Received: 12 January 2025 | Revised: 4 May 2025 | Accepted: 7 May 2025

https://doi.org/10.1016/j.jtha.2025.05.008

ORIGINAL ARTICLE

Multicenter study to improve clinical interpretation of anticardiolipin and anti-β2-glycoprotein I antibody test results for diagnosis of antiphospholipid syndrome

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Abstract

Background: Anticardiolipin (aCL) and anti-β2-glycoprotein I ($a\beta_2$GPI) antibodies are laboratory markers important for antiphospholipid syndrome (APS) diagnosis and classification. There is an important interassay variation among aCL and $a\beta_2$GPI assays.

Objectives: This study aimed to harmonize aCL and $a\beta_2$GPI test result interpretation across assays.

Methods: Commercial aCL immunoglobulin (Ig) G/IgM and $a\beta_2$GPI IgG/IgM assays from 3 different diagnostic companies (Thermo Fisher Scientific, Orgentec, and Werfen)

Manuscript handled by: Rolf Urbanus

Final decision: Rolf Urbanus, 7 May 2025

Angela Tincani, Savino Sciascia, and Xavier Bossuyt share senior authorship.

were evaluated using 176 diagnostic samples from patients with APS and 433 diseased controls. International APS reference materials (Harris/Louisville, Koike/Sapporo, National Institute of Biological Standards and Controls 21/266) were analyzed to evaluate traceability. Reference values were verified using samples from 120 healthy controls.

Results: Using the manufacturers' proposed cutoffs, there was large variability in diagnostic sensitivity and specificity among assays. Thresholds corresponding to 97.5% and 99.5% specificity in diseased controls were used to delimit test result intervals (negative [<97.5% specificity threshold], weak positive, and high positive [>99.5% specificity threshold]). Test result interval-specific likelihood ratios (LRs) were concordant across the different aCL and aβ_2GPI assays. For all assays, the LR for APS increased with increasing antibody level. Higher LRs were found for IgG than for IgM assays and for double and triple antibody positivity. The added diagnostic value of aβ_2GPI IgM was limited.

Conclusion: Defining thresholds for antibody levels and assigning test result interval-specific LRs allows alignment of clinical interpretation of aCL and aβ_2GPI assays.

KEYWORDS
antiphospholipid antibodies, antiphospholipid syndrome, harmonization, likelihood ratios

INTRODUCTION

Antiphospholipid syndrome (APS) is an autoimmune disease clinically characterized by thromboembolic events and/or pregnancy morbidity and the presence of antiphospholipid (aPL) antibodies [1,2]. Since the symptoms associated with APS are common in the general population, there is great need for reliable diagnostic aPL assays [3,4]. Anti-cardiolipin (aCL) and anti-β2-glycoprotein I (aβ_2GPI) antibodies are 2 of 3 aPL antibodies necessary for APS diagnosis [5,6]. They are also included in the APS classification criteria [2] and need to test positive on ≥2 occasions with a minimum period of 12 weeks in between to be relevant in APS. Diagnosis differs fundamentally from classification. Diagnostic guidelines provide a set of signs, symptoms, and tests for use in routine clinical care to support diagnosis and to guide clinical decision making in individual patients, whereas classification criteria define homogeneous cohorts of patients for clinical research [6–8].

APS classification criteria [2], diagnostic guidelines [5,6], and European Alliance of Associations for Rheumatology (EULAR) recommendations on APS management [9] recognize that the likelihood for APS depends on (i) the aPL isotype (immunoglobulin [Ig] G > IgM), (ii) the aPL antibody titer, and (iii) combined (double or triple) aPL antibody positivity [2,5]. The most recent APS classification criteria state that presence of aCL and aβ_2GPI IgG/IgM should be determined by standardized ELISAs [2]. Such restriction is contrary to the current trend to increasingly use automated, high throughput aPL immunoassays in clinical laboratories [8,10].

There is poor agreement in quantitative test results obtained by different aPL immunoassays [11,12]. This important interassay variation is caused by various factors (including differences in technology, calibration, nature and source of antigen, and detection antibody) and is observed despite traceability to international available aPL reference materials [4,13–17]. In addition, test result interpretation is hampered by the heterogeneity in the way the cutoff for positivity is set [11,12].

Likelihood ratios (LRs) have been suggested to be useful tools to help with the interpretation of autoantibody test results [17,18]. LRs give information on how much a test result changes the probability of having a disease. They provide information for test result intervals, moving away from a dichotomous (positive/negative) approach [18,19]. The aim of this international multicenter study was to harmonize the interpretation of commonly used commercially available aCL IgG/IgM and aβ_2GPI IgG/IgM assays by applying the concept of LR, which is a unit-independent method to express test results.

2 | MATERIALS AND METHODS

2.1 | Patients and samples

The *APS patient cohort* (n = 176) consisted of newly diagnosed APS patients, provided by 2 referral centers for APS included in the European Reference Network on Rare and Complex Connective Tissue and Musculoskeletal Diseases (University of Brescia, Italy; n = 68, and San Giovanni Bosco Hospital, University of Turin, Italy; n = 108). APS classification was based on the 2006 Sydney criteria [1], including lupus anticoagulant (LA) testing performed according to the International Society on Thrombosis and Haemostasis (ISTH) guidelines [20] and in-house aCL IgG/IgM and aβ_2GPI IgG/IgM ELISA assays. Most

(64.8%) of the APS patients had thrombotic manifestations, 16.9% had obstetric manifestations and 18.1% had a combination of both.

The *diseased control cohort* (DCG) (n = 433) consisted of consecutively included patients for whom aPL antibody tests were requested but no APS diagnosis was established. The samples for the DCG group were collected in 2 Belgian hospitals (ZAS Hospital Antwerp; n = 152, and AZORG Hospital Aalst; n = 281). Clinically, 17.3% of the DCG patients presented with obstetric morbidity, 28.2% with thrombotic manifestations, 53.8% with rheumatic disorders (detailed description in Supplementary Data S1), and 0.7% with a combination of rheumatic and thrombotic manifestations.

In addition, 120 *healthy individuals* (HCs) were recruited. All subjects were asymptomatic and did not show clinical signs of thrombosis, pregnancy morbidity, tumors, infection, and/or autoimmune disease. Sample collection complied with the World Medical Association's Declaration of Helsinki (as revised in 2013). Ethical approval was granted by the Institutional Review Board of AZORG Hospital Aalst (Belgium registration number B1262023000003). Informed consent was obtained if applicable for individuals included in this study.

An overview of the demographic features of the different study cohorts is included in Supplementary Data S1.

2.2 | Reference materials

Traceability was evaluated by analysis of available international aPL reference materials, ie, (i) the polyclonal 2020 GM-300 Louisville standards traceable to the original Harris standards [13,14], (ii) the monoclonal HCAL (aCL IgG) and EY2C9 (aCL IgM) Koike or Sapporo standards [15], and (iii) the first World Health Organization-declared 2022 aβ_2GPI IgG standard (National Institute of Biological Standards and Controls [NIBSC] 21/266) [16,17,21], produced by European Commission-Joint Research Centre in collaboration with the Medicines and Healthcare products Regulatory Agency (formerly NIBSC).

2.3 | aCL IgG/IgM and aβ_2GPI IgG/IgM assays

All samples were analyzed using 4 assays (aCL IgG, aCL IgM, aβ_2GPI IgG, aβ_2GPI IgM) from 3 different manufacturers most commonly used in Europe: QUANTA Flash chemiluminescent immunoassay (CLIA) performed on BIOFlash (Inova Diagnostics/Werfen), the EliA fluorescent enzyme immunoassay (FEIA) performed on Phadia250 (Thermo Fisher Scientific, Phadia AB), the QUANTA Lite ELISA (Inova Diagnostics/Werfen) performed on a QUANTA-Lyser 2 instrument (Inova Diagnostics/Werfen), and the Orgentec ELISA, which was manually performed.

