ARTICLE IN PRESS

Pathology xxx (xxxx) xxx

Contents lists available at ScienceDirect

Pathology

journal homepage: www.pathologyjournal.rcpa.edu.au

IMMUNOPATHOLOGY / HAEMATOLOGY

A contemporary analysis of solid-phase antiphospholipid antibody testing: implications for classification or diagnosis of antiphospholipid syndrome

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ARTICLE INFO

Key words:
antiphospholipid syndrome
anticardiolipin
anti-beta-2-glycoprotein
diagnosis
classification
external quality assessment
enzyme-linked immunosorbent assay

ABSTRACT

Antiphospholipid antibody (aPL) testing is required for diagnosis of antiphospholipid syndrome (APS). Although they have different uses, clinicians sometimes (inappropriately) use APS classification criteria as diagnostic criteria. We evaluated interlaboratory performance of testing for anticardiolipin (aCL) and anti-beta-2-glycoprotein (aβ2GPI) from participants of the Royal College of Pathologists of Australasia Quality Assurance Programs Immunology program over the last 3 years, from information in the final reports. We analysed data for aCL immunoglobulin (Ig)G, aCL IgM and aβ2GPI IgG testing, as representing the main aPL assays. These were assessed according to reagent type and subtyped into enzyme-linked immunosorbent assay (ELISA) versus non-ELISA solid-phase assays. We assessed results for interlaboratory consensus regarding aPL-positive versus aPL-negative samples and quantitative data, including mean, median, coefficient of variation (CV), and standard deviation. Universal consensus was only achieved in approximately one-third (39%) of samples over the reporting period, while 80% participant agreement occurred in 87.5% of tests overall, with results comparable between analytes (22/24=92% IgG aCL; 19/24=79% IgM aCL; 22/24=92% IgG aβ2GPI). CVs were often above 50% for aPL-positive samples for most ELISA assays, but often <20% for non-ELISA methods, with the poorest overall harmonisation for aCL IgM testing. There was an emerging trend for reduced usage of ELISA-based assays, with currently fewer than 25% of participants using these assays. In conclusion, variability persists in the reporting of aCL and aβ2GPI, with limited consensus and an emerging trend to uptake of non-ELISA solid-phase reagents, with reduced CV trends, highlighting the disconnect between APS classification and diagnostic criteria. Further harmonisation of solid-phase testing for real-world diagnostic applications is also required.

1. Introduction

Antiphospholipid syndrome (APS) was described over 40 years ago and is a clinicopathological diagnosis.[1,2] APS remains a challenging diagnosis for both laboratory staff and clinicians. Diagnosis requires the demonstration of a positive antiphospholipid antibody (aPL) profile in the correct clinical context (generally thrombosis and/or pregnancy morbidity). Testing for aPL includes clot-based assays for lupus anticoagulant (LA), and immunological 'solid-phase' assays for anticardiolipin (aCL) and anti-beta-2-glycoprotein (aβ2GPI). Furthermore, updated recommendations for the classification of APS have been recently published with joint support by the European Alliance of Associations for Rheumatology (EULAR) and American College of Rheumatology (ACR), including laboratory criteria and expanded clinical criteria, and a focus on optimising specificity for use in observational studies and trials.[3] As explained in this publication and arising commentaries, classification guidelines, as distinct from diagnostic criteria, are not intended to capture the entire population of potential patients with APS, and caution should be used if so applied.[3,4] Diagnostic criteria include an expanded clinical and laboratory criteria set, and APS diagnosis should instead be guided by expert guidelines such as the International Society on Thrombosis and Haemostasis (ISTH) Scientific and Standardisation Committee (SSC) for LA/aPL.[5–10]

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https://doi.org/10.1016/j.pathol.2025.07.003
Received 30 January 2025; Received in revised form 30 June 2025; Accepted 16 July 2025
Available online xxxx

Please cite this article as: Thirunavukarasu C et al., A contemporary analysis of solid-phase antiphospholipid antibody testing: implications for classification or diagnosis of antiphospholipid syndrome, Pathology, https://doi.org/10.1016/j.pathol.2025.07.003

A variety of immunoassays are available for assessment of aPL, including new automated platforms and historical or classic enzyme-linked immunosorbent assay (ELISA) testing. As an example of the mismatch between classification criteria and diagnostic criteria, only ELISA testing is considered for characterisation of 'definite' APS in the classification criteria,[3] although newer methodologies can of course be used for APS diagnosis and indeed now comprise the main methodologies in use.[4] In Australasia, according to recent data from the Royal College of Pathologists of Australasia Quality Assurance Programs (RCPAQAP), laboratories use a mix of ELISA-based and non-ELISA-based solid-phase testing, with ELISA-based testing now accounting for a minority of testing modalities.[4] Recently introduced automated platforms include variations of solid-phase (e.g., magnetic microparticles, microspheres) and various detection systems [e.g., chemiluminescence (CLIA), multiplex flow immunoassay (MFI), flow cytometry] with several theoretical advantages, including harmonised test conditions, strict protocols, more rapid and less labour-intensive throughput, and reduced interlaboratory variability.[11] Furthermore, most laboratories restrict testing to a core panel including immunoglobulin (Ig)G aCL, IgM aCL and IgG aβ2GPI[12,13] for practical purposes, including recognised diagnostic utility, but also because these comprise the main so-called 'classification criteria' aPL.[3,14–16]

The current report provides an assessment of testing for aCL and aβ2GPI, and presents findings from the RCPAQAP Immunology program, evaluating laboratory performance over the last 3 years (2022–2024). Based on these findings, we will also provide recommendations to improve the processes for these immunological aPL assays, which are integral in optimising the quality of test results and in the diagnosis and management of APS.

2. Method

2.1. Setting

This study assesses data submitted by participants of the RCPAQAP Immunology program from the first survey in 2022 until the end of 2024. This 3-year period was chosen as it provides a reasonable period of contemporary testing. Specifically, results from this external quality assessment (EQA) program for aCL and aβ2GPI antibody testing were evaluated from finalised RCPAQAP reports. These reports outline participant numbers and tests performed. Although the RCPAQAP EQA covers antibody testing for aCL and aβ2GPI for all major Ig subtypes (IgG, IgM, and IgA), participant numbers are highest (i.e., >50) for aCL (IgG and IgM) and aβ2GPI (IgG), with participant numbers for other isotypes being much lower (aCL and aβ2GPI IgA both <10; aβ2GPI IgM <30). Accordingly, this study restricts analysis to the core panel of antibody testing comprising aCL IgG, aCL IgM, and aβ2GPI IgG, as comprising sufficiently large data sets for analysis.

