Review:
Laboratory Evaluation of the Antiphospholipid Syndrome

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Abstract. The antiphospholipid syndrome (APS) was first described in 1986. The original association of this hypercoagulable state with anticardiolipin antibodies (aCL) resulted from the synthesis of evidence stemming from laboratory findings in systemic lupus erythematosus (SLE), ie, the frequent occurrence of false-positive VDRL tests and the paradoxical observation of the so-called “lupus anticoagulant” (LA), an increase in phospholipid (PL)-dependent clotting times. By the early 1990s, it was clear that a co-factor was involved in the reaction of antibodies to PL (aPL) in SLE patients with secondary APS and that this was a hitherto-obscure protein, beta-2 glycoprotein I (β2GPI). In the intervening years, it has been established that β2GPI and other PL-binding proteins such as prothrombin (PT) are relevant antigens in APS and assays for these antigens have been developed, standardized, and applied to subjects with both primary and secondary APS. Measurement and confirmation of LA activity is based on a stepwise approach and should follow the recommendations of the International Society of Thrombosis and Haemostasis. Although antibodies to various PL-binding proteins have been suggested as diagnostic targets for APS, the current (2006) consensus guidelines recognize only LA, aCL, and anti-β2GPI for the classification of APS.

Keywords: antiphospholipid syndrome, Hughes’ syndrome, lupus anticoagulant, anticardiolipin antibodies, phospholipid-binding proteins, beta-2 glycoprotein I, systemic lupus erythematosus

Introduction

Clinicians treating patients with systemic lupus erythematosus (SLE) had long been aware that SLE patients frequently exhibited a so-called “lupus anticoagulant” (LA), ie, the paradoxical in vitro prolonged clotting time in the absence of a concomitant in vivo effect. These patients also reacted positively with serologic tests for syphilis that used cardiolipin (CL) as an antigen, eg, the VDRL test, when more definitive tests demonstrated that the patients were, indeed, not infected. It remained for Hughes et al in 1985 [1] to describe the autoimmune, hypercoagulable state now known as anticardiolipin syndrome, Hughes’ syndrome, or, most commonly, antiphospholipid syndrome (APS). The constellation of symptoms in patients with APS secondary to SLE (Table 1) was found, additionally, to occur in some subjects who did not have SLE, a condition termed primary APS [2]. Since the development of a widely-used anticardiolipin antibody (aCL) enzyme-linked immunosorbent assay (ELISA), described by Louizou et al [3] in 1985, the diagnostic armamentarium for APS has evolved in keeping with fundamental biochemical and immunologic discoveries about APS. This review evaluates those findings as they relate to current diagnostic testing for APS.
ELISA Detection of Relevant Antibodies in APS

Laboratories worldwide have developed ELISAs for APS testing with varying degrees of standardization. Several ELISAs for APS have been translated into commercially-available assay kits, although these, too, vary considerably [4] in their response to serum antibodies from patients with APS. In the following sub-sections, a hierarchical approach, based on an overall assessment of utilization and usefulness of these assays, will be taken in an effort to examine their value in the clinical laboratory. This portion of the review will focus on methodologic aspects, with assessments of the clinical utility of antibody ELISAs, as well as LA tests, in a later section.

Anticardiolipin antibodies (aCL). The ELISA for aCL was the first [3], and today remains, the most widely used ELISA for APS. In fact, the utility of the aCL assay stems from the current recognition that patients with APS exhibit antibodies to a variety of phospholipid-binding proteins (PLBPs) [4]. Thus, when fetal-calf serum is used as the blocking agent and is used in the washing solution for the aCL ELISA, the calf serum PLBPs, which exhibit substantial homology with their human counterparts, provide a variety of established, as well as putative, antigens for reactivity. These PLBPs are capable of acting as antigens for relevant APS autoantibodies when bound to anionic PL other than CL, leading some investigators to examine the use of phosphatidylserine as the lipid matrix [5,6] for ELISA testing in APS. Nevertheless, the aCL ELISA remains the standard for PL-based solid-phase antibody assays.