An overview of the different assays and their specific test characteristics is provided in Supplementary Data S2. The tests were performed on serum samples according to the manufacturers' instructions and ISTH guidelines [22] by experienced laboratory technicians of the AZORG Hospital Aalst. Results are expressed in the arbitrary units of the respective assays (Supplementary Data S2).

2.4 | Statistical analysis

Within-laboratory imprecision was determined using the manufacturer's internal quality control materials, a patient sample pool with low and high aCL/aβ_2GPI IgG/IgM concentrations [23]. The materials were analyzed in duplicate measurements in 10 consecutive analysis runs.

For *analytical method comparison*, Spearman's rank correlation coefficients (ρ) (and 95% CI) were calculated between aCL/aβ_2GPI IgG/IgM methods [24].

Reference materials were reconstituted according to the providers' recommendations and measured in singlet during 3 separate analysis runs [25].

The *99% upper reference limits* (URL) were calculated for every aCL/aβ_2GPI IgG/IgM assay, using the results from the HCs. Right-sided Grubb's test was applied for outlier exclusion on logarithmic transformed data. Shapiro–Wilk test was used for evaluation of Gaussian data distribution. As there was a non-Gaussian distribution, a right-sided, nonparametric percentile method was applied for defining the reference limits [26]. Given the non-Gaussian distribution of the data (right-sided Grubb's applies for Gaussian distributed data), outliers were confirmed as far-out outliers on the box-whisker plots and by applying the modified Z-score [27].

Diagnostic performance was evaluated by analysis of sensitivity, specificity, receiver operating characteristic curve (ROC) analysis, LR, and test result interval-specific LR as previously described [19,28]. All analyses were based on the test results of the APS and DCG cohorts.

Statistical analysis was performed using MedCalc (version 22.021; MedCalc Software Ltd), with statistical significance defined as P value < 0.05.

3 | RESULTS

3.1 | Technical performance

3.1.1 | Imprecision

An overview of the within-laboratory imprecision results for both the manufacturer internal quality control and the patient pools is presented in Supplementary Data S3 and was evaluated according to the criteria stated by the ISTH Scientific and Standardization Committee subcommittee on Lupus Anticoagulant/Antiphospholipid Antibodies, ie, a maximum imprecision of 20% for ELISA and 10% for automated systems [22]. The imprecision ranged from 3.0% to 23.0% and was the highest for the ELISA assays. For the automated methods, the EliA ACA IgG revealed the highest imprecision of 13.9% for the low-level patient pool and the QUANTA Flash ACA IgG the lowest imprecision (3.1%) for the high-level patient pool. In general, the imprecision

was higher for the double-layered aCL assays than the mono-layered aβ$_2$GPI assays.

3.1.2 | Correlation between assays

Correlation graphs revealed a low-to-moderate numerical agreement among test results obtained by the different aPL methods (Supplementary Data S4). Spearman rank correlation ρ ranged from 0.451 to 0.648 for aCL IgG, from 0.503 to 0.780 for aCL IgM, from 0.435 to 0.673 for aβ$_2$GPI IgG, and from 0.619 to 0.738 for aβ$_2$GPI IgM assays.

3.1.3 | Traceability to reference materials

The test results of the 3 reference materials with the different aPL assays are presented in box-whisker plots in Supplementary Data S5. If available, the target concentration assigned by the reference organization is presented as well. Despite the use of the standardized IgG phospholipid (GPL) or IgM phospholipid (MPL) units for most of the aCL IgG and aCL IgM methods (excluding QUANTA Flash), the box-whisker plots revealed a wide variation in quantitative level of the same reference material across the different antigen-specific assays using their assay-specific calibration curve.

For the Harris standards, aCL IgG EliA and QUANTA Lite and aCL IgM QUANTA Flash and QUANTA Lite were close to the target value. For the other aCL assays and for the aβ$_2$GPI assays, test results of the reference materials significantly differed from the target values. Within the same manufacturer, the deviation toward the target value varied (ie, was not consistently higher or lower). For the Koike standards, EliA and QUANTA Flash aCL IgG were closest to the target value. The Orgentec and QUANTA Lite ELISAs did not react with the Koike/Sapporo standards (Supplementary Data S5), even though the inserts of these ELISAs claim traceability to the Koike/Sapporo standards (Supplementary Data S2). Although loss of reactivity of this reference material has recently been communicated [29], QUANTA Flash IgG, EliA IgG, and and QUANTA Flash IgM gave measurable titers for both aCL and aβ$_2$GPI.

The target value of 200 IU/mL of the NIBSC 21/266 aβ$_2$GPI IgG standard was higher than the measuring range of the QUANTA Flash and QUANTA Lite assays. Therefore, additionally, 1:2 dilution of the standard was analyzed with all aCL IgG and aβ$_2$GPI IgG methods. A significant deviation toward the target value was observed for most of the aβ$_2$GPI IgG methods (for EliA, 142.0 U/mL; QUANTA Flash, 2999 CU; Orgentec, 57.9 U/mL; QUANTA Lite, 98.8 U/mL).

A 1:2 dilution of the standard was also analyzed with all aCL IgG assays. For the aCL IgG assays, results obtained were close to the target value of 100 IU/mL (for EliA, 118.7 GPL; Orgentec, 100.5 GPL; and QUANTA Lite, 94.3 GPL).

3.2 | 99% URL in healthy controls versus manufacturer's cutoff

The aPL test results of 120 HCs are presented in Figure 1 and summarized in the table in Supplementary Data S6. For the Orgentec ELISAs, the 99% URL determined in 120 HCs closely corresponded to the cutoff proposed by the manufacturer (Figure 1). For the other assays, however, the 99% URL differed from the cutoffs proposed by the manufacturer. The difference between the URL and the cutoff was not consistent. For example, the 99% URL obtained for QUANTA Flash aCL IgG was 1.9 times (ie, higher than) the manufacturer's cutoff, whereas the 99% URL for aβ$_2$GPI IgG and aβ$_2$GPI IgM were both 0.6 times (ie, lower than) the manufacturer's cutoff.

3.3 | Diagnostic performance

Figure 1 shows aPL results obtained with 4 different assays using samples from APS patients and controls. The cutoff of the manufacturer and the 99% URL obtained for HCs are indicated. Based on the cutoff values set by the manufacturer, the sensitivity and specificity for APS of the different aPL assays were calculated (Supplementary Data S7). The sensitivity ranged from 21.0% to 54.0% for aCL IgG, from 12.5% to 47.7% for aCL IgM, from 20.5% to 64.2% for aβ$_2$GPI IgG, and from 19.3% to 35.8% for aβ$_2$GPI IgM. The corresponding specificity ranged from 97.2% to 99.5% for aCL IgG, 94.9% to 98.6% for aCL IgM, 96.3% to 99.1% for aβ$_2$GPI IgG, and 97.2% to 99.5% for aβ$_2$GPI IgM. Consequently, there was a marked variation in corresponding LRs for APS between assays: the coefficient of variation in LR was 48.4%, 31.8%, 52.2% and 63.9% for aCL IgG, aCL IgM, β$_2$GPI IgG, and aβ$_2$GPI IgM, respectively.