Additionally, the reports provide basic statistical analysis of individual methods [i.e., median, mean, standard deviation (SD), coefficient of variation (CV%), when there are ≥4 participants]. Accordingly, data analysis is mainly restricted to methods where data are available for ≥4 participants.

2.2. Samples

The RCPAQAP distributed 24 samples during the data capture period, and all samples were generated in-house from patient material donated from various source laboratories or else from normal plasma/serum. In general, neat serum or neat defibrinated plasma was provided to survey participants as small aliquots. Samples comprised a sequential mix of normal and antibody-positive samples. Some repeat samples were issued, and these can help assess comparability between surveys and inter- and intra-laboratory performance over time. Eight samples were sent per 12-month period, comprising separate proficiency challenges with positive and normal samples distributed in a semi-random fashion. Furthermore, some samples were positive for single or multiple antibodies, as potentially representative of variable 'real-world' test patterns.

2.3. Data analysis

As per the RCPAQAP Immunology program, all participants submit numerical values for the aPL tests they perform. Participants are also required to submit their interpretation of data in terms of whether test results are normal, elevated, or equivocal, which then allows comparison with an interpretation target, as based on consensus among ≥80% of participants for that particular analyte. This process enables participants to interpret results in a similar way to real-world patient testing and also allows further analysis of test results. The RCPAQAP does not assess reported numerical values, instead assessing the reported interpretations, which are marked as either concordant or not concordant, as assessed against the target interpretation. We analyse and report

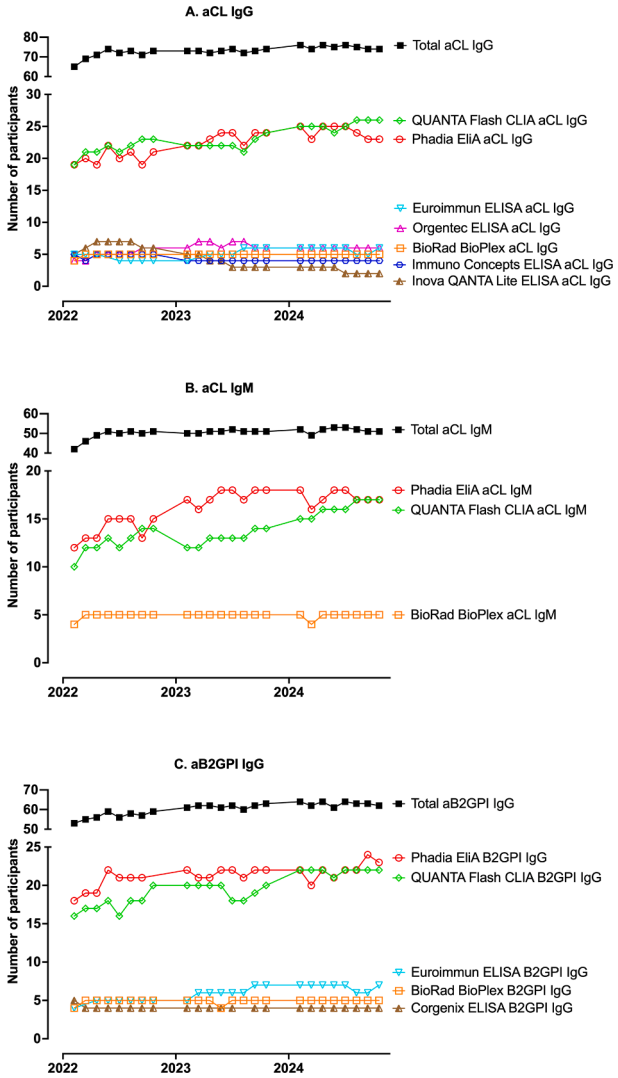

Fig. 1. Total number of participants in the RCPAQAP Immunology program according to analyte and reagents. (A) IgG aCL testing, (B) aCL IgM testing, (C) aB2GPIb IgG testing, with various reagents including Phadia EliA, Inova Quanta Flash CLIA, Euroimmun ELISA, Orgentec ELISA, Bio-Rad Bio-Plex, Immuno Concepts ELISA, and Inova Quanta Lite ELISA. aCL, anticardiolipin antibodies; CLIA, chemiluminescence immunoassay; ELISA, enzyme-linked immunosorbent assay; EliA, enzyme-linked immunosorbent assay (Phadia platform); Ig, immunoglobulin; RCPAQAP, Royal College of Pathologists of Australasia Quality Assurance Programs.

Table 1
Summary of patient clinical information and/or features of APS, including interlaboratory consensus on whether sample was aPL positive or negative

Specimen no. (IM-AP)	Age (years)	Sex	Clinical notes	Clinical features of APS?	Donor repeats?	Consensus[a]					
						IgG aCL	100% agreement	IgM aCL	100% agreement	IgG aβ2GPI	100% agreement
2022/1	60	M	APS	Yes	IM-AP-22-05	**Yes**	**Yes**	**Yes**	**Yes**	**Yes**	No
2022/2	41	M	APS	Yes		**Yes**	No	**Yes**	**Yes**	**Yes**	No
2022/3	34	F	Not available	N/A		**Yes**	No	No	No	**Yes**	No
2022/4	56	M	Hyperferritinaemia, metabolic syndrome	No		**Yes**	**Yes**	No	No	**Yes**	**Yes**
2022/5	60	M	APS	Yes	IM-AP-22-01	**Yes**	No	**Yes**	**Yes**	**Yes**	**Yes**
2022/6	54	M	Triple positive APS, recurrent thromboses, stroke. SLE. Meds: rituximab, HCQ, mycophenolic acid, warfarin, aspirin, colchicine, allopurinol	Yes		**Yes**	**Yes**	**Yes**	No	**Yes**	**Yes**
2022/7	41	M	APS	Yes		**Yes**	No	**Yes**	No	**Yes**	No
2022/8	50	F	History of elevated ferritin and constitutional symptoms including arthralgia. Treatment: regular venesections	No		**Yes**	No	**Yes**	No	**Yes**	No
2023/1	60	M	APS	Yes		**Yes**	No	**Yes**	No	**Yes**	Yes
2023/2	60	M	Non-HFE iron overload syndrome. Treatment: regular venesections. Meds: telmisartan, Cartia	No	IM-AP-23-08	**Yes**	**Yes**	**Yes**	**Yes**	**Yes**	**Yes**
2023/3	34	F	Not available	N/A	IM-AP-23-07	**Yes**	**Yes**	No	No	**Yes**	No
2023/4	41	M	APS	Yes		**Yes**	**Yes**	**Yes**	No	**Yes**	No
2023/5	42	F	APS (stroke, Libman-Sacks endocarditis, thrombocytopenia). Meds: warfarin, atorvastatin, previous rituximab exposure	Yes		**Yes**	**Yes**	**Yes**	No	**Yes**	**Yes**
2023/6	65	F	SLE with secondary Sjogren syndrome. APS. Meds: mycophenolic acid, prednisone, warfarin, metformin hydrochloride, perindopril, gliclazide	Yes		**Yes**	No	**Yes**	**Yes**	Yes	No
2023/7	34	F	Not available	N/A	IM-AP-23-03	**Yes**	No	No	No	**Yes**	No
2023/8	60	M	Non-HFR iron overload.	No	IM-AP-23-02	**Yes**	**Yes**	**Yes**	No	**Yes**	**Yes**
2024/1	41	M	APS	Yes		**Yes**	**Yes**	**Yes**	**Yes**	No	No
2024/2	75	F	APS. Meds: acetylsalicylic acid, minoxidil, calciferol	Yes		No	No	**Yes**	**Yes**	No	No
2024/3	45	M	APS	Yes		**Yes**	No	**Yes**	No	**Yes**	No
2024/4	53	M	Haemochromatosis. Treatment: regular venesections	No		**Yes**	No	**Yes**	No	**Yes**	No
2024/5	34	F	Normal defibrinated plasma	No		**Yes**	No	No	No	**Yes**	No
2024/6	59	M	APS	Yes		No	No	**Yes**	No	**Yes**	**Yes**
2024/7	45	M	APS	Yes		**Yes**	**Yes**	**Yes**	No	**Yes**	**Yes**
2024/8	35	M	Hereditary haemochromatosis (homozygous C282Y mutation). Treatment: regular venesections	No		**Yes**	**Yes**	**Yes**	No	**Yes**	**Yes**