Although aCL ELISAs are useful, they suffer from a lack of specificity. Various diseases (primarily infectious) [7] have been shown to produce antibodies to CL in protein-independent systems. Moreover, CL is sensitive to oxidation [8], leading to reactivity with oxidized low density lipoproteins [9] as well as PLBP-oxidized CL complexes from patients with APS [10]. Thus, the presence of aCL as an incidental finding in a patient with neither clear clinical evidence of APS nor the presence of other laboratory findings should suggest an alternative diagnosis.

Antibodies to β2 glycoprotein I (anti-β2GPI). In 1990, it was discovered [21,22] that patients with APS have antibodies that react with a protein component of the aCL ELISA assay system, β2 glycoprotein I (β2GPI). Although, initially, there was some question as to whether the involvement of β2GPI was artifactual [13], Matsuura et al [23] demonstrated convincingly that β2GPI, adsorbed on irradiated plastic surfaces (resulting in the formation of oxygen-bearing surface functional groups), would bind aCL from patients with APS.

Table 1. Major clinical manifestations of APS.

<table>
<thead>
<tr>
<th>Manifestation</th>
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<tbody>
<tr>
<td>Venous thrombosis</td>
</tr>
<tr>
<td>Arterial occlusion</td>
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<tr>
<td>Recurrent fetal loss</td>
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<tr>
<td>Thrombocytopenia with or without hemolytic anemia</td>
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<tr>
<td>Livido reticularis</td>
</tr>
<tr>
<td>Transient cerebral ischemia</td>
</tr>
<tr>
<td>Migraine</td>
</tr>
<tr>
<td>Chorea</td>
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<tr>
<td>Transverse myelitis</td>
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</table>

Much of the effort toward standardization of the aCL ELISA is due to Nigel Harris and colleagues who instituted a series of workshops as early as 1986 and continuing until the late 1990s [11-14]. These efforts resulted in dissemination of aCL reference calibrators standardized in GPL, MPL, and APL units for antibodies of the IgG, IgM, and IgA classes, respectively (the so-called Louisville standards). Later, monoclonal antibodies for aCL, primarily targeting β2 glycoprotein I (β2GPI), were developed, these being HCAL, a chimeric IgG [15], and EY2C9, an IgM antibody [16]. These standards were used in a multi-center European study of aCL assays [17] and, when used in conjunction with a standardized method, were found to reduce inter-laboratory variability. A common strategy for minimizing the imprecision of aCL assays is the use of a semi-quantitative nominal scale for rating laboratory results for aCL. Thus, a recent study used the following designations: negative, equivocal, moderate-positive, medium-positive, and high-positive [18], although other studies have used fewer categories [19]. Currently, the College of American Pathologists’ (CAP) proficiency testing program for anticardiolipin antibodies [20] grades participants only on the semi-quantitative result.
Keil et al [24] found that some sera from subjects with aCL activity reacted with β2GPI bound to surface-modified polystyrene, the surface of which was modified [25] to allow the protein to be chemisorbed. By 1995, it became apparent from clinical studies that β2GPI was a relevant antigen for antibodies from patients with APS [26–28].

Numerous laboratories developed in-house anti-β2GPI ELISA assays. Arvieux et al [29] described an anti-β2GPI ELISA as early as 1991 and other investigators soon followed with similar assays [23,30,31]. To provide some standardization and other investigators soon followed with similar ELISA for anti-β2GPI, based on the absorbance signal at 450 nm from an ELISA for anti-β2GPI, i.e., $A_{450}$ x titer\(^1\). This was followed by the description of a standardized ELISA for IgG anti-β2GPI and a reference interval based on 204 adult subjects [33]. In 1998, anti-β2GPI ELISA methods were described for all 3 of the major immunoglobulin classes [34] and reference intervals were established, based on standard units: SGU (noted above), SMU (for IgM), and SAU (for IgA). These assay systems were precise, linear, and correlated well with aCL; they formed the basis for the first FDA-approved, commercially-available anti-β2GPI ELISA kits.

However, the issue of standardization of anti-β2GPI ELISA methods remains unresolved. The Standardization Group of the European Forum on Antiphospholipid Antibodies reported results of a multi-center evaluation using both commercially-available and in-house assay systems for IgG and IgM anti-β2GPI [35]. They found that only at higher titers (measured in Forum Units, FU), was agreement among the participating centers good. In a recent study, Audrain et al [36] evaluated 5 ELISA systems (4 commercial and 1 in-house) for anti-β2GPI. Contingency table analysis demonstrated considerable homogeneity of results for anti-β2GPI; these authors also found the best concordance among the higher titers.

