

2023 American College of Rheumatology/European League Against Rheumatism antiphospholipid syndrome classification criteria solid phase–based antiphospholipid antibody domain—collaborative efforts of Antiphospholipid Syndrome Alliance for Clinical Trials and International Networking and ISTH SSC to harmonize enzyme-linked immunosorbent assay and non–enzyme-linked immunosorbent assay antiphospholipid antibody tests: communication from the ISTH SSC Subcommittee on Lupus Anticoagulant/Antiphospholipid Antibodies

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1 | INTRODUCTION

Antiphospholipid syndrome (APS) is characterized by the presence of persistent antiphospholipid antibodies (aPLs), which are linked to an increased risk of thrombosis- and pregnancy-related morbidity. In APS, where diagnosis heavily centers on aPL testing, the choice of the aPL assay and the correct interpretation of the results is of paramount importance.

As per the previous revised Sapporo and recently published 2023 American College of Rheumatology (ACR)/European League Against Rheumatism (EULAR) APS classification criteria, the laboratory requirement of solid phase assays, ie, “moderate” or “high” level anticardiolipin (aCL) and anti- β_2 -glycoprotein-I (a β_2 GPI) antibodies, has been based on the enzyme-linked immunosorbent assay (ELISA) to ensure homogeneity in research [1,2] with the majority of the available studies primarily using ELISA technology justifying this choice [3,4]. However, alternative automated solid phase platforms are becoming more commonly used in routine because of their reproducibility and feasibility compared with other solid phase assays [5]. Some of these automated platforms display a different dynamic range than other solid phase platforms, which may give much higher analytical values. This is true for chemiluminescent immunoassays (CLIAs), which are becoming one of the most common platforms. While there is poor quantitative agreement in aCL or a β_2 GPI analytes between ELISA and automated platforms, there is, fortunately, categorical (positive/negative) clinically relevant concordance between the different platforms when considering the results of the 4 aPL assays together [6]. In addition, “moderate” or “high” aCL/a β_2 GPI levels in automated platforms have not been widely established. It is, therefore, that this numerical divergence among results necessitates a standardized approach to harmonize the interpretation of the results of the ELISA and non-ELISA solid phase aPL assays used in daily practice and clinical research [7–15].

With this intent, an international collaborative effort between Antiphospholipid Syndrome Alliance for Clinical Trials and International Networking and the International Society on Thrombosis and Haemostasis Scientific and Standardization Committee Subcommittee on Lupus Anticoagulant/Antiphospholipid Antibodies has been initiated. The objectives of this first report of the working group are to describe (a) how the chosen laboratory solid phase-based assays for APS classification criteria impact routine clinical practice and (b) the efforts and opportunities to improve harmonization between ELISA and non-ELISA solid phase aPL assays.

2 | REAL LIFE SCENARIO CHALLENGES REGARDING ELISA AND NON-ELISA SOLID PHASE APL ASSAYS

There is generally good categorical concordance among assays based on different technical platforms. This is true particularly when the sample displays medium-to-high aPL titers, but low values represent the problem [7–15].

One of the reasons is that the new solid phase techniques employ detection systems that can affect the range of result values. For example, CLIA displays a different dynamic range than other solid phase platforms, which may give results with much higher numerical values than other solid phase assays. For example, the same patient sample can result in 77 IgG phospholipid units for aCL immunoglobulin G ELISA and 475 chemiluminescence units with a non-ELISA automated system, eg, CLIA [5]. While a significant correlation between the assays has been reported for strongly positive and clearly negative results, the difference in analytic values obtained with the 2 methods can create misunderstandings. For instance, clinicians might interpret a low titer in non-ELISA tests as a high titer when using ELISA assays. This bias could lead to overestimating the patient's risk of experiencing APS clinical events, potentially resulting in more aggressive treatment and possible side effects. To avoid this risk, physicians should consider the type of technique used to detect aPL. This aspect is particularly relevant and more evident when aPL results are in low titers [7–15].