aβ2GPI, anti-β2-glycoprotein I antibodies; aCL, anticardiolipin antibodies; APS, antiphospholipid syndrome; HCQ, hydroxychloroquine; Ig, immunoglobulin; IM-AP, sample code; SLE, systemic lupus erythematosus.

[a] Consensus defined as ≥80% agreement of positive or normal; 100% consensus defined as unanimous agreement of positive or normal. Summary for 80% consensus: overall 63/72=87.5%; 22/24 samples=92% (IgG aCL); 19/24=79% (IgM aCL); 22/24=92% (IgG aB2GPI). Summary for 100% consensus: overall 28/72=39%; 11/24=46% (IgG aCL); 7/24=29% (IgM aCL); 10/24=42% (B2GPI IgG).

summaries of these qualitative and quantitative results of testing from the finalised survey reports.

3. Results

3.1. Method representation

We reviewed data from the RCPAQAP Immunology EQA for the past 3 years (from the first survey of 2022 to the end of 2024). Participant numbers varied according to reagent and analyte but averaged from 42 (IgM aCL) to 76 (IgG aCL) (Fig. 1). Table 1 provides relevant patient clinical information and/or features of APS for patient samples: determination of interlaboratory consensus on whether sample was aPL positive or negative (consensus defined as ≥80% positive or ≥80% negative) and also identifies test samples where testing demonstrated 100% participant agreement. A summary of results from all 24 samples distributed during this period is presented in Table 2. Fourteen of 24 samples (58.3%) yielded median aPL values >20 units, with the remainder yielding median aPL values ≤20 units (Table 2).

3.2. Qualitative concordance

Complete consensus was usually not attained, and it accounted for approximately one-third (39%) of tests across all analytes (aCL IgG, aCL IgM, aβ2GPI IgG) for 24 distributions. Interestingly, 100% participant agreement was more common for IgG aCL (11/24 samples, 46%) compared with aCL IgM and IgG aβ2GPI [7/24 (29%) and 10/24 (42%), respectively]. Using the RCPAQAP's minimally defined criteria of 80% participant agreement, consensus was attained in 87.5% of testing overall. Comparison between analytes demonstrated similar 80% consensus results in all groups (22/24=92% IgG aCL; 19/24=79% IgM aCL; 22/24=92% IgG aβ2GPI) (Table 1).

In terms of testing modality, ELISA solid-phase testing was only used by a quarter of survey participants, with a trend towards decreasing use of ELISA testing over the study period (Fig. 1). ELISA-based testing ranged between 32% and 33% for IgG aCL, 27% and 32% for IgM aCL, and 20% and 25% for IgG aβ2GPI. The remainder of test methods comprised non-ELISA immunological solid-phase platforms (Fig. 1). Substantial variability in terms of median results, data range, and CV data were present regardless of ELISA versus solid-phase testing reagent subtype (Fig. 2–5).

3.3. Quantitative comparison

Inter-laboratory CVs often exceeded 50% for ELISA-based methods, reflecting widely variable numerical test values for the different aPL analytes from participants (Fig. 5, Tables 1 and 2). In contrast, for the majority of samples with median aPL values >20 units, CVs were often <20% for non-ELISA methods (Table 3). Standard deviations (SDs) also frequently exceeded 100 aPL 'units' for samples with median aPL values >20 units, with multiple cases of SDs being >1000 units in some survey samples (Table 2).

3.4. Method variation comparison

Fig. 2–4 examine analyte levels per survey according to reagent. Positive results (moderate to high level according to the current classification criteria[3]) are represented by red bars (≥40 units) compared with lower results (<40 units) in green.

Fig. 2 represents aCL IgG testing with reagents including non-ELISA solid-phase platforms comprising Bio-Rad Bio-Plex, Phadia EliA, and Inova Quanta Flash CLIA, and ELISA assays including Euroimmun ELISA, Immuno Concepts ELISA, Inova Quanta Lite, and Orgentec ELISA. There was harmony for some (but not all) samples, with uniform reporting of results ≥40 units versus <40 units, respectively, according to reagent type across surveys. The samples not demonstrating harmonised findings (i.e., 23/6, 24/2, 24/6), could generally also, be identified as those with a lower chance of achieving consensus in Table 1.

Fig. 3 represents aCL IgM testing, with a fewer number of methods comprising ≥4 participants, namely Bio-Rad Bio-Plex, Inova Quanta Flash CLIA, and Phadia EliA, all of which were non-ELISA-based platforms. In contrast to aCL IgG results, there was marked variability amongst qualitative testing results, with several samples (i.e., 22/3, 23/3, 23/7, 24/3, 24/6, 24/7) yielding results ≥40 units versus <40 units according to the method. Again, these generally aligned to limited consensus data in Table 1.