CAP includes anti-β2GPI in its anticardiolipin proficiency testing survey [20], but currently does not grade the results for laboratory accreditation.

**Antibodies to protrombin and protrombin-phosphatidylserine complex (a-PRO/PS).** Although protrombin (PRO) deficiency had long been associated with the LA phenomenon [37], Edson et al [38] suggested in 1984 that aPRO might be implicated as a mechanism for PRO deficiency in SLE patients. In 1988, Fleck et al [39] found evidence of aPRO in 38 of 40 patients with either prolonged activated partial thromboplastin time (aPTT, 24 of 25 patients) or prolonged PRO time (PT) (14 of 15 patients). Additionally, in 3 subjects with SLE, Fleck et al [39] were able to adsorb the antibodies on immobilized PRO. Bevers et al [40] demonstrated the lipid requirement for binding in the PRO-aPRO system and suggested that the Ca\(^{2+}\)-mediated binding of anionic PL generates an epitope for aPRO. This finding was confirmed by Oosting et al [41], who suggested that other PLBPs, i.e., protein C (ProC) and protein S (ProS), might be mediators of an anticoagulant system that involves inhibition of endothelial protrombinase.

ELISA methodologies include the use of PRO, alone, as the antigen, or the use of PRO bound to phosphatidylserine (PS) as the antigenic substrate [42,43]. The former are generally referred to as aPRO; the latter as aPRO/PS or a similar designation that recognizes that the complex may be involved in presentation of the epitope. Donohoe et al [44] demonstrated variable binding of aPRO under various assay conditions, leading them to develop an aPRO ELISA with excellent analytical performance characteristics. Similarly, Matsuda et al [45] optimized an aPRO/PS ELISA and compared the results of the assay with an ELISA for aPRO. They concluded that in SLE patients, aPRO and aPRO/PS are different antibodies exhibiting, in some cases, partial identity.

Although there are several commercially available aPRO ELISA kits, the FDA website (http://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfCLIA/results.cfm) currently lists those (for IgG and IgM classes) from only 1 manufacturer as approved for diagnostic use. This ELISA is not included in CAP’s proficiency testing program [20] and is considered as investigational (not covered) by at least 2 major health care insurance carriers (http://aetna.com/cpb/data/CPBA0662.html) and (http://www.IBX.com/pdfs/providers/policies_guidelines_pubs/coding_guidelines/04_665_IBC_CGPV_winter.pdf).
Antibodies to annexin V. Annexin V (A5), perhaps most widely recognized as an in vitro marker of apoptosis, is a PLBP with anticoagulant properties due to its ability to prevent the degradation of Factor Va [46]. Within the past 10 years, it has been investigated as a potential marker for complications of pregnancy in women with APS, i.e., recurrent spontaneous abortion and fetal death.

In 1994, Rand et al [47] demonstrated that placental villi-bound A5 was significantly lower in pregnant women with APS than in those without APS. Shortly thereafter, reports of assays being used to detect anti-A5 in autoimmune states began to appear [48,49]. Nevertheless, recent studies, both retrospective [50] and prospective [51], have concluded that, despite reduced A5 concentrations in plasma of pregnant women with APS, anti-A5 measurements are not useful as markers of recurrent fetal loss.

Several ELISA kits for assay of IgG and IgM-class anti-A5 are available (http://www.ibl-america.com/elisa.html and http://www.vincibiochem.it/ELISA-A-B.htm), but are not approved for diagnostic use.

Antibodies to protein C and protein S. The activation of ProC is a key anticoagulant regulatory mechanism. ProC is converted to activated ProC (APoC) by thrombin-thrombomodulin complex on the endothelial surface, and it, in turn, inactivates Factor Va and combines with a cofactor, ProS, on the platelet surface to inactivate Factor Va and Factor VIIIa [52] (Fig. 1).