At a threshold corresponding to a specificity of 97.5%, the sensitivity ranged from 38.1% to 53.4% for aCL IgG, 19.9% to 36.9% for aCL IgM, 34.7% to 67.0% for aβ$_2$GPI IgG, and 31.3% to 38.6% for aβ$_2$GPI IgM (Supplementary Data S8). The range of sensitivities associated with a predefined specificity was narrower than the range of sensitivities associated with the cutoffs proposed by the manufacturer. Consequently, the difference in corresponding LRs for APS between assays was lower when predefined specificity was used than when the manufacturer's cutoff was used: the coefficient of variation was 14.3% for aCL IgG, 24.0% for aCL IgM, 32.4% for aβ$_2$GPI IgG, and 8.7% for aβ$_2$GPI IgM assays.

The ROC curves of the different aPL assays are presented in Figure 2 and reveal good alignment of the diagnostic performance between ELISA, CLIA, and FEIA aPL methods. Areas under the curve (AUCs) were significantly higher for aCL IgG (0.832-0.890) than for aCL IgM (0.751-0.814) (Figure 2A, B) and for aβ$_2$GPI IgG (0.866-0.911) than for aβ$_2$GPI IgM (0.636-0.791) (Figure 2C, D). Independent of the assay technology, the diagnostic performance of aβ$_2$GPI IgG mostly tended to be higher than the performance of aCL IgG and aCL IgM, which

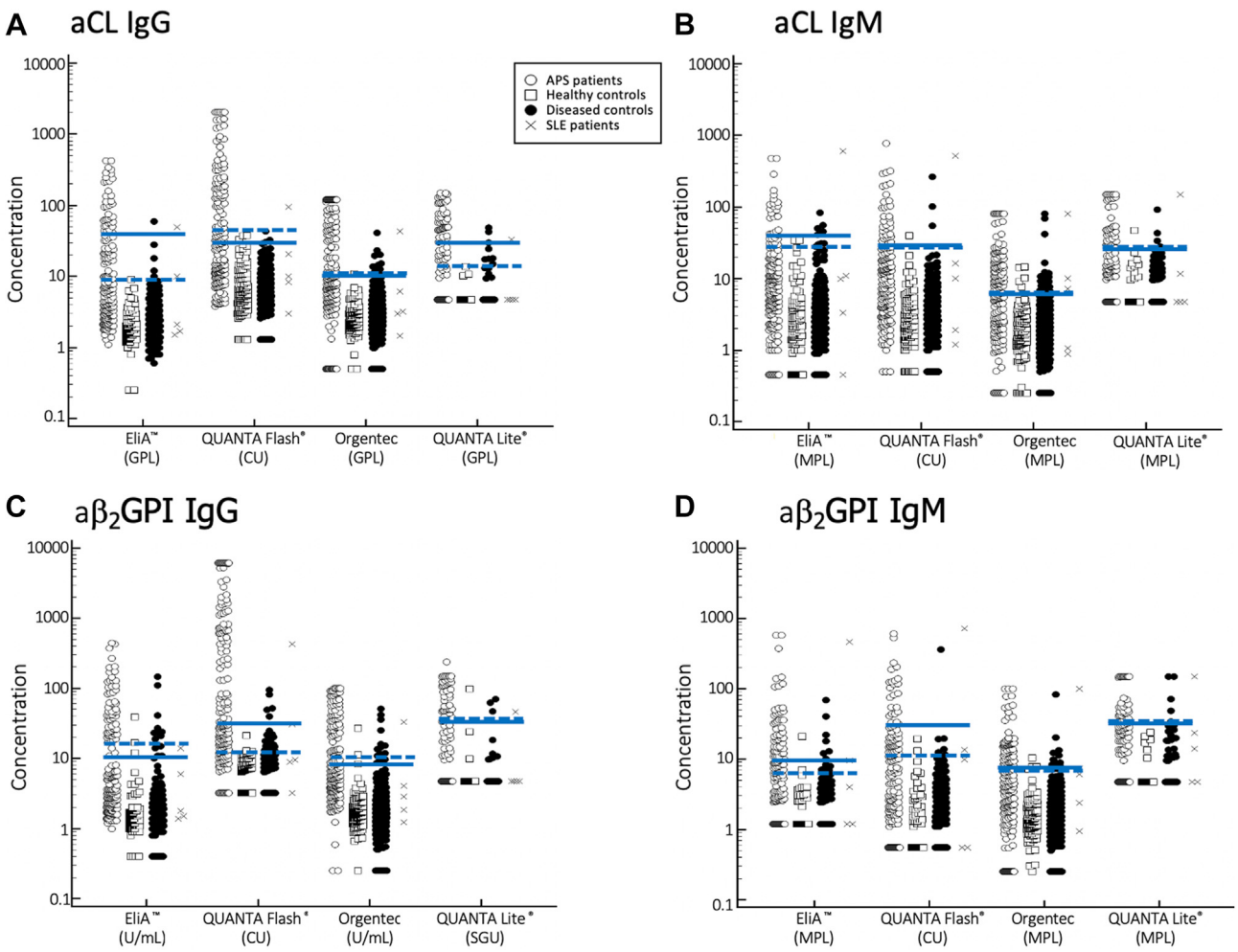

FIGURE 1 Logarithmic transformed dot plots of (A) aCL IgG, (B) aCL IgM, (C) aβ$_2$GPI IgG and aβ$_2$GPI IgM test results in patients with APS, diseased control patients, and healthy controls for the 4 different aPL assays. The cutoffs assigned by the manufacturers are presented in full lines and the 99% upper reference limits are presented in dashed lines. A threshold of 100% "specificity" for APS could be obtained for aCL IgG and aβ$_2$GPI IgG for EliA, QUANTA Flash, Orgentec, and QUANTA Lite at 59 GPL, 94 CU, 43 GPL, 48 GPL, and 146 U/mL, 429 U/mL, 51 U/mL, 70 SGU, respectively. aβ$_2$GPI, anti-β2-glycoprotein I antibody; aCL, anticardiolipin antibody; aPL, antiphospholipid antibody; APS, antiphospholipid syndrome; CU, chemiluminescent unit; GPL, IgG phospholipid unit; Ig, immunoglobulin; SLE, systemic lupus erythematosus.

outperformed aβ$_2$GPI IgM. Subanalysis of the diagnostic performance of the aPL assays for the obstetric and thrombotic subpopulations revealed no significant differences (Supplementary Data S9).

3.4 | Harmonized specificity-based thresholds

To determine test result intervals across assays in a consistent way, we defined intervals that are delimited by thresholds that correspond to predefined specificities (90.0%, 92.5%, 95.0%, 97.5%, 99.0%, and 99.5%). Table 1 shows the LRs for APS associated with the various predefined specificities. LRs for APS increased with increasing antibody levels for aCL IgG, aCL IgM, and aβ$_2$GPI IgG, but less so for aβ$_2$GPI IgM. For all aPL tests except EliA aCL IgM, a significant LR >10 was obtained for antibody levels >97.5% specificity threshold.

In addition, for almost all manufacturers and assays, the 99.0% specificity threshold determined in the HC cohort (see above) aligned with the 97.5% specificity threshold determined in the DCG (Supplementary Data S6). A threshold of 100% "specificity" for APS could be obtained for aCL IgG and aβ$_2$GPI IgG with EliA, QUANTA Flash, Orgentec, and QUANTA Lite at 59 GPL, 94 CU, 43 GPL, and 48 GPL and 146 U/mL, 429 U/mL, 51 U/mL, and 70 SGU, respectively.