Table 2
Summary of results from all 24 samples

Specimen no. (IM-AP)	Median (aPL units)			Mean (aPL units)			Standard deviation (aPL units)			Coefficient of variation (%)		
	IgG aCL	IgM aCL	IgG aβ2GPI	IgG aCL	IgM aCL	IgG aβ2GPI	IgG aCL	IgM aCL	IgG aβ2GPI	IgG aCL	IgM aCL	IgG aβ2GPI
2022/1	110.2	119.5	167.0	648.3	204.5	167.0	1034.0	195.0	2309.7	159.5	95.3	152.0
2022/2	111.0	472.0	103.0	111.0	472.0	131.3	204.0	537.0	122.0	99.0	103.0	92.9
2022/3	3.3	32.8	3.1	4.8	32.8	8.2	5.12	16.1	7.9	107.2	45.8	96.7
2022/4	2.6	6.0	1.4	2.6	11.5	3.8	2.9	86.2	4.1	96.7	86.2	107.0
2022/5	101.2	115.2	169.0	101.2	190.6	1740.0	968.2	177.0	2458.4	148.8	92.9	141.0
2022/6	342.5	5.0	442.0	724.4	5.0	2468.0	802.5	3.98	3587.7	110.8	79.1	145.0
2022/7	95.2	472.0	124.4	180.3	557.1	131.2	162.4	705.0	114.0	90.0	127.0	86.9
2022/8	2.8	1.5	2.0	4.4	5.3	6.8	204.6	406.0	22.0	204.6	406.0	323.0
2023/1	110.7	122.8	178.0	727.9	203.5	1723.0	1107.0	194.0	2381.8	152.0	95.4	138.0
2023/2	2.1	1.9	1.6	2.7	2.6	1.6	2.94	3.13	3.5	109.9	122.0	102.0
2023/3	3.7	20.1	3.3	4.3	20.1	8.5	3.06	15.5	7.6	70.9	51.5	89.3
2023/4	120.0	472.0	101.2	211.6	655.1	117.9	180.0	877.0	104.0	85.1	134.0	88.2
2023/5	120.5	5.8	100.2	282.9	7.5	478.7	284.6	4.46	621.0	100.6	59.6	130.0
2023/6	71.4	1.5	238.8	176.4	1.6	1415.0	170.9	1.18	1844.1	96.9	72.4	130.0
2023/7	4.0	23.0	3.0	5.1	28.3	8.4	5.64	15.2	8.3	110.0	53.7	98.5
2023/8	3.1	1.9	1.6	3.1	4.1	3.9	3.13	13.3	4.1	100.1	322.0	106.0
2024/1	94.3	527.4	98.8	190.6	489.5	127.2	161.4	82.6	117.0	84.7	82.6	91.7
2024/2	23.6	111.3	19.3	27.3	160.1	28.7	13.86	102.0	26.0	50.8	64.0	91.1
2024/3	430.0	80.0	155.0	457.4	93.6	1136.0	291.0	66.1	1426.2	63.6	70.6	126.0
2024/4	2.0	1.0	2.9	2.6	1.7	3.8	2.8	2.8.0	3.2	107.1	162.0	83.9
2024/5	2.7	18.8	3.2	3.8	21.1	6.1	3.1	12.1	5.2	57.5	57.5	84.0
2024/6	90.1	47.5	112.8	297.3	67.8	389.9	357.4	59.8	430.5	120.2	88.3	110.4
2024/7	419	80.0	150.0	481.8	103.6	1167.5	317.1	82.0	1476.9	65.8	79.1	126.5
2024/8	3.0	2.6	2.5	4.4	3.5	3.7	3.6	3.2	3.4	83.1	91.2	91.1

aβ2GPI, anti-β2-glycoprotein I antibodies; aCL, anticardiolipin antibodies; IM-AP, sample code; aPL, antiphospholipid antibody; Ig, immunoglobulin.

Fig. 4 demonstrates aβ2GPI testing according to reagent, with non-ELISA-based solid-phase reagents (Bio-Rad Bio Plex, Inova Quanta Flash CLIA, and Phadia EliA) and few ELISA-based reagents (Corgenix and Euroimmun). Again, although most methods showed concordance in results ≥40 units versus <40 units for most samples, there was discordance for some samples (i.e., 22/2, 22/7, 23/6, 24/1, 24/2), with some platforms (e.g., Phadia EliA) showing greater discordance to other methods.

Finally, Fig. 5 shows assay variability (as CVs) according to the level of aPL for the main methods as well as overall. In general, CVs were highest for lowest (generally negative) levels, but high CVs were also evident for high levels. Some methods (e.g., Inova Quanta Flash CLIA) appeared to have lower CVs than other methods.

4. Discussion

We present data from the past 3 years of aCL and aβ2GPI testing from RCPAQAP Immunology program reports, with utility for the diagnostic evaluation of patients with aPL/APS.[5–8] Participant numbers have increased slightly over this period, and as last reported.[14] However, we also note a trend for decreasing use of ELISA-based reagents across all analytes, as also highlighted in the previous report.[14] Indeed, in 2024, non-ELISA (especially Phadia EliA and Inova Quanta Flash CLIA) platforms were the dominant methods used across all analytes. In contrast, ELISA-based reagents account for approximately one-quarter of reagents and are increasingly absent for some analytes (e.g., aCL IgM; Fig. 1 and 3), where some ELISA-based platforms had fewer than four