Although most thrombotic manifestations of dysregulation in this anticoagulant pathway are due to APoC resistance (of which an estimated 95% are due to arg506gln phenotype, the Factor V Leiden mutation) [53], anti-ProC and anti-ProS have been described in serums from patients with manifestations of APS [54]. Earlier work [55], examining the activity of β2GPI-anti-β2GPI on the ProC system, suggested that ProC binding to antibodies may be via an interaction with β2GPI. Data of Icko et al [56] and Izumi et al [57] supported this hypothesis. Inhibition studies have clarified the specificity of anti-ProS, and an analysis of the frequency of these antibodies in 184 SLE patients and 99 controls showed strong association of anti-ProS with venous and arterial thrombosis [58].

Other putative antigen specificities. Several other protein antigens have been implicated as targets for APS antibodies and have been measured primarily by ELISA. They include thrombin [59,60] and plasmin [61], which share 3 highly homologous sequences of amino acids with PRO, which might lead to cross-reactivity among some of these antibodies [61]. However, despite the structural homology between PRO and thrombin, anti-thrombin antibodies appear to be distinguishable from aPRO and more closely associated with aCL and anti-β2GPI [60]. Few data are available on autoantibodies to protein Z, a regulatory co-factor for the control of Factor Xa [62], and to endothelial protein C receptor (anti-EPCR) [63]. However both have been implicated as independent risk factors for complications of pregnancy and for fetal death [63,64].

Immunoglobulin class and sub-class distributions of antibodies in APS. Antibodies of the 3 major Ig classes, IgA, IgG, and IgM, have been found to react with PLBPs in patients with APS. IgA, however has been controversial. The presence of true IgA aCL was questioned by Selva-O’Callaghan et al [65]. However, further studies provided strong evidence that IgA aCL, as well as IgA anti-β2GPI, are found in patients with APS [66]. In fact, Galazka et al [67] found that 15 of 39 APS patients had IgA that bound to β2GPI immobilized on an activated polystyrene surface at levels >5 standard
deviations above the mean level of binding in a control group of 50 subjects.

There is evidence that SLE patients of African heritage (African-Caribbean or African-American) have increased prevalence of IgA aCL or anti-β2GPI [68,69]. However, the presence of IgA class antibodies was not associated with a substantial risk of APS symptoms in these groups.

Despite the observation of Ig class association with specific manifestations of APS [70] and data suggesting that IgA anti-β2GPI enhances the sensitivity of APS detection [71], the preponderance of recent data demonstrates that IgA antibodies co-occur with those of the IgG and IgM classes, leading to the conclusions that the clinical efficiency of assays for IgA aCL and anti-β2GPI is low [72-74] and that IgA aPRO and aPRO/PS may not even be present in patients with APS [72]. As discussed later in this review, the current consensus guidelines include IgG and IgM anti-β2GPI and aCL, but not IgA anti-β2GPI or IgA aCL.

There are few data on IgG subclass distributions of antibodies in APS. Guerin et al [75] found IgG anti-β2GPI to be more specific and sensitive for APS; although all 4 IgG subclasses were implicated as anti-β2GPI, IgG2 was predominant.

Alternative antibody assays. There are few alternatives to the standard microtiter–plate ELISA format for antibodies in APS. A novel approach was taken by Stewart et al [76], who developed a flow cytometric assay for aPL using polystyrene microspheres coated with various PL. The promise of this assay system appears not to have been fulfilled, based on the paucity of reports on the application of this method.

Functional Assays of Lupus Anticoagulants

The basis for functional assays to identify the presence of a lupus anticoagulant dates back to 1952, when Conley and Hartmann noted that a circulating in vitro anticoagulant in systemic lupus erythematosus patients was associated with false positive tests for syphilis [77]. This LA was later found paradoxically to increase the thrombotic tendencies of in vivo models [78]. In 1980, the activity of LA was found to be caused by antibodies against negatively charged PL [79]. Since then, due to a better understanding of the biology of the LA, coagulation assays play an important role in the diagnosis of APS.

The LA antibodies are heterogeneous. Thus, their identification is enhanced when more than 1 coagulation assay is used to assure a proper diagnosis. Over the last 20 years, numerous screening tests and confirmatory tests have become available. Their methodology and rationale for use in identifying the presence of a LA is based on an international consensus that requires the following: (a) prolongation of a PL-dependant clotting time, (b) evidence of an inhibitor as shown by mixing studies, (c) confirmation of PL dependence, and (d) the exclusion of specific inhibition of any 1 coagulation factor [80,81] An algorithm for the laboratory assessment of LA is outlined in Table 2.