3.5 | Test result interval-specific LRs

Based on the LRs associated with specificity-based thresholds, we defined (i) negative aPL antibody results for levels <97.5% specificity threshold, (ii) medium-positive aPL antibody results for levels between the 97.5% and 99.5% specificity thresholds, and (iii) high-positive aPL antibody for antibody levels >99.5% specificity. Test result interval-specific LRs were calculated for every antibody level interval (Table 2). The LR for APS <97.5% specificity threshold varied between 0.48 and 0.64 for aCL IgG, 0.65 and 0.82 for aCL

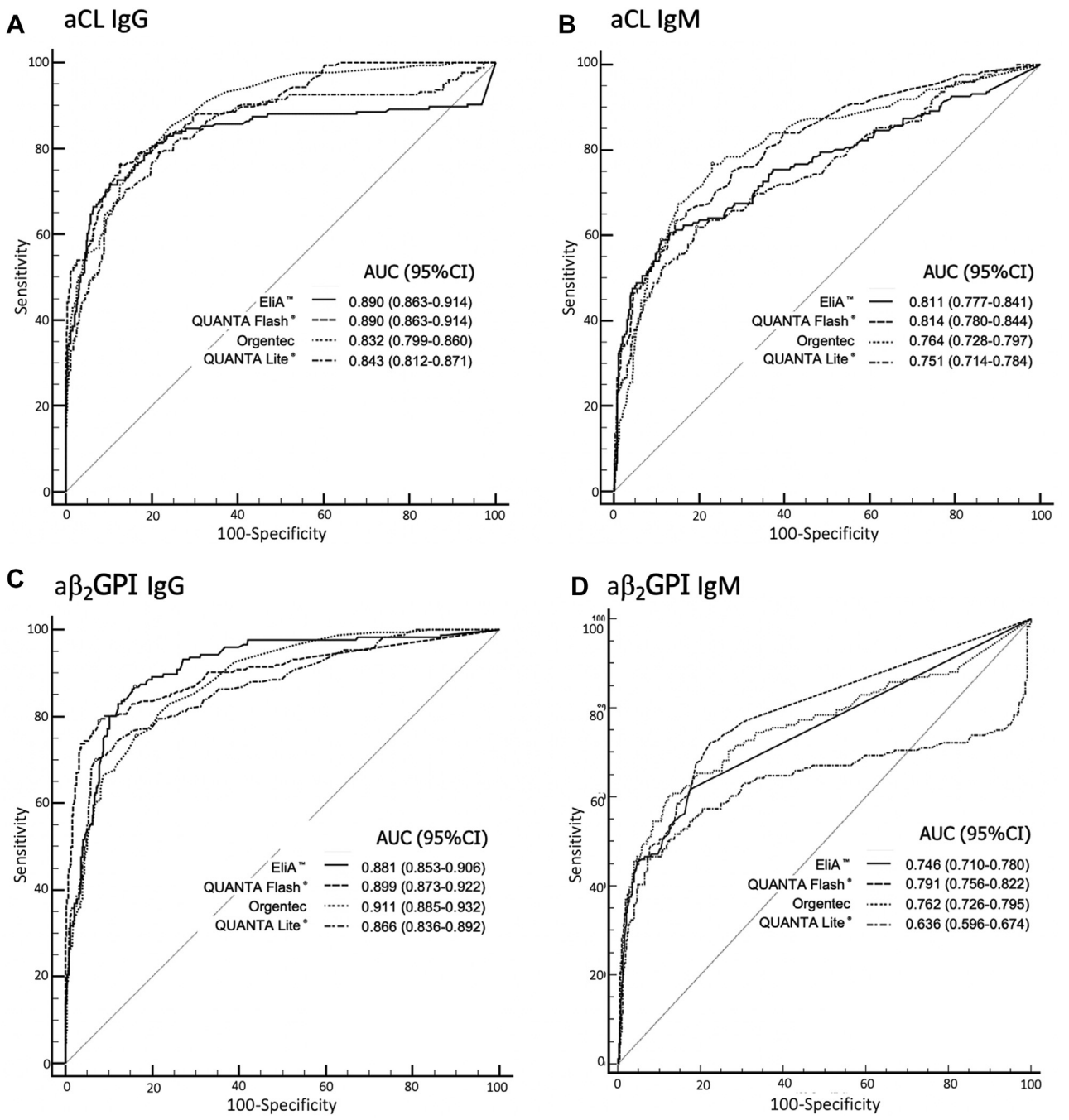

FIGURE 2 ROC curves and AUC (95% CI) for (A) aCL IgG, (B) aCL IgM, (C) aβ_2GPI IgG, and (D) aβ_2GPI IgM. aβ_2GPI, anti-β2-glycoprotein I antibody; aCL, anticardiolipin antibody; AUC, area under the curve; Ig, immunoglobulin; ROC, receiver operating characteristic.

IgM, 0.34 and 0.67 for aβ_2GPI IgG, and 0.63 and 0.70 for aβ_2GPI IgM. The LRs for the antibody interval delimited by thresholds corresponding to specificities of 97.5% and 99.5% ranged between 4.4 and 10.9 for aCL IgG, 4.9 and 15.3 for aCL IgM, 7.4 and 13.9 for aβ_2GPI IgG, and 8.7 and 16.0 for aβ_2GPI IgM. Finally, the LRs for antibody levels higher than the threshold, corresponding to >99.5% specificity, ranged from 36.1 to 64.0 for aCL IgG, 7.4 to 19.7 for aCL IgM, 27.9 to 54.9 for aβ_2GPI IgG, and 9.8 to 29.5 for aβ_2GPI IgM, which were significantly higher for the IgG isotype than the IgM isotype. In addition, LRs for IgG, independent of the method, were significantly increased from the medium-positive range to the high-positive range, which was not observed for IgM. For all methods and for both IgG and IgM, the LRs for the medium-positive interval (defined by 97.5%-99.5% specificity thresholds) were ≤10, in contrast to the high-positive interval (>99.5% specificity threshold) all LRs for IgG, which far exceeded >10 [18].

TABLE 1 Likelihood ratios for antiphospholipid syndrome of anticardiolipin antibodies and anti-β2-glycoprotein I antibodies at specificity-based thresholds.