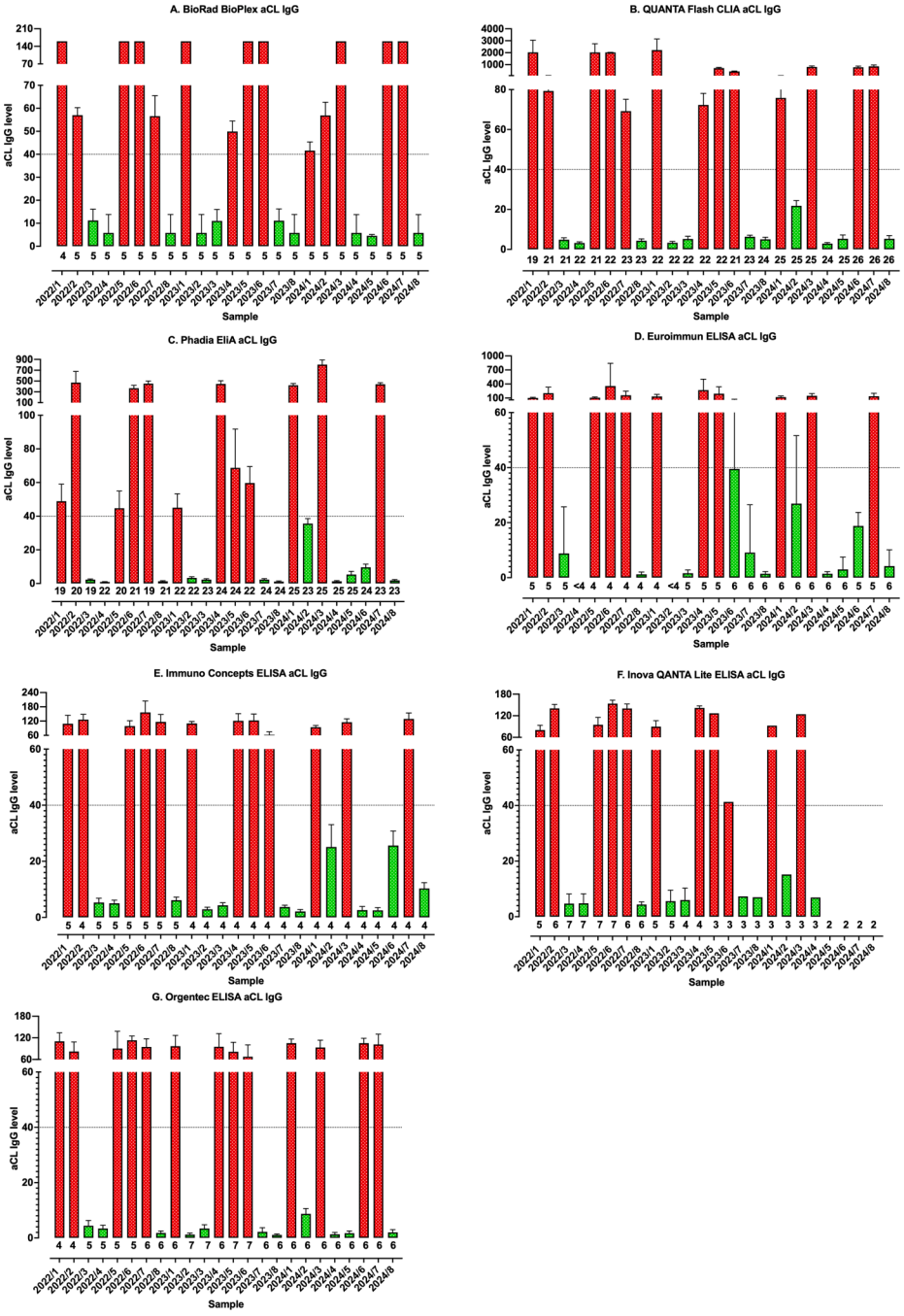

Fig. 2. Variations in numerical data submitted by participants of the RCPAQAP Immunology program for IgG aCL per analyte including Bio-Rad Bio-Plex, Immuno Concepts ELISA, Euroimmun ELISA, Inova Quanta Flash CLIA, Inova Quanta Lite ELISA, Orgentec ELISA, and Phadia EliA. Green bars represent quantitative result <40 units; red bars represent quantitative results ≥40 units (dotted horizontal line at 40 units). Results shown as means ± standard deviation. Numbers below bars = number of reported results. aCL, anticardiolipin antibodies; CLIA, chemiluminescence immunoassay; ELISA, enzyme-linked immunosorbent assay; EliA, enzyme-linked immunosorbent assay (Phadia platform); Ig, immunoglobulin; RCPAQAP, Royal College of Pathologists of Australasia Quality Assurance Programs.

users per survey, thereby eliminating these methods from robust statistical evaluation. This highlights the disconnect between diagnostic versus classification criteria for APS. In agreement with our findings, a recent United Kingdom National External Quality Assessment Service EQA review published similar results with comparison of methodologies for antiphospholipid testing. The study had a larger sample size of approximately 1500 and 2600 for aCL and aβ2GPI testing, respectively, revealing a trend towards use of fluorescence enzyme immunoassay (FEIA)/CLIA and with reduced ELISA use.[17]

Thus, if the newest ACR classification guidelines[3] were used as diagnostic criteria and followed in terms of requirements to use ELISA-based methods, most real-world patients would not receive a diagnosis of aPL/APS. Furthermore, there is inconsistency in test results according to methods; thus, using the ACR classification guidelines would mean different patients would receive a classification as moderate-level aPL (40–79 units) or high-level aPL (>80 units) depending on the ELISA method used. This is also contrary to ISTH SSC subcommittee advice, which cautions against semi-quantitative reporting, particularly when comparing ELISA to other solid-phase assay reagents.[8] Furthermore, newer solid-phase systems such as CLIA have an expanded dynamic test range and can report much higher antibody levels than ELISA, raising further issues on how to categorise results into semi-quantitative sets of low, moderate or high positive and compare results between assays.[18]

Test samples repeated in different challenges yielded similar results, including median and SDs, which is consistent with previous findings[14] (see Tables 1 and 2). Further, qualitatively, results were more consistent between laboratories in terms of identified concordance (Table 1, Fig. 2–4). Consensus was overall more consistent with IgG aCL testing followed by IgG aβ2GPI; however, significant variability still exists with aCL IgM testing. Overall, there were also fewer laboratories reporting results for this analyte, with only three solid-phase reagents used by ≥4 participants to enable statistical analysis by the RCPAQAP Immunology program. Whilst the overall reported rate of 100% consensus among participants was relatively low at 39%, it was higher than that reported in an earlier study where only 15.3% achieved 100%.[16] Explanations for reduced overall 100% concordance include the possibility of reporting error or transcription error, and test method variability; accordingly, the low proportion of 100% concordance is not unexpected. However, the trend for improved overall concordance compared with past studies is encouraging and may reflect reduction in variability due to improved methodologies and improved adherence to Australian consensus guidelines.[12,15] Furthermore, the trend away from semi-quantitative reporting was also noted in a prior review of RCPAQAP data.[14] Although at odds with recent classification guideline recommendations, this may account for some reduction in variability, particularly between ELISA and solid-phase reagents, again highlighting the discordance between research ('classification') and 'real-world' ('diagnosis') focus on testing outcomes. However, limitations of conformity remain in the context of transcription error, method variability, and intrinsic challenges with pre- and post-analytical issues.

