Antiphospholipid Antibody Testing: When to Trust the Results

Written by The Life Science Feed Editorial Team · Edited by Mara Voss

Antiphospholipid syndrome (APS) presents a diagnostic challenge, often requiring a precise understanding of laboratory testing to confirm a clinical suspicion. The presence of antiphospholipid antibodies (aPL) is central to diagnosis, but their transient nature and varying titres complicate interpretation. Clinicians must navigate the nuances of testing protocols to accurately identify patients at risk for thrombosis and pregnancy morbidity, distinguishing true APS from incidental antibody presence.

KEY TAKEAWAYS

- The Pivot: Antiphospholipid antibody testing demands strict adherence to timing and repeat testing guidelines to differentiate transient positivity from persistent, clinically significant antibodies.
- The Data: Persistent positivity, defined by two positive tests at least 12 weeks apart, is a critical diagnostic criterion for antiphospholipid syndrome.
- The Action: Do not diagnose antiphospholipid syndrome based on a single positive antibody test; always confirm with repeat testing after a minimum 12-week interval.

Antiphospholipid syndrome is an autoimmune disorder characterised by arterial or venous thrombosis and/or pregnancy morbidity, occurring in the presence of persistently elevated antiphospholipid antibodies. These antibodies include lupus anticoagulant (LA), anticardiolipin antibodies (aCL), and anti-β2-glycoprotein I antibodies (anti-β2GPI). The clinical manifestations are diverse, ranging from deep vein thrombosis and pulmonary embolism to recurrent miscarriages and stroke. Accurate diagnosis hinges on both clinical criteria and laboratory evidence of persistent aPL positivity.

The initial evaluation for suspected APS typically involves testing for all three antibody types. Lupus anticoagulant is a functional assay, detecting antibodies that interfere with phospholipid-dependent coagulation tests. Anticardiolipin and anti-β2GPI antibodies are detected via enzyme-linked immunosorbent assays (ELISA), measuring IgG and IgM isotypes. The choice of assay and the specific cut-off values used by laboratories are important for interpretation, as these can vary and influence results. For a comprehensive overview of rheumatological conditions, including autoimmune disorders, the Oxford Handbook of Rheumatology (5th ed) offers a practical reference.

The Importance of Timing and Thresholds

A single positive antiphospholipid antibody test is rarely sufficient for a definitive diagnosis of APS. Transient elevations of aPL can occur in various conditions, including infections, malignancies, and in response to certain medications. These temporary elevations do not typically confer the same thrombotic risk as persistent positivity. Therefore, current guidelines mandate repeat testing to confirm persistence.

The standard recommendation is to repeat positive aPL tests at least 12 weeks after the initial positive result. This interval is essential for differentiating transient antibody production from sustained autoimmune activity. If the antibodies remain positive at the second testing, and meet established titre thresholds, then the laboratory criterion for APS is fulfilled. Without this confirmation, a diagnosis of APS should be approached with caution.

Thresholds for positivity also matter. For aCL and anti-β2GPI antibodies, medium to high titres (typically >40 GPL or MPL units, or >99th percentile for the laboratory) are considered clinically significant. Low titres, while detectable, are often not associated with the same level of thrombotic risk and may not meet diagnostic criteria for APS, even if persistent. Laboratories should clearly state their cut-off values and reference ranges to aid in interpretation. This level of detail is essential for avoiding overdiagnosis and subsequent overtreatment, which carries its own risks.

Navigating Discordant Results and Repeat Testing

Discordant results, where one antibody is positive and another is negative, or where titres fluctuate, are not uncommon. In such cases, clinical context becomes paramount. A patient with a clear thrombotic event and persistent, high-titre aCL antibodies, but a negative LA, might still warrant a diagnosis of APS. Conversely, a patient with no clinical events but persistent low-titre antibodies may not meet full diagnostic criteria, though they may still require careful monitoring. The complexity of these scenarios highlights the need for expert interpretation, often involving collaboration between haematologists, rheumatologists, and obstetricians.