	EliA		QUANTA Flash		Orgentec ELISA		QUANTA lite ELISA	
Specificity-based threshold	Threshold (GPL)	LR (95% CI)	Threshold (CU)	LR (95% CI)	Threshold (GPL)	LR (95% CI)	Threshold (GPL)	LR (95% CI)
aCL IgG								
>90.0%	3.8	7.0 (5.1-9.6)	11.6	7.2 (5.3-9.8)	5.1	7.1 (5.3-9.6)	7.9	6.6 (4.8-8.9)
>92.5%	4.9	8.2 (5.7-11.9)	12.7	8.8 (6.2-12.6)	5.7	9.4 (6.6-13.4)	8.3	6.9 (4.8-10.0)
>95.0%	5.5	11.5 (7.4-17.8)	16.6	11.6 (7.5-18.0)	7.1	12.1 (7.7-18.9)	8.7	10.0 (6.3-15.7)
>97.5%	7.3	18.8 (10.3-34.4)	20.6	21.0 (11.5-38.3)	10.7	17.2 (9.4-31.6)	10.9	15.0 (8.1-27.7)
>99.0%	12.0	40.0 (14.8-108.1)	28.6	51.7 (19.2-138.7)	20.3	37.5 (13.9-101.6)	24.6	30.8 (11.3-83.9)
>99.5%	28.0	54.1 (13.3-220.9)	32.3	96.0 (23.8-386.2)	23.1	73.8 (18.2-298.7)	33.0	59.1 (14.5-240.3)
aCL IgM								
>90.0%	6.7	5.7 (4.1-7.9)	7.6	5.4 (4.0-7.5)	5.3	5.6 (4.1-7.7)	9.3	4.9 (3.6-6.8)
>92.5%	8.5	6.4 (4.4-9.2)	8.7	6.8 (4.7-9.7)	6.2	6.8 (4.8-9.9)	10.1	6.1 (4.2-8.8)
>95.0%	11.0	7.0 (4.4-11.2)	11.4	10.1 (6.4-15.9)	7.6	9.8 (6.3-15.4)	12.9	7.9 (5.0-12.4)
>97.5%	25.0	8.4 (4.2-16.6)	15.4	13.6 (7.4-25.3)	10.2	14.5 (7.9-26.9)	18.2	10.5 (5.6-19.8)
>99.0%	45.0	11.1 (3.8-32.3)	21.4	28.9 (10.6-79.0)	16.5	25.8 (9.4-71.0)	28.0	24.6 (8.9-67.7)
>99.5%	55.0	20.9 (4.9-89.6)	101.0	13.5 (3.0–60.4)	69.0	11.1 (2.4-50.7)	43.3	29.5 (7.1-123.6)
aβ$_2$GPI IgG								
>90.0%	2.5	6.8 (5.0-9.1)	11.5	8.4 (6.3-11.4)	3.5	7.8 (5.8-10.5)	7.2	7.3 (5.4-9.8)
>92.5%	3.0	8.2 (5.7-11.6)	12.2	10.3 (7.4-14.4)	4.5	8.7 (6.1-12.3)	7.3	9.5 (6.7-13.4)
>95.0%	4.3	10.1 (6.5-15.7)	13.6	15.3 (10.0-23.5)	5.9	11.0 (7.1-17.1)	7.5	10.9 (7.1-16.7)
>97.5%	14.0	14.8 (7.7-28.2)	18.3	26.3 (14.6-47.6)	11.0	14.5 (7.9-26.9)	9.2	15.2 (8.2-28.1)
>99.0%	24.0	27.7 (10.1-75.8)	49.1	48.5 (18.0-130.4)	25.1	28.9 (10.6-79.0)	18.4	33.8 (12.5-91.9)
>99.5%	41.0	39.4 (9.5-162.5)	82.1	82.2 (20.4-332.0)	36.1	44.3 (10.8-182.0)	46.8	43.1 (10.5-177.1)
aβ$_2$GPI IgM								
>90.0%	3.7	4.8 (3.5-6.6)	3.5	5.0 (3.6-6.9)	3.7	5.6 (4.1-7.7)	8.7	4.8 (3.5-6.7)
>92.5%	4.3	6.7 (4.6-9.8)	4.6	6.3 (4.4-9.1)	4.3	6.8 (4.8-9.9)	9.0	6.2 (4.3-9.0)
>95.0%	4.8	9.4 (6.0-14.7)	6.1	9.7 (6.2-15.4)	5.1	9.6 (6.2-15.0)	11.2	8.3 (5.3-13.1)
>97.5%	7.6	14.3 (7.7-26.5)	9.5	15.2 (8.2-28.1)	7.8	15.0 (8.1-27.7)	21.7	12.3 (6.6-22.9)
>99.0%	18.0	19.7 (7.1-54.8)	13.8	30.8 (11.3-83.9)	12.3	27.7 (10.1-75.8)	48.2	12.3 (4.3-35.5)
>99.5%	40.0	19.7 (4.6-84.7)	19.6	44.3 (10.8-182.0)	20.2	16.0 (3.4-70.1)	70.9	9.8 (2.8-34.5)

aβ$_2$GPI, anti-β2-glycoprotein I antibody; aCL, anticardiolipin antibody; CU, chemiluminescent unit; GPL, IgG phospholipid unit; LR, likelihood ratio; MPL, IgM phospholipid unit; SGU, standard IgG unit; SMU, standard IgM unit.

3.6 | LRs of combined aPL antibody positivity

Finally, we performed the LR calculations for various combinations of negative (ie, <97.5% specificity threshold) and positive aCL and aβ$_2$GPI results for the different assays included in the study (Table 3). Venn diagrams, visualizing combined aPL positivity, are provided in Supplementary Data S10.

LRs for APS significantly increased by combining aCL with aβ$_2$GPI positive test results and were higher for IgG than for IgM (Table 3). By combining positive aCL IgG, aCL IgM, and aβ$_2$GPI IgG positive test results, the highest LRs for APS were obtained. When only the thrombotic and obstetric control cohorts were considered (ie, excluding the autoimmune cohort), the combination of positive aCL IgG, aCL IgM, and aβ$_2$GPI IgG resulted in an infinite LR for APS for 3 of the 4 manufacturers (Table 4). Such combination was found in 4.5% to 19.3% of the APS patients. The added diagnostic value of aβ$_2$GPI IgM was limited. The combination of negative aCL IgG/IgM and aβ$_2$GPI IgG/IgM lowered the LR to 0.22 to 0.41 (Table 3), which does not

TABLE 2 Test result interval-specific likelihood ratios for antiphospholipid syndrome.

Specificity-based threshold intervals	EliA	QUANTA Flash	Orgentec ELISA	QUANTA lite
aCL IgG				
<97.5%	92/421 **0.54 (0.47-0.62)**	82/421 **0.48 (0.41-0.56)**	99/421 **0.58 (0.51-0.66)**	109/421 **0.64 (0.57-0.72)**
[97.5-99.5%]	40/9 **10.9 (5.4-22.1)**	16/9 **4.4 (2.0-9.7)**	17/9 **4.6 (2.1-10.2)**	19/9 **5.2 (2.4-11.3)**
≥99.5%	44/3 **36.1 (11.4-114.7)**	78/3 **64.0 (20.5-200.0)**	60/3 **49.2 (15.6-154.8)**	48/3 **39.4 (12.4-124.7)**
aCL IgM				
<97.5%	141/421 **0.82 (0.76-0.89)**	115/421 **0.67 (0.60-0.75)**	111/421 **0.65 (0.58-0.73)**	129/421 **0.75 (0.69-0.83)**
[97.5-99.5%]	18/9 **4.9 (2.3-10.7)**	49/9 **13.4 (6.7-26.7)**	56/9 **15.3 (7.7-30.3)**	23/9 **6.3 (3.0-13.3)**
≥99.5%	17/3 **13.9 (4.1-47.0)**	12/3 **9.8 (2.8-34.5)**	9/3 **7.4 (2.0-26.9)**	24/3 **19.7 (6.0-64.5)**
aβ$_2$GPI IgG				
<97.5%	115/421 **0.67 (0.60-0.75)**	58/421 **0.34 (0.27-0.42)**	111/422 **0.65 (0.58-0.73)**	108/421 **0.63 (0.56-0.71)**
[97.5-99.5%]	27/9 **7.4 (3.5-15.4)**	51/9 **13.9 (7.0-27.7)**	29/8 **8.9 (4.2-19.1)**	32/9 **8.8 (4.3-17.9)**
≥99.5%	34/3 **27.9 (8.7-89.6)**	67/3 **54.9 (17.5-172.4)**	36/3 **29.5 (9.2-94.6)**	36/3 **29.5 (9.2-94.6)**
aβ$_2$GPI IgM				
<97.5%	112/421 **0.66 (0.59-0.73)**	108/421 **0.63 (0.56-0.71)**	111/422 **0.65 (0.58-0.73)**	121/421 **0.70 (0.64-0.78)**
[97.5-99.5%]	48/9 **13.1 (6.6-26.2)**	32/9 **8.7 (4.3-17.9)**	52/8 **16.0 (7.8-33.0)**	43/9 **11.8 (5.9-23.6)**
≥99.5%	16/3 **13.1 (3.9-44.5)**	36/3 **29.5 (9.2-94.6)**	13/3 **10.7 (3.1-37.0)**	12/3 **9.8 (2.9-37.6)**

Medium-positive results are defined by results between the thresholds defined by 97.5% specificity and 99.5% specificity. High-positive results are defined by results exceeding the 99.5% specificity threshold. Results are given for aCL IgG, aCL IgM, aβ$_2$GPI IgG, and aβ$_2$GPI IgM. Plain numbers indicate the number of patients and controls (patients/controls), and the numbers in bold indicate the LR (± 95% CI). See Table 1 for information on the assay-specific thresholds.

aβ$_2$GPI, anti-β2-glycoprotein I antibody; aCL, anticardiolipin antibody; LR, likelihood ratio.

allow exclusion of APS. Even if only aPL immunoassays were considered (ie, isolated LA-positive APS patients were excluded), the LRs remained relatively high-ranging (0.07-0.26 for combined negative aCL IgG/IgM and aβ$_2$GPI IgG/IgM).

