testing

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The detection of antiphospholipid antibodies is a cornerstone requisite practice in the evaluation and diagnosis of antiphospholipid syndrome (APS). APS is commonly characterized by the detection of persisting antiphospholipid antibodies in the clinical context of thrombotic or obstetric manifestations. The 2006 Sydney Criteria outline clinical criteria and define anticardiolipin (aCL), anti-β-2 glycoprotein-1 (β2GP1), and the lupus anticoagulant in classifying definite APS.[1] Other antibodies, including anti-phosphatidylserine-prothrombin (aPSPT), have been identified and linked to APS though their clinical utility remains an area of uncertainty and subject to ongoing research.

In the ELISA (enzyme-linked immunosorbent assay) detection of APS antibodies, the use of serum is thought preferable over plasma according to international consensus bodies and guidelines. The cited concerns include the dilution effects of the citrate anticoagulant needed in plasma sampling, possible matrix effects from fibrinogen, and potential for interference from platelets.[2–4]

There are few studies suggesting that paired serum and plasma ELISA testing for antiphospholipid antibodies produce comparable quantitative values.[5–7] Further, there is a lack of replicating studies on a well-defined clinical population in a real-world clinical setting. Therefore, we sought to evaluate the reproducibility of ELISA detection of antiphospholipid antibodies in an all-comer clinical population undergoing medical evaluation for APS.

A cross-section of all patients from our institution from June 2017 to December 2018 was identified undergoing serologic evaluations for suspected or previously diagnosed APS. The cohort underwent serum tests for immunoglobulin G and immunoglobulin M isotypes of aCL and β2GP1 which are recognized in the current 2006 Sydney APS classification criteria. Serologic testing used commercially available ELISA assays (QUANTA Lite, Inova Diagnostics, San Diego, California, United States) in a CLIA (Clinical Laboratory Improvement Amendments)-accredited medical laboratory. The same ELISA tests were repeated once from the same patient on the same day, platelet-poor citrated plasma. aPS-PT antibodies, which are not a part of current Sydney APS classification criteria, were also found to be tested in a small minority of patients and thus presented opportunity for

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Table 1 Reproducibility of aCL, β2GP1, and aPS-PT antibody levels in paired serum versus plasma

	N samples	Quantitative levels			Intersample agreement of reference value intervals
		Median difference (IQR)[a]	Mean difference (SD)[a]	*p*-Value	Weighted κ coefficient (95% CI)
IgG aCL	134	0.0 (0.0–0.0)	1.4 (11.8)	<0.001	0.86 (0.78–0.94)
IgM aCL	134	0.0 (0.0–2.1)	2.1 (9.4)	<0.001	0.85 (0.76–0.93)
IgG β2GP1	110	0.0 (0.0–0.0)	0.8 (5.6)	0.018	1.00 (1.00–1.00)
IgM β2GP1	110	0.0 (0.0–0.05)	1.8 (11.6)	<0.001	0.80 (0.68–0.92)
IgG aPS-PT	7	0.0 (0.0–0.0)	1.6 (4.3)	>0.10	[b]
IgM aPS-PT	7	0.0 (−1.4 to 0.0)	−0.9 (1.9)	>0.10	[b]

Abbreviations: aCL, anticardiolipin; aPS-PT, anti-phosphatidylserine-prothrombin; IgG, immunoglobulin G; IQR, interquartile range; SD, standard deviation; β2GP1, anti-β2 glycoprotein-1.
Note: Reference value intervals of negative, weakly positive, or positive.
[a](Serum)–(plasma) in units of GPL, MPL, or U/mL.
[b]Sample number threshold not met for statistical testing.

repeat testing by plasma (QUANTA Lite, Inova Diagnostics, San Diego, California, United States).

Plasma samples were stored at −80°C and tested within 3 weeks. Quantitative antibody levels were determined for each isotype without citrate volume correction and then stratified into the following reference value intervals for aCL and β2GP1: negative (<15.0 GPL/MPL or U/mL), weakly positive (15.0–39.9), or positive (≥40.0). For aPS-PT, the following reference value intervals were used: negative (≤30 U), borderline (30.1–39.9), or positive (≥40.0).

Patient demographics and clinical characteristics were summarized using descriptive statistics (medians, percentages, etc.). Paired differences of antibody levels were compared using Wilcoxon paired signed-rank tests. Inter-sample reliability of resulted reference value intervals was compared using kappa coefficients. Analyses were performed using SAS version 9.4 (SAS Institute, Cary, North Carolina, United States).

