

Optimising antiphospholipid antibody testing: real-world performance of chemiluminescent immunoassays and revised clinical cut-offs in a hospital setting

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INTRODUCTION

Although the 2023 EULAR/ACR classification criteria¹ recommend ELISA for antiphospholipid antibody detection, many laboratories have transitioned to automated chemiluminescent immunoassays (CLIA) due to advantages such as faster turnaround times and reduced manual handling. However, manufacturer-defined cut-offs for CLIA platforms are derived from healthy donor populations, which may not reflect the clinical complexity of hospital cohorts and can lead to misclassification. Current guidelines advise that laboratories using non-ELISA methods establish their own clinical decision points to ensure diagnostic accuracy.

AIM

To evaluate the performance of the Werfen AcuStar CLIA platform for anticardiolipin (aCL) IgG and anti-β₂ glycoprotein I (β₂GPI) IgG and determine revised cut-offs based on the 99th centile of APS-negative hospital patients.

METHOD

We conducted a retrospective audit of aCL IgG and β₂GPI IgG tests performed on AcuStar over 12 months (August 2024–July 2025) at Northern Pathology Victoria, Australia. Positive results and clinical findings were adjudicated by two clinicians to confirm a diagnosis of antiphospholipid syndrome (APS).

CONCLUSIONS

- Manufacturer cut-offs for CLIA-based APS testing may overestimate positivity in hospital populations.
- Revised 99th centile thresholds substantially improve PPV without compromising sensitivity. We recommend adopting revised cut-offs with an “equivocal” category and confirmatory testing for new positives to enhance diagnostic accuracy.

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RESULTS

789 samples were included in the analysis, including 19 from patients who met criteria for APS.

- 93 aCL IgG and 57 β₂GPI IgG tests were positive at ≥20 CU (Table 1).
- Using manufacturer cut-offs, aCL IgG demonstrated 89.5% sensitivity and 90.1% specificity, while β₂GPI IgG showed 100% sensitivity and 95.1% specificity.
- However, positive predictive value (PPV) was low (18.3% aCL IgG; 33.3% β₂GPI IgG), indicating a high rate of false positives.
- Revised thresholds were adjusted to the 99th centile (≥65 CU for aCL IgG and ≥90 CU for β₂GPI IgG), derived from the 770 APS-negative patients across inflammatory, thrombotic and obstetric presentations.
- PPV improved to 66.7% for aCL IgG and 68.0% for β₂GPI IgG, with only a modest reduction in sensitivity (Table 2).
- Combined antibody positivity using revised cut-offs yielded a sensitivity of 94.7%, specificity of 98.4%, and PPV of 60.0%.
- Importantly, only one APS case would have been missed under the revised strategy, but it remained detectable by lupus anticoagulant testing.

- Based on these findings, locally derived reporting thresholds have been defined (Table 3):
- Equivocal: results between the manufacturer’s cut-off and locally defined positive threshold, of uncertain significance and less likely to be associated with APS.
- Positive: >99th centile of APS-negative patients.
- High positive: defined according to thresholds reported by Vandavelde et al.²

aCL/β ₂ GPI level (CU)	aCL IgG (n)	β ₂ GPI IgG (n)*
<20	696	731
20–40	58	23
40–80	16	8
>80	19	26

Table 1. Distribution of results for aCL IgG and β₂GPI IgG tests performed on the Werfen AcuStar CLIA platform. *1 patient sample was not processed. CU – chemiluminescent units.

Cut-off strategy	Sensitivity (%)	Specificity (%)	PPV (%)
aCL ≥20 CU	89.5	90.1	18.3
aCL ≥65 CU	84.2	99.0	66.7
β ₂ GPI ≥20 CU	100.0	95.1	33.3
β ₂ GPI ≥90 CU	89.5	99.0	68.0
Combined (revised cut-offs)	94.7	98.4	60.0

Table 2. Sensitivity and specificity for manufacturer and 99th centile revised cut-offs. The combined revised cut-off values were determined based on aCL and/or β₂GPI IgG positivity. PPV – positive predictive value.

Definition	aCL IgG (CU)	β ₂ GPI IgG (CU)
Negative	<20	
Equivocal	20–65	20–90
Positive	≥65	≥90
High Positive	≥770	≥2800

Table 3. Study-derived local cut-offs.

CONTACT INFORMATION

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