This divergence in the range of the scale of values is a distinction vital for research, a setting that requires a standardized approach for selecting appropriate participants for clinical studies. Ensuring a uniform study population with similar laboratory and clinical profiles is essential for the validity and reliability of the research findings. On the other hand, we should also have a standardized approach to interpret and compare these assays in routine practice and clinical settings. Indeed, determining “moderate” and “high” thresholds of non-ELISA aCL/a β_2 GPI testing platforms is high-priority in the research agenda to guide the future update of the 2023 ACR/EULAR APS classification criteria) [2].

3 | APS CLASSIFICATION VS DIAGNOSIS

The introduction of the 2023 ACR/EULAR APS classification criteria underscores the importance of distinguishing candidate patients for research vs receiving a diagnosis in clinical practice [2]. From this perspective, there are a few points worth mentioning.

Primarily, classification criteria are intended for use in research settings. They implement a consistent method for selecting participants in clinical studies, ensuring a uniform study population in terms of the condition under investigation. This uniformity, which includes comparable laboratory and clinical characteristics, is crucial for ensuring the credibility and dependability of the study outcomes. Diagnosis, in contrast, is a healthcare provider's clinical decision to identify a specific disease or condition in a particular patient. In the clinical context, diagnosing APS should involve a comprehensive assessment of the patient's symptoms, medical history, physical examination, and ultimately, laboratory test results, ie, aPL profile. Most importantly, excluding other possible conditions by conducting a thorough and comprehensive differential diagnosis is paramount in clinical practice [16]. Furthermore, given the abundant presence of β_2 GPI in the placenta, compared with negligible levels in the resting endothelium [17], the incorporation of aPL profile in the diagnostic/risk stratification might be different for thrombotic vs obstetric APS [18].

Although classification criteria can inform the diagnostic process, they should not replace it as classification and diagnosis are not interchangeable. Clinicians must weigh additional factors and apply their clinical judgment, which may not be fully addressed by these criteria. Diagnosis is tailored to each patient and encompasses a more extensive evaluation of their condition. This is crucial as it guides the specific management and treatment strategies to follow.

4 | EFFORTS TO IMPROVE HARMONIZATION BETWEEN ELISA AND NON-ELISA

Despite dealing with identical or very similar antigen molecules, achieving complete harmonization across various techniques has been and remains a challenge. Besides the matter of assay sensitivity, variations in antigen/matrix interaction, such as those seen with bead-based techniques, may differ across different solid phase platforms. These variances can potentially lead to conformational alterations in the antigenic molecule and/or affect binding efficiency, leading to different epitope expressions, eventually affecting the autoantibody binding.

However, despite these differences, new technological platforms offer unique benefits, as outlined earlier. Therefore, the primary objective for all stakeholders moving forward should be to guarantee the clinical comparability of ELISA and non-ELISA aPL solid phase assays. Achieving this goal requires conducting focused studies involving a significant number of patients and control subjects. Additionally, using internationally validated reference materials could provide added value [19,20]. These materials aid in accurately setting up the laboratory assays and eventually establish conversion strategies essential for interpreting results from different assays.

5 | CONCLUSION

The ELISA requirement for the solid phase aCL and $\alpha\beta_2$ GPI assays in APS classification criteria should be viewed in the context of a clear separation between disease classification (for research) and disease diagnosis (clinical practice). This requirement should not impact the routine practice of some diagnostic laboratories that efficiently employ different platforms other than ELISA. Most importantly, from a practical point of view, physicians caring for aPL-positive patients should be aware of the different ranges of ELISA and non-ELISA solid phase-based aCL/ $\alpha\beta_2$ GPI assays for a proper and accurate interpretation of the results.

With this in mind, we hope that the Antiphospholipid Syndrome Alliance for Clinical Trials and International Networking and the International Society on Thrombosis and Haemostasis Scientific and Standardization Committee Subcommittee on Lupus Anticoagulant/Antiphospholipid Antibodies collaboration and, advantageously, the combined effort with the diagnostic companies interested in aPL assays will offer the right tools for harmonizing the ELISA and non-ELISA solid phase aPL assays.

AUTHOR CONTRIBUTIONS

All authors should have made substantial contributions to drafting the article or revising it critically for important intellectual content and to the final approval of the version to be submitted.

DECLARATION OF COMPETING INTERESTS

There are no competing interests to disclose.

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