Proportion of those with autoimmune diseases were enumerated as determined by the related specialty physician. Particularly, patients diagnosed with APS met 2006 Sydney Criteria (revised 1999 Sapporo Criteria) and systemic lupus erythematosus (SLE) met 2012 SLICC (Systemic Lupus International Collaborating Clinics) criteria.

One hundred fifty patients were identified for study with 134 aCL, 110 β2GP1, and 7 aPS-PT unique serum samples eligible for subsequent paired plasma testing. The cohort mean age was approximately 49 ± 17 years. Approximately 69% were female and 87% were white. Approximately 51% had one or more autoimmune condition. Notably, 21% had APS which included both newly diagnosed disease related to testing within the study's time frame and previously established disease. Approximately 13% met the criteria for SLE.

As shown in ►**Table 1**, median differences between serum and plasma were 0.0 units across all isotypes tested. There was a tendency for serum quantitative levels to have slightly higher values than plasma, and the differences were technically statistically significant. However, the mean differences were small and ranged within 2.1 units. There was strong inter-sample agreement in resulted laboratory reference interval categories with kappa coefficients ranging from 0.80 to 1.00.

To our knowledge, we present the largest study examining the reproducibility of antiphospholipid antibody detection on paired serum and plasma samples in a real-world, all-comer clinical setting. The study was able to detect small differences between serum and plasma antiphospholipid antibody levels. There was a tendency for antibody levels from serum to be of slightly higher value when compared with plasma. The small differences detected may very well reflect previously cited concerns of citrate dilution and matrix effects. These effects, singly or in combination, would have a negative bias and underestimate the true result. However, the quantitative differences between the paired samples are notably small at no more than 2.1 units and are likely not of significant clinical relevance in the medical practice setting ultimately. This notion is corroborated by the strong inter-sample agreement between serum and plasma in the resulting reference intervals stratifying into negative, weakly positive, and positive.

Strengths of this study includes the large sample size which has aided in detection of very small differences. Compared with prior studies, this study's use of real-world clinical patients with commercially available laboratory kits improves generalizability to the clinical setting. Though it should be noted that this study cohort is derived from a predominantly white North American tertiary referral population which may have other limitations in applying to other ethnic populations. There have been reports of differences in APS antibody distribution and resulting clinical phenotypes that may need additional considerations in interpreting and applying this study's data.[8]

In conclusion, this study finds small differences in the detection of APS antibodies between serum and plasma which do not appear clinically significant. These findings suggest that plasma could reasonably be used interchangeably in lieu of serum for ELISA detection of antiphospholipid antibodies.

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Conflict of Interest
None declared.

References

1 Miyakis S, Lockshin MD, Atsumi T, et al. International consensus statement on an update of the classification criteria for definite antiphospholipid syndrome (APS). J Thromb Haemost 2006;4 (02):295–306
2 Wong RC, Favaloro EJ, Adelstein S, et al. Consensus guidelines on anti-beta 2 glycoprotein I testing and reporting. Pathology 2008; 40(01):58–63
3 Andreoli L, Malacarne F, Ceribelli A, Kutschera I, Tincani A. Anti-cardiolipin and anti-β2-glycoprotein I antibodies: performance of new commercial ELISA kits. Ann N Y Acad Sci 2007;1109(01): 531–537
4 Wong RCAustralasian aCL Working Party. Consensus guidelines for anticardiolipin antibody testing. Thromb Res 2004;114 (5–6):559–571
5 Lewis D, Pound M, Ortel T. The reactivity of paired plasma and serum samples are comparable in the anticardiolipin and anti-β2-glycoprotein-1 ELISAs. Reply to a rebuttal. J Thromb Haemost 2006;4(06):1435–1437
6 Dier KOL, Fink C, Lopez L. Antiphospholipid antibody recovery in serum and citrated plasma by 4 different assays. Paper presented at: AACC/ASCLS/CSCC Annual Meeting; July 31–August 3, 2001; Chicago, Illinois, United States
7 Lewis DA, Pound ML, Ortel TL. The reactivity of paired plasma and serum samples are comparable in the anticardiolipin and anti-β2-glycoprotein-1 ELISAs. J Thromb Haemost 2006;4(01): 265–267
8 Uthman I, Khamashta M. Ethnic and geographical variation in antiphospholipid (Hughes) syndrome. Ann Rheum Dis 2005;64 (12):1671–1676