4 | DISCUSSION

Currently, the gold standard for confirmation of the clinical diagnosis of APS is the finding of aPLs [5]. According to a recent Italian study, the prevalence of aPLs were 1.0% to 4.9% in the healthy population and 5.6%, 9.9%, 10.9%, and 17.0% in patients with pathological pregnancy, venous thromboembolism, myocardial infarction, and stroke, respectively [30]. In our study, we focused on aCL IgG/IgM and aβ$_2$GPI IgG/IgM test results, which are integrated in both APS diagnostic guidelines [5,6], classification criteria [2], and management recommendations [9]. Both aCL and aβ$_2$GPI IgGs were confirmed as markers of thrombosis in a recent metanalysis [31]. Nevertheless, we have confirmed the great variability and low-to-moderate numerical agreement among test results obtained by different aCL and aβ$_2$GPI methods (Supplementary Data S4a and S4b) [3,11,32,33]. These interassay differences result in a wide variation in antibody titers among assays, also observed in external proficiency testing [10,34] and literature [11,35]. A first factor attributing to this interassay variation is the assay composition. Commercial aCL assays are bilayer assays, all using β$_2$GPI as cofactor to bind cardiolipin as antigen (Supplementary Data S2). aβ$_2$GPI assays are monolayer assays using β$_2$GPI directly as antigen. For both aCL and aβ$_2$GPI immunoassays,

TABLE 3 Likelihood ratios for antiphospholipid syndrome of combined antiphospholipid antibody positivity, with the 97.5% specificity threshold as cutoff for positivity.

Specificity-based threshold	EliA	QUANTA Flash	Orgentec ELISA	QUANTA lite ELISA
aCL IgG and aβ$_2$GPI IgG				
<97.5%	88/413 **0.52 (0.45-0.61)**	53/412 **0.32 (0.25-0.40)**	99/419 **0.58 (0.51-0.66)**	95/411 **0.57 (0.50-0.65)**
≥97.5%	57/4 **35.1 (12.9-95.2)**	89/3 **73.0 (23.4-227.5)**	65/8 **20.0 (9.8-40.8)**	54/2 **66.4 (16.4-269.5)**
aCL IgM and aβ$_2$GPI IgM				
<97.5%	100/414 **0.59 (0.52-0.68)**	103/415 **0.61 (0.54-0.69)**	104/418 **0.61 (0.54-0.69)**	105/412 **0.63 (0.55-0.71)**
≥97.5%	23/5 **11.3 (4.3-29.3)**	56/6 **23.0 (10.1-52.3)**	58/8 **17.8 (8.7-36.6)**	31/3 **25.4 (7.9-82.1)**
aCL IgG/M and aβ$_2$GPI IgG				
<97.5%	65/402 **0.40 (0.33-0.48)**	39/402 **0.24 (0.18-0.32)**	71/408 **0.43 (0.35-0.51)**	75/401 **0.46 (0.39-0.55)**
≥97.5%	8/1 **19.7 (2.5-156.2)**	34/1 **83.6 (11.5-606.4)**	28/2 **34.4 (8.3-143.0)**	18/1 **44.3 (6.0-329.2)**
aCL IgG/M and aβ$_2$GPI IgG/M				
<97.5%	45/396 **0.28 (0.22-0.36)**	36/397 **0.22 (0.17-0.30)**	67/406 **0.41 (0.34-0.49)**	59/393 **0.37 (0.30-0.46)**
≥97.5%	5/1[a] **12.3 (1.5-104.5)**	32/1[a] **78.7 (10.8-571.7)**	21/2[a] **25.8 (6.1-109.0)**	10/1[a] **24.6 (3.2-190.8)**

Plain numbers indicate the number of patients and controls (patients/controls) and the numbers in bold indicate the LR (± 95% CI). See Table 1 for information on the assay-specific thresholds.

aβ$_2$GPI, anti-β2-glycoprotein I antibody; aCL, anticardiolipin antibody; aPL, antiphospholipid antibody; LR, likelihood ratio.

[a] Systemic lupus erythematosus patient in diseased control group tested positive with all aPL tests of 3 methods; 1 thrombotic control group patient tested positive with all aPL tests of Orgentec ELISA.

antigens are not exclusively of human origin (Supplementary Data S2). Although the differences in diagnostic performance of the aPL assays in our multicenter study (Supplementary Data S8) were not related to antigen origin, ISTH experts recommend the use of antigens from human sources over those of animal origin (eg, bovine) to avoid false-positive reactivity [4,22]. In addition, aCL and aβ$_2$GPI assays differ in the way the antigen is fixed to the solid phase, which has an impact on the epitopes that are available for antibody detection. β$_2$GPI is a circular polypeptide consisting of 5 domains (I-V) with 2 disulfide bonds in each domain and an additional disulfide bond in domain V. Phospholipid binding by domain V of the protein results in a conformational change from the circular (closed) form to an open J-elongated conformation, exposing the "cryptic epitope" Gly40 to Arg43 in the first domain of β$_2$GPI [36,37]. Antibodies to domain I are associated with a high-risk APS profile (both thrombotic and obstetric [36,38]). The exposure of the epitope of domain I has been shown to vary across commercial aβ$_2$GPI IgG assays [39]. On the other hand, domain V, involved in the binding of β$_2$GPI to CL, will no longer be exposed for antibody binding in aCL assays, explaining aβ$_2$GPI positive results combined with aCL negative results. Clinically, aPL asymptomatic carriers display a preferential polarization profile toward antibodies directed to domains IV to V [36,38]. In addition, a recent systematic review by Jiang et al. [40] revealed no conclusive evidence for the association of isolated aβ$_2$GPI positivity and clinical APS events.

Positive aCL in the absence of aβ$_2$GPI should also be interpreted with caution, especially at lower titers [41,42]. Isolated aCL are usually not associated with APS-related clinical symptoms. If aβ$_2$GPI antibodies are negative, aCL antibodies are either cofactor independent or bind through cofactors different from β$_2$GPI with unknown significance. Conclusively, combinatorial testing of aCL and aβ$_2$GPI is of added diagnostic value [35].

Traditionally, aCL and aβ$_2$GPI antibodies have been detected by ELISA. In the past decades, newer aPL assays using automated analyzers became commercially available and are increasingly used, as shown by several external proficiency providers [10]. Automated systems make use of an alternative solid phase (eg, magnetic beads), use alternative detection methods (ie, CLIA, FEIA) and apply technique-specific calibration algorithms with consequent differences in antigen-binding techniques and therefore different antigen exposures [43]. Automated methods are more efficient, easier to use, less prone to interoperator variation, and show less interlaboratory variation, as confirmed by our imprecision findings (Supplementary Data S3). They allow the simultaneous detection of various single autoantibodies, achieving quantitative results with significantly shorter

TABLE 4 Likelihood ratios for antiphospholipid syndrome of combined antiphospholipid antibody positivity, with the 97.5% specificity threshold as cutoff for positivity applied on thrombotic/obstetric control group only (n = 197).