Repeat testing should ideally be performed using the same laboratory and assay methods to ensure comparability of results. Switching laboratories or assay platforms between initial and confirmatory tests can introduce variability, making accurate assessment of persistence more challenging. If a different laboratory must be used, clinicians should be aware of potential differences in methodology and reference ranges. This is particularly relevant for lupus anticoagulant testing, which is a highly sensitive functional assay susceptible to pre-analytical variables and interference from anticoagulants. For instance, monoclonal antibodies may complicate transfusion testing, and similar interferences can affect aPL assays.

Patients already on anticoagulation pose a specific challenge for LA testing. Many direct oral anticoagulants (DOACs) and warfarin can interfere with LA assays, leading to false-positive results. If LA testing is critical for diagnosis, it may be necessary to temporarily discontinue anticoagulation, if clinically safe, or to use LA assays that are less susceptible to anticoagulant interference, such as dilute Russell viper venom time (DRVVT) with specific neutralisation steps. This requires careful planning and communication with the patient and the laboratory.

Clinical Context and Diagnostic Pitfalls

The clinical presentation must always guide the interpretation of aPL test results. A positive antibody test in isolation, without any history of thrombosis or pregnancy morbidity, does not equate to APS. Such individuals are often referred to as asymptomatic aPL carriers. While some debate exists regarding the management of these carriers, current guidelines generally do not recommend prophylactic anticoagulation in the absence of clinical events. The risk of future thrombotic events in asymptomatic carriers is lower than in those with established APS, but it is not zero. Regular clinical review is therefore prudent.

One common pitfall is testing for aPL too soon after a thrombotic event. Acute thrombosis can sometimes induce transient aPL positivity, making it difficult to distinguish from persistent antibodies. It is generally recommended to wait at least 6 weeks after an acute thrombotic event before performing initial aPL testing, and then to follow the 12-week repeat

testing protocol. This delay helps to minimise false positives due to acute phase reactions. Similarly, testing during acute infections or inflammatory flares can also yield transient positive results, further emphasising the need for careful timing.

Another consideration is the use of aPL testing in specific populations, such as children or pregnant women. In paediatric patients, the interpretation of aPL can be more complex due to different prevalence rates of autoimmune conditions and the potential for transient positivity following infections. For pregnant women, accurate and timely diagnosis of APS is important for guiding management to prevent adverse pregnancy outcomes. However, the same principles of repeat testing and careful interpretation apply. Our previous coverage on monoclonal antibodies protecting infants from severe RSV highlights the importance of precise antibody identification in vulnerable populations.

The diagnostic criteria for APS require at least one clinical criterion (thrombosis or pregnancy morbidity) and one laboratory criterion (persistent aPL positivity). The laboratory criteria specify the type of antibody (LA, aCL IgG/IgM, anti-β2GPI IgG/IgM), the titre (medium-high for aCL/anti-β2GPI), and the timing of persistence (two positive tests at least 12 weeks apart). Adherence to these criteria is paramount for accurate diagnosis and appropriate management. Deviations can lead to misclassification, with potential consequences ranging from unnecessary lifelong anticoagulation to missed opportunities for preventing serious clinical events.

The field continues to evolve, with ongoing research into novel aPL antibodies and their clinical significance. For example, anti-phosphatidylserine/prothrombin antibodies (aPS/PT) are gaining recognition as potentially useful markers, particularly in cases where standard aPL tests are negative but clinical suspicion for APS remains high. But these newer assays are not yet universally included in diagnostic criteria and their interpretation requires even greater expertise. The challenge lies in integrating these emerging markers responsibly into clinical practice, ensuring they add value without increasing diagnostic confusion. This is a common theme in diagnostics, as seen with Afirma molecular testing in thyroid disease, where new markers refine existing paradigms.

Where the Data Falls Short

The primary limitation in interpreting aPL testing often stems from the inherent variability of the assays themselves. Lupus anticoagulant testing, being a functional assay, is particularly sensitive to pre-analytical factors such as sample collection, processing, and storage. Different reagents and methodologies across laboratories can also yield inconsistent results, making standardisation a persistent challenge. This lack of perfect standardisation can lead to discrepancies between laboratories, complicating the confirmation of persistent positivity.