There remains ongoing interlaboratory variability regarding median data range and CV data. Unfortunately, CVs frequently still exceed 50% for ELISA assays, which parallel previous local analyses.[14,16] However, pleasingly, CVs were often <20% for non-ELISA assays for positive aPL samples (Table 3). A review of recent RCPAQAP reports for other more common immunological analytes (for example, elevated deamidated gliadin IgG antibodies, and elevated thyroid peroxidase antibodies) showed CVs in the same region as for aPL. The CV reflects a ratio of SD to the mean and is illustrated in QAP survey reports when analysing method-specific quantitative results, and is a means to assess interlaboratory precision. CV values thus provide information on error, bias, and imprecision and allow laboratories to monitor, improve, and establish good laboratory practice and address discrepancies in reporting compared to their peer group using the same methodology. However, comparison of CVs when results are reported in low concentrations is of reduced applicability due to mathematical limitations in the applied formula.

Possible explanations for ongoing variability between laboratories include different assay platforms, lack of standardisation between

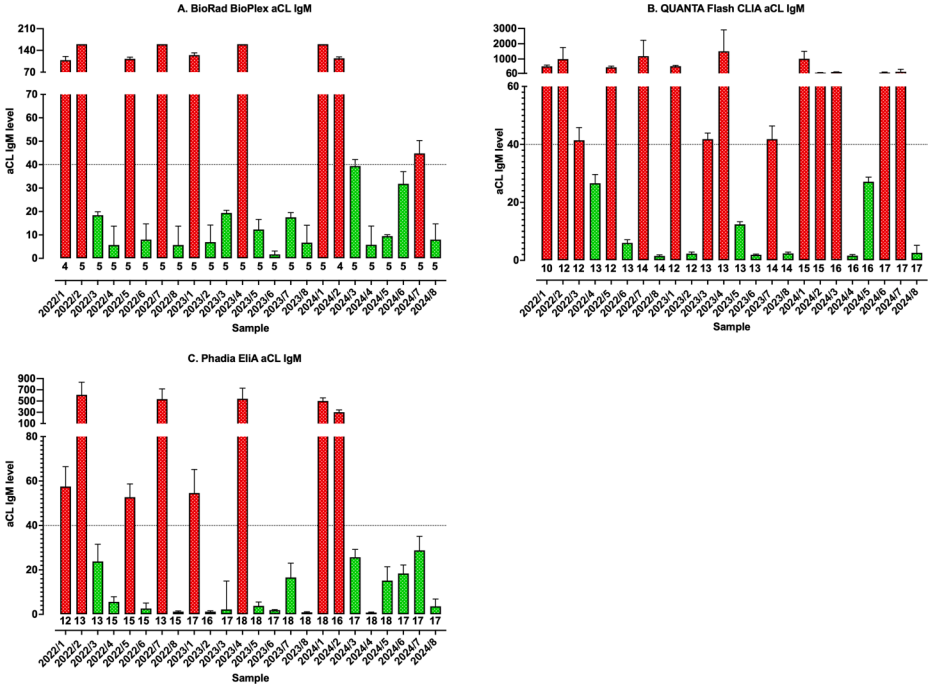

Fig. 3. Variations in numerical data submitted by participants of the RCPAQAP Immunology program for IgM aCL per analyte, including Bio-Rad Bio-Plex, Inova Quanta Flash CLIA, and Phadia EliA. Green bars represent quantitative result <40 units; red bars represent quantitative results ≥40 units (dotted horizontal line at 40 units). Results shown as means ± standard deviation. Numbers below bars = number of reported results. aCL, anticardiolipin antibodies; CLIA, chemiluminescence immunoassay; EliA, enzyme-linked immunosorbent assay (Phadia platform); Ig, immunoglobulin; RCPAQAP, Royal College of Pathologists of Australasia Quality Assurance Programs.

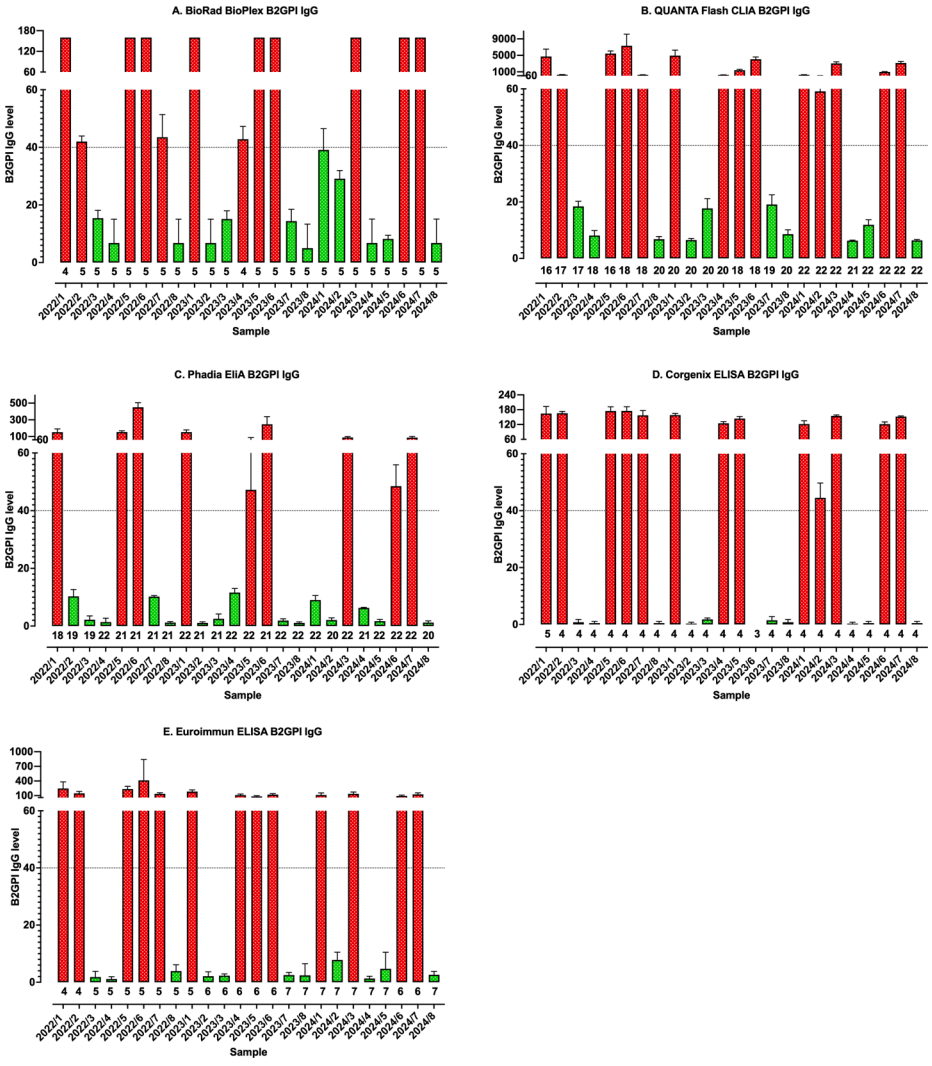

Fig. 4. Variations in numerical data submitted by participants of the RCPAQAP Immunology program for IgG aβ2GPI per analyte, including Bio-Rad Bio-Plex, Immuno Concepts ELISA, Euroimmun ELISA, Inova Quanta Flash CLIA, Inova Quanta Lite ELISA, Orgentec ELISA, and Phadia EliA. Green bars represent quantitative results <40 units; red bars represent quantitative results ≥40 units (dotted horizontal line at 40 units). Results shown as means ± standard deviation. Numbers below bars = number of reported results. aβ2GPI, anti-β2-glycoprotein I antibodies; CLIA, chemiluminescence immunoassay; ELISA, enzyme-linked immunosorbent assay; EliA, enzyme-linked immunosorbent assay (Phadia platform); Ig, immunoglobulin; RCPAQAP, Royal College of Pathologists of Australasia Quality Assurance Programs.