Specificity-based threshold	EliA	QUANTA Flash	Orgentec ELISA	QUANTA lite ELISA
aCL IgG and aβ_2GPI IgG				
<97.5%	88/190 **0.52 (0.45-0.60)**	53/184 **0.32 (0.26-0.41)**	99/193 **0.57 (0.50-0.66)**	95/189 **0.56 (0.49-0.65)**
≥97.5%	57/1 **63.8 (8.9-455.9)**	89/1 **99.6 (14.0-707.5)**	65/4 **18.2 (6.8-48.9)**	54/0 **∞**
aCL IgM and aβ_2GPI IgM				
<97.5%	100/190 **0.59 (0.52-0.67)**	103/191 **0.60 (0.53-0.69)**	104/191 **0.61 (0.54-0.69)**	105/191 **0.61 (0.54-0.70)**
≥97.5%	23/1 **25.7 (3.5-188.7)**	56/2 **31.3 (7.8-126.5)**	58/2 **32.5 (8.0-131.0)**	31/1 **34.7 (4.8-251.5)**
aCL IgG/M and aβ_2GPI IgG				
<97.5%	65/185 **0.39 (0.32-0.48)**	39/181 **0.24 (0.18-0.32)**	71/189 **0.42 (0.35-0.50)**	75/186 **0.45**
≥97.5%	8/0 **∞**	34/0 **∞**	28/1 **31.3 (4.3-228.0)**	18/0 **∞**
aCL IgG/M and aβ_2GPI IgG/M				
<97.5%	45/183 **0.28 (0.21-0.36)**	36/179 **0.23 (0.17-0.30)**	67/188 **0.40 (0.33-0.48)**	59/183 **0.36 (0.29-0.45)**
≥97.5%	5/0 **∞**	32/0 **∞**	21/1[a] **23.5 (3.2-173.0)**	10/0 **∞**

Plain numbers indicate the number of patients and controls (patients/controls) and the numbers in bold indicate the LR (± 95% CI). See Table 1 for information on the assay-specific thresholds.

aβ_2GPI, anti-β2-glycoprotein I antibody; aCL, anticardiolipin antibody; aPL, antiphospholipid antibody; LR, likelihood ratio.

[a] 1 thrombotic control group patient tested positive with all aPL tests of Orgentec ELISA.

execution times (30-40 minutes). Moving from the batch analysis of ELISA to a random-access method contributes to shifting aPL testing to the current laboratory scenario.

Supposedly, standardization relies on the use of the same reliable and stable reference material by the various assays. This should enable consistency of assay results across different methods and laboratories over time [44]. Aiming at standardization among aCL assays, several expert organizations were provided international aCL reference materials, ie, original Harris standard (including matched sets of serum calibrators) [13,14] and monoclonal Koike or Sapporo standards [15,45]. However, despite the use of (i) (the same) international reference materials as a calibrator (Supplementary Data S2) and (ii) standardized GPL or MPL units for reporting test results (even after using different reference materials as calibrator; Supplementary Data S2), large variation in aCL titers remained (Supplementary Data S5). In contrast to the instructions for use, no reactivity was found toward HCAL for Orgentec and QUANTA Lite ELISAs and toward EY2C9 for EliA aCL assay. During the preparation of this manuscript, the availability and distribution of the monoclonal reference materials (by Plasma Services Group) has been discontinued because of loss of reactivity [29], which might explain our results. Recently, the first international β_2GPI IgG reference (NIBSC 21/266) [16], originating from 2 APS patients, was cleared by the World Health Organization. The reference material is available from the Joint Research Center [21]. The lack of a β_2GPI IgG reference material until now could (partly) explain the variability observed among the currently available assays (Supplementary Data S5c). As none of the available reference materials completely mimics the polyclonal and heterogenous nature of aPL, standardization among aPL immunoassays remains an unachieved goal [46].

With respect to clinical performance, ROC analysis revealed comparable AUCs for ELISA, CLIA, and FEIA aPL methods (Figure 2), with a higher diagnostic accuracy for the IgG than IgM isotype for both aCL and aβ_2GPI assays. Even though the American College of Rheumatology/EULAR APS classification criteria state that single positivity for aCL/aβ_2GPI IgM is insufficient to fulfill the criteria, we obtained a LR >10 for nearly all aPL for antibody levels exceeding the 97.5% specificity threshold. Single positivity aCL/aβ_2GPI IgM was observed in 0 to 4.5% and 1.7% to 11.4% of the APS patients and 0.9% to 1.8% and 0.5% to 1.8% of the DCG patients, respectively (Supplementary Data S10). Although very rare, an occasional false single aPL positive in the DCG group may result from natural statistical outliers and/or from innate analytical impression around the cutoff.

A limitation of our study is the inability to assess the diagnostic performance of aCL and aβ_2GPI antibodies separately in obstetric and thrombotic cohorts. Future studies involving larger cohorts will be necessary to conduct subanalyzes that distinctly address the obstetric and thrombotic manifestations of this condition, enabling a more nuanced understanding of the diagnostic value of assays.

Not only the isotype but also the level of aPL antibodies defines the risk of vascular and obstetric features in APS patients [2,5,38]. Our findings confirmed that increasing levels of aPL are associated with an increased likelihood for APS, which was more pronounced for the IgG than for the IgM isotype (Tables 1 and 2). Therefore, reporting nominal aPL test results and adding the corresponding LR is of additional clinical value [18,33]. Despite the difference in quantitative levels, the LRs obtained for the different specificity-based intervals aligned the clinical interpretation of aPL test results across tests from different manufacturers [18].

Taken together, our multicenter study confirmed (i) the lack of standardization among aPL assays, including "standardized" ELISAs; (ii) the low correlation of nominal aPL test results; and (iii) the wide variation in manufacturers' defined aPL cutoff values, obviously impacting the diagnostic performance of the aPL assays (Supplementary Data S7).

Fundamental to obtaining a correct and harmonized aPL test result interpretation is the definition of a "positive aPL test result." In the 2023 APS classification criteria [2], positivity for aCL and aβ_2GPI is considered as moderate (40-79 units) or high (≥80 units) when detected by standardized ELISA [2]. The choice of 40 GPL/MPL was based on its correlation with APS-related symptoms [47]. Recent multicenter studies relied on the moderate/high titer classification to align test result interpretations [33,48]. The ISTH guidance on laboratory diagnosis of APS, however, defines positivity of aCL and aβ_2GPI as any titer greater than the 99th percentile cutoff value of HCs and currently advises against an indication of low/moderate/high titer, awaiting more studies exploring the semiquantitative reporting [5,6]. Semiquantitative interpretation of aCL and aβ_2GPI IgG levels may be clinically valuable, provided that assay-specific thresholds are clearly defined [11]. In line with previous autoantibody harmonization initiatives [28,49–51], we therefore evaluated the impact of using specificity-based thresholds on aPL test result interpretation among different manufacturers (Supplementary Data S8; Tables 1 and 2). The 97.5% specificity thresholds (based on DCG) was associated with a LR for APS > 10 for most of the aPL tests (Table 1) and were highly comparable with the 99% URL evaluated in the HC cohort, justifying the use of this threshold to define aPL positive patients according to diagnostic guidelines [1,2,5,6] and ensuring a more genuine APS classification in research studies. The use of specificity-based thresholds reduced the interassay variation in diagnostic performance of aCL IgG/IgM and aβ_2GPI IgG/IgM assays (Supplementary Data S8), thereby aligning the clinical interpretation among different manufacturers (Table 2).