But the clinical utility of low-titre aPL remains an area of ongoing debate. While current diagnostic criteria focus on medium to high titres, some clinicians observe thrombotic events in patients with persistently low-titre antibodies. Whether these patients represent a distinct subgroup or if their events are coincidental is not fully resolved. The current guidelines provide clear thresholds, but real-world clinical scenarios do not always fit neatly into these categories. This gap in understanding means some patients may be left in a diagnostic grey area, without clear guidance on risk stratification or management.

The long-term risk of thrombosis in asymptomatic aPL carriers, particularly those with only one type of antibody or low titres, is also not fully quantified. While general recommendations advise against prophylactic anticoagulation, the precise risk factors that might tip an asymptomatic carrier towards a thrombotic event are still being investigated. This uncertainty leaves clinicians with a difficult decision: monitor closely and risk an event, or intervene with anticoagulation

and risk bleeding complications. The next generation of studies needs to provide clearer stratification tools for these patients.

CLINICAL IMPLICATIONS

The persistent challenge with antiphospholipid antibody testing is not merely about getting a positive result, but about interpreting its true clinical meaning. Clinicians must resist the urge to diagnose APS based on a single, isolated antibody detection, especially in the absence of clear clinical events. The 12-week repeat testing rule is not a suggestion; it is a fundamental safeguard against misdiagnosis and the lifelong anticoagulation that follows.

The variability in laboratory assays, particularly for lupus anticoagulant, means that consistency in testing protocols is paramount. Sending repeat samples to the same laboratory, or at least ensuring comparable methodologies, can prevent unnecessary diagnostic confusion. Ignoring these details can lead to patients being labelled with a chronic autoimmune condition and subjected to potentially harmful treatments without sufficient evidence.

For patients, an accurate diagnosis of APS carries significant implications, from managing thrombotic risk to navigating pregnancy. But an incorrect diagnosis can lead to unnecessary anxiety, invasive investigations, and the risks associated with long-term anticoagulation. The onus is on the clinician to apply the diagnostic criteria rigorously, understanding that a positive test is only one piece of a complex puzzle, not the entire picture.

REFERENCES

1. 1. Devreese KMJ, Bertolaccini ML, Branch DW, et al. An update on laboratory detection and interpretation of antiphospholipid antibodies for diagnosis of antiphospholipid syndrome: guidance from the ISTH-SSC Subcommittee on Lupus Anticoagulant/Antiphospholipid Antibodies. J Thromb Haemost. 2025;23(2):731-744. doi:10.1016/j.jtha.2024.10.022
2. 2. Cohen H, Werring DJ, Chandratheva A, Mittal P, Devreese KMJ, Isenberg DA. Survey on antiphospholipid syndrome diagnosis and antithrombotic treatment in patients with ischemic stroke, other brain ischemic injury, or arterial thromboembolism in other sites: communication from ISTH SSC Subcommittee on Lupus Anticoagulant/Antiphospholipid Antibodies. J Thromb Haemost. 2023;21(10):2963-2976. doi:10.1016/j.jtha.2023.06.020
3. 3. Efthymiou M, Bertolaccini ML, Cohen H. Viewpoint: Lupus anticoagulant detection and interpretation in antiphospholipid syndrome. Rheumatology (Oxford). 2024;63(SI):SI54-SI63. doi:10.1093/rheumatology/kead623
4. 4. Masucci M, Li Kam Wa A, Shingleton E, Martin J, Mahir Z, Breen K. Point of care testing to monitor INR control in patients with antiphospholipid syndrome. EJHaem. 2022;3(3):899-902. doi:10.1002/jha2.522
5. 5. Kamali S, Dave M, Raheja P, Sivapalaratnam S, Platton S. Evaluation of International Council for Standardization in Haematology Recommendations on Activated Partial Thromboplastin Time Mixing Tests Using an Automated Haemostasis Analyser. Int J Lab Hematol. 2025;47(6):1178-1185. doi:10.1111/ijlh.14537
6. 6. Moore GW, Foxton E, Platton S, Yartey N, White D, MacDonald SG. Triple-positive antiphospholipid syndrome does not guarantee positivity in each lupus anticoagulant assay. J Thromb Haemost. 2023;21(12):3539-3546. doi:10.1016/j.jtha.2023.08.009

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