internationally produced commercial kits, differences in antibody affinity according to coating method used for solid phase, differential sources of β2GPI used in assays, and lack or differential use of universal standards for assay calibration. Challenges for standardisation pertaining to solid-phase methodologies, including different reporting units [e.g., chemiluminescent units (CU) versus IgG phospholipid units (GPL) and IgM phospholipid units (MPL)], dynamic detection ranges, and non-standardised cut-off values for the detection of positive versus negative aPL between methods are also relevant, as noted in the prior RCPAQAP review.[14] Furthermore, clinical disease phenotype may be relevant to diagnostic performance, with improved concordance of solid-phase versus ELISA testing noted in one large obstetric cohort[19] and solid-phase reagent and isotype-specific variability in another large multicentre obstetric study.[20]

Numerical compatibility is an additional limitation in comparing method performance and proficiency between methods. Lack of universally accepted reference standards for measuring aCL and aβ2GPI remains an issue from a quality control and EQA. Furthermore, challenges exist in the standardisation of polyclonal versus monoclonal reagents used to characterise individual patient antibodies. In particular, variability exists in aβ2GPI assays due to variable exposure of cryptic targeted epitopes such as Glycine40-Arginine43 (G40-R43), leading to variability in results between assays and risk of false-negative results.[21,22]

Attempts to overcome issues in standardisation of calibration have been historically addressed with polyclonal patient-derived calibrators for aCL with the 'Harris standard' in the 1980s with serial dilutions of highly positive aCL patient samples.[23] The concentrations are expressed in GPL and MPL, where 1 unit is equivalent to 1 μg/mL. Subsequent attempts at calibration were developed, including the 'Koike standards' and 'Sapporo standards', using monoclonal antibody (MAb) standards for aCL and aβ2GPI. MAb concentrations for aCL can also be expressed in GPL/MPL units if validated against the original Harris standards, but there is assay variability in aPL for detection of the MAb standards. Difficulty also remains in the reporting of aβ2GPI, as no international unit is available for comparison.[24] For example, using Inova diagnostics method, ELISA and non-ELISA solid-phase assay both have different correlations to the Koike standards. Phadia EliA is standardised to the original Harris method but not to the Koike standard. Ideally, universal reporting of both aCL and aβ2GPI using IU/mL as the universal unit could overcome some difficulties in standardisation; however, at this point they remain unstandardised.[24] We suggest that further efforts need to be applied to harmonise testing and improve precision and accuracy of aPL testing. Areas for improvement include uniform adoption of

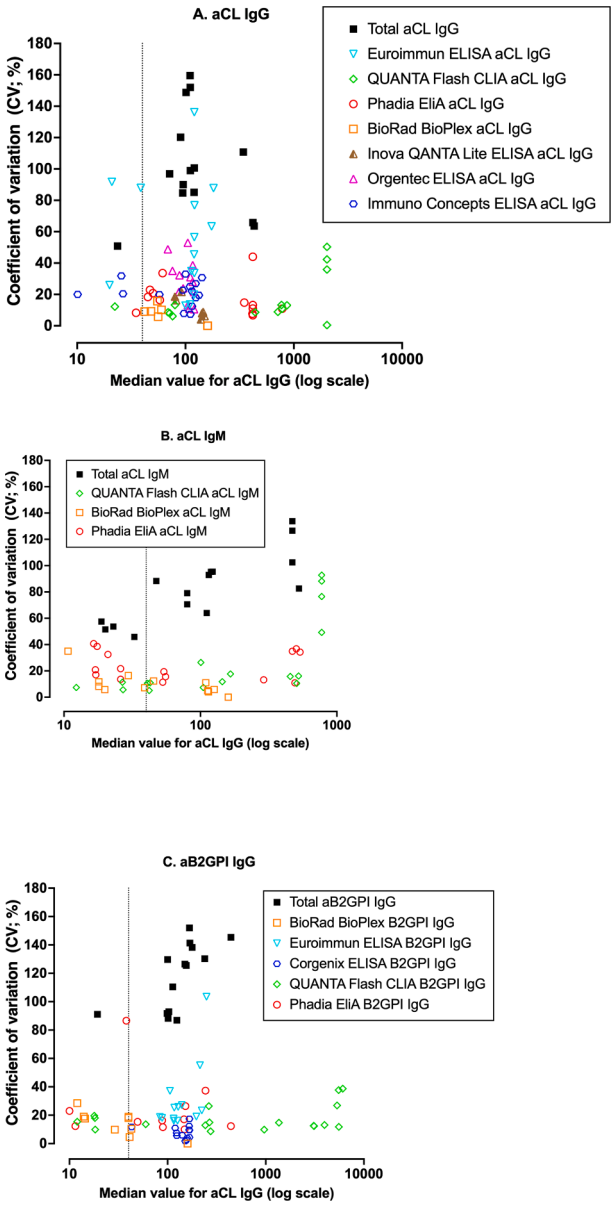

Fig. 5. Variation in numerical data submitted by participants of the RPCAQAP Immunology program for IgG aCL, IgM aCL, and IgG aβ2GPI, respectively, for all 24 samples distributed over the period of data analysis (2022–2024 inclusive), shown as interlaboratory coefficients of variation (CV) for each sample plotted against median reported value. Data restricted to samples where median values ≥10, since values <10 have doubtful clinical relevance, and CVs increase considerably for these samples, which is consistent with the derived mathematical calculations (smaller denominators in CV calculation). Nevertheless, CVs were frequently >50%. Dotted vertical line shown at 40 units. aβ2GPI, anti-β2-glycoprotein I antibodies; aCL, anticardiolipin antibodies; CLIA, chemiluminescence immunoassay; ELISA, enzyme-linked immunosorbent assay; EliA, enzyme-linked immunosorbent assay (Phadia platform); Ig, immunoglobulin; RCPAQAP, Royal College of Pathologists of Australasia Quality Assurance Programs.