Of note, however, is that there is some discrepancy between the 99% URLs obtained in our study and those obtained in the studies by the Kaneshige et al. [35], Zigon et al. [48], Cabrera–Marante et al. [52], and Manukyan et al. [53] (Supplementary Data S11). Differences in statistical evaluation (parametric vs nonparametric, outlier exclusion vs no outlier exclusion), inclusion/exclusion criteria and demography (age, sex, ethnicity), and (the limited) numbers of individuals included can be addressed as most probable causes. The method for outlier detection used and exclusion of the outliers has a major impact on the accurateness of the 99% URLs obtained [54–56]. Therefore, similar to other autoimmune serology tests [28,49–51], the use of specificity-based thresholds is a clinically valuable alternative and probably a more profound approach to align test result interpretation across manufacturers.

However, the 97.5% specificity thresholds in the cohort reported by Vandevelde et al. [33] are significantly higher compared with our results for all included assays. In this respect, the composition of the DCG is very important. Our multicenter control cohort comprised consecutive diseased patients (detailed composition summarized in Supplementary Data S1), directly mirroring our daily routine care. Among the DCG, 53.8% were autoimmune patients, of which only 1.4% (6/433) had systemic lupus erythematosus (SLE) without secondary APS. As the incidence of APS in SLE ranges from 7% to 51% [57], the presence of aPL antibodies will be higher in a control group that contains a substantial proportion of SLE patients. This was also illustrated by the markedly increased LRs for APS after exclusion of the autoimmune control cohort (Table 3 vs Table 4). Vandevelde et al. [33] did not include consecutive patients tested for aPL antibodies but included preselected cohorts of (i) autoimmune disease controls (47% SLE), (ii) hospitalized controls lacking clinical APS criteria, (iii) thrombotic controls without the presence of aPL antibodies, and (iv) obstetric controls who did not fulfill laboratory criteria for APS (Supplementary Data S11).

For diagnostic studies the composition of the APS patient group is also important. In our multicenter APS cohorts, all APS patients fulfilled the APS Sydney classification criteria [1] with laboratory diagnostics based on (i) LA measurement according to the ISTH guidelines [20] and (ii) presence of aCL IgG/IgM and aβ_2GPI IgG/IgM using a noncommercial in-house ELISA. A minority (13.8%) of the APS patients tested negative in all aPL antibody commercial assays, and these patients were all isolated LA positive at primary diagnosis.

As a limitation of our study, we acknowledge that the specificity-based thresholds and the resulting test result interval-specific APS LRs should be confirmed by additional studies mimicking routine APS diagnostics.

We found that use of multiple aPL antibody reactivity increased the LRs for APS (Table 3). This conforms with the APS diagnostic guidelines [5,6], classification criteria [2], and management guidelines [9] and the fact that multiple positive aPL antibody test results have the strongest association with thrombotic manifestations of APS [37]. Triple positivity (LA, aCL, and aβ_2GPI) is associated with recurrent thrombosis, and if detected in asymptomatic individuals, is associated with an important risk for primary thrombotic event [38,58]. In this study, combined positivity for aCL IgG, aCL IgM, and aβ_2GPI IgG in all assays resulted in a higher LR for APS (Table 3). By the exclusion of the autoimmune control cohort, infinite LRs for APS

were obtained by most manufacturers. There was no added diagnostic value of detecting aβ$_2$GPI IgM, probably due to the fact that our APS cohort was mainly composed of individuals with thrombotic events. The combination of negative aCL IgG/IgM and aβ$_2$GPI IgG/IgM lowered the LR for APS from 0.48 to 0.82 and 0.34 to 0.70 (Table 2) to 0.22 to 0.41 (Table 3). Of note, only LRs <0.1 are regarded sufficient to reliably exclude APS diagnosis [19]. Lower thresholds should have been defined to identify test result intervals associated with LR <0.1. Based on current evidence, simultaneous testing of aCL IgG/IgM and aβ$_2$GPI IgG/IgM should be performed to obtain the optimal diagnostic sensitivity in women suspected of having obstetric APS. However, in thrombotic patients suspected of APS, testing for IgM is not considered essential as a first line of testing [41]. Nevertheless, it is useful in risk stratification because the presence of IgM, along with IgG and LA, increases the risk for thrombotic complications [38]. The availability of automated assays allows simultaneous detection of multiple types of aPL antibodies for improved diagnosis of APS [35,41,59].

Although the recent 2023 APS classification criteria restrict the detection of aCL and aβ$_2$GPI IgG/IgM to "standardized" ELISAs, our study revealed no superiority of the ELISA assays over automated assays, either analytically (ELISAs have higher imprecision, lower Spearman rank correlation, lack of traceability) or diagnostically (ELISAs have lower AUC of ROC vs CLIA and/or FEIA methods). In 2018 and again more recently, the ISTH Scientific and Standardization Committee on Lupus Anticoagulant/Antiphospholipid Antibodies published a guidance document for the laboratory diagnosis of APS including the use of non-ELISA assays [5,6,60]. Interestingly, the authors underlined how we need to raise awareness of the different ranges of ELISA and non-ELISA solid phase-based aCL/aβ$_2$GPI assays for a proper and accurate interpretation of the results. In fact, the differences among methods can create misunderstanding, such as a clinical interpretation of a low titer in non-ELISA tests as a high titer when using ELISA with a consequent overestimation of the patient's risk, potentially resulting in more aggressive treatment and possible side effects.

In conclusion, harmonization in the clinical interpretation of aPL antibody test results could be obtained by the postanalytical use of uniform thresholds based on predefined specificity, irrespective of the assay technology [10,33]. To confirm our specificity-based thresholds, additional studies mimicking routine APS diagnostics are warranted. Ideally, specificity-based thresholds should be evaluated for thrombotic and obstetric cohorts separately.

ACKNOWLEDGMENTS

We thank all participating diagnostic companies for in-kind provision of the assays, technical training and support and the constructive discussions. Furthermore, we are very thankful to the laboratory technicians of the AZORG Hospital Aalst for their most appreciated assistance in performing the antiphospholipid assays. All participating diagnostic companies in-kind provided the antiphospholipid assays, technical training, and support free of charge (see competing interests).

AUTHOR CONTRIBUTIONS

L.V.H., M.I., and X.B. contributed to the study conception and design. Patient samples were selected by L.V.H., E.B., B.M., S.B., S.S., S.P., E.M.G., and A.T. Material preparation and data collection was performed by B.M., S.B., E.B., S.V.D.B., and L.V.H. and data analysis by L.V.H. and X.B. Manuscript was prepared by L.V.H. and X.B. and commented upon by all authors. The final manuscript was read and approved by all authors. All authors have accepted responsibility for the entire content of this manuscript and approved its submission.

DECLARATION OF COMPETING INTERESTS

X.B. and L.V.H. have received speaker fees from Thermo Fisher Scientific and Werfen and have been consultants for Thermo Fisher Scientific and Werfen. S.P. has received speaker fees from Thermo Fisher Scientific. S.S. and M.I. have been consultants for Werfen. All participating diagnostic companies (Thermo Fisher Scientific, Phadia AB; Inova Diagnostics/Werfen; Orgentec) in-kind supported with aPL antibody assays and technical training. S.P., E.B., M.R., E.M.G., B.M., S.B., S.V.D.B., B.V.C., K.M.D., and A.T. have no conflicts of interest to declare.

DATA AVAILABILITY

All data relevant to the study are included in the article or uploaded as supplementary information.

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SUPPLEMENTARY MATERIAL

The online version contains supplementary material available at https://doi.org/10.1016/j.jtha.2025.05.008.