validated reference standards available to all manufacturers and for transferable use across methods.[25,26] An ISTH SSC subcommittee has recently proposed the adoption of interval-specific likelihood ratios (IS-LR) for determining semi-quantitative thresholds for low, moderate, and high-positive antibody levels across different solid-phase platforms; however, further validation is required.[9]

We note several limitations in our study, including the study design being based on retrospective analysis, and the limitations to data

Table 3
Summary of CV data for current study (only samples with median aPL >20 units)

Sample ID	Analyte and assay type[a]																			
	aCL IgG							aCL IgM							aβ2GPI IgG					
	A	B	C	D	E	F	G	D	E	F	G	H	A	E	F	G				
2022/1	**12.9**	33	**16.3**	21.5	**0**	50.3	20.8		**11**	**16.1**	**15.6**	**17.4**	55.2	**0**	37.7	26.4				
2022/2	63.4	**17.9**	**8**	35	**5.8**	**13.3**	44		**0**	76.5	36.8	**4.4**	27.1	**4.6**	26.4	23				
2022/5	**13.6**	22.7	21.6	52.9	**0**	35.9	22.9		**4.9**	**15.8**	**11.4**	**9.7**	23.3	**0**	**11.8**	**10**				
2022/6	136.2	30.7	**6.2**	**10.6**	**0**	**0.4**	**14.8**	49.8	83.9	**18.5**	94.9	**9.5**	103.5	**0**	38.7	**12.3**				
2022/7	56.5	26.9	**8.9**	23.9	**15.7**	**8.6**	**11**	**0**	**0**	88.2	34.3	**12.3**	**16.2**	**18**	**8.6**	**15**				
2023/1	34.9	**7.4**	**18.6**	31	**0**	42.3	**18.4**	51.2	**5.9**	**10.5**	**19.4**	**3.9**	**19**	**0**	26.9	**17.1**				
2023/4	87.8	25	**4.1**	38.6	**9.2**	**8**	**13.2**	**0**	**0**	92.8	34.9	**5.6**	**17.6**	**10.4**	**12.9**	**12.3**				
2023/5	76.8	22.1		32.2	**0**	**8.9**	33.6	26.7	35	**7.4**	46.3	**5.9**	**18.8**	**0**	**14.7**	86.6				
2023/6	87.9	**19.9**		48.8	**0**	**8.6**	**16.4**	39.8	84.7	**13.1**	**13.1**		**16.1**	**0**	**13.1**	37.3				
2024/1	**19.9**	**7.8**		**11**	**9.1**	**6.1**	**7.7**	**0**	**0**	49.2	**10.9**	**11**	37.2	**18.9**	**14.9**	**17.6**				
2024/2	91.8	31.8		22.2	**10.1**	**12.2**	**8.3**	**19.4**	**4.1**	**7.4**	**13.2**	**11.7**	34.8	**9.8**	**13.6**	34.9				
2024/3	33.6	**12.4**		21.6	**0**	**10.9**	**10.9**	**4**	**7.2**	**11.8**	**13.6**	**2.6**	25.9	**0**	**12.4**	**11.6**				
2024/6	26	20.4		**13**	**0**	**13.3**	**19.8**	**18.8**	**16.4**	**26.4**	**20.8**	**7.5**	**18.1**	**0**	**9.8**	**15.3**				
2024/7	45.5	**19.5**		28	**0**	**13.1**	**6.7**	**12**	**12.3**	**17.7**	**21.7**	**1.8**	25.3	**0**	**12.5**	**16.3**				

aβ2GPI, anti-β2-glycoprotein I antibodies; aCL, anticardiolipin antibodies; aPL, antiphospholipid antibody; CV, coefficient of variation; Ig, immunoglobulin.
Bold values are CVs under 20%.
Methods with majority of samples (i.e., >7/14 samples) with CVs <20%: E, Bio-Rad Bio-Plex (aCL IgG, aCL IgM, B2GPI IgG); F, Inova Quanta Flash CLIA (aCL IgG, aCL IgM, B2GPI IgG); G, Phadia EliA (aCL IgG, aCL IgM, B2GPI IgG); H, Corgenix ELISA (B2GPI IgG).
[a] Assay type: A, Euroimmun ELISA; B, Immuno Concepts ELISA; C, Inova Quanta Lite ELISA; D, Orgentec ELISA; E, Bio-Rad Bio-Plex; F, Inova Quanta Flash CLIA; G, Phadia EliA; H, Corgenix ELISA.

availability associated with a review of RCPAQAP reports rather than the original data, as well as a relatively small data capture period (3 years) and sample size (n=24). Nevertheless, our report represents the most contemporary analysis of the current state of aPL testing and should permit improvements in laboratory testing and harmonisation initiatives.

5. Conclusion

In summary, our results demonstrate similar trends to prior local evaluations of the RCPAQAP Immunology program data.[14–16] Inter-laboratory variability still persists, especially for ELISA assays, despite availability of Australian consensus guidelines;[15,27] however, the consistency in qualitative reporting appears improved compared with prior evaluations. This is perhaps driven by the uptake of non-ELISA aPL methods.

Finally, our report highlights the discordance between classification and diagnostic criteria suggested in the ACR guidelines. Restricting reporting of aCL and aβ2GPI antibodies to using ELISA-based solid-phase assays (as in the classification criteria) would result in most patients not receiving a diagnosis for APS. Also, adopting classification criteria to identify patients as having moderate (>40) or high (>80) aPL levels would result in different outcomes based on assays performed, since assays (especially non-ELISA assays) reflect higher dynamic ranges. Although greater work is still required to harmonise the results of solid-phase assays, restricting testing to ELISA solid-phase assays is arguably not a feasible real-world approach. One can also argue that the non-ELISA-based methods provide reduced variability and greater dynamic range. Solid-phase antibody testing remains an important part of the diagnostic algorithm for APS; however, results should continue to be interpreted with caution, considering need to further optimise accuracy and precision, improve inter-laboratory variability, and to further harmonise testing.

Author contributions

CT wrote the original draft manuscript. All authors contributed to revision and approved its submission for publication. EF and LP assisted with conceptualisation, data curation, investigation, methodology, and supervision. EF assisted with formal analysis, and resources, including PRISM software.

Conflicts of interest and sources of funding

The authors state that there are no conflicts of interest to disclose. This study did not receive any formal funding.

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