**Prolongation of PL-dependent clotting time.** The diagnosis of a LA generally begins with a screening test that demonstrates prolongation of a PL-dependent coagulation reaction. This screening test needs to be as sensitive as possible to avoid false negatives. However, due to the heterogeneous nature of PLs, no single test is 100% sensitive. Numerous screening assays exist, including the PTT, DRVVT, KCT, TTI, and many others. Each of these tests has its advantages and disadvantages, and knowledge about each is essential. Hence, each of the major screening functional assays will be discussed.

<table>
<thead>
<tr>
<th>Step</th>
<th>Prolongation of phospholipid-dependant clotting time</th>
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<tbody>
<tr>
<td>1</td>
<td>PTT</td>
</tr>
<tr>
<td></td>
<td>PT</td>
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<tr>
<td></td>
<td>DRVVT</td>
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<tr>
<td></td>
<td>Other snake venom assays</td>
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<tr>
<td></td>
<td>KCT</td>
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<td></td>
<td>TTI</td>
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<tr>
<td>Step 2</td>
<td>Mixing assays</td>
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<tr>
<td></td>
<td>PNP</td>
</tr>
<tr>
<td></td>
<td>Platelet-derived vesicles</td>
</tr>
<tr>
<td></td>
<td>Hexagonal-phase phospholipids</td>
</tr>
<tr>
<td></td>
<td>High-phospholipid confirmatory reagent</td>
</tr>
<tr>
<td>Step 3</td>
<td>Confirmation of phospholipid dependence</td>
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<td></td>
<td>Step 4</td>
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</table>

*Table 2. Diagnostic algorithm for the detection of LA.*
Activated partial thromboplastin time (aPTT). The aPTT is a screening test for the intrinsic and common pathways of the coagulation cascade, the endpoint of which is formation of a fibrin clot that can be detected visually, by densitometry, or by electro-mechanical means. The LA antibody may cause prolongation of the aPTT by preventing the assembly of the prothrombinase complex. The PL component of the PTT reagent is critical in determining the test sensitivity. Reagents vary in the type and concentration of PL, thus causing inconsistency [82,83]. Additionally, conditions causing acute phase reactants (ie, infection, pregnancy) associated with increased fibrinogen and factor VIII, may shorten the aPTT and possibly mask a weak LA. Sensitive modifications of the aPTT, such as the dilute aPTT, can verify the presence of LA in patients who are receiving anticoagulant therapy or who have congenital or acquired factor deficiencies [84].

Prothrombin time (PT). The PT assay assesses the extrinsic and common pathways of the coagulation cascade. Normally, patients with a LA will have a normal PT unless they are receiving oral anticoagulants or they develop an inhibitor to PRO. Variations of the PT assay give rise to various tests used in the diagnosis of LA. These include the dilute Russell's viper venom time, kaolin clotting time, and tissue thromboplastin time.

Dilute Russell’s viper venom time (DRVVT). Russell’s viper venom activates factor X, which in the presence of PL, calcium, and factor V activates PRO, leading to the formation of a fibrin clot. In the DRVVT, dilution of the venom yields a clotting time in which concentration of the PL reagent is the rate-limiting step. Any inhibition of this PL by a LA manifests in a prolonged DRVVT [85]. Many commercial DRVVT assays are available, but they vary in sensitivity due to the concentration of PL. If the PL concentration is too high, the tests become insensitive to weak LA.

Other snake venom assays. Other snake venoms are used for LA testing. Taipan (Oxyuranus scutellatus) venom activates PRO in the presence of PL and Ca²⁺. Textarin (the venom from Psuedonaja textiles) acts similarly but also requires the presence of factor V. Specificity of both of these tests can be improved by mixing tests and/or a confirmation test such as the use of ecarin, an enzyme purified from the venom of Echis carinatus, in conjunction with the Textarin test (86).

Kaolin clotting time (KCT). The most sensitive screening tests for LA contain little PL and are sensitive to the bypass effect of the procoagulant platelet PL [87]. For this reason, the KCT, where no additional PL is used, is very sensitive [88]. The sensitivity of the KCT depends on the influence of residual red cell membranes and plasma lipids on coagulation. A LA is identified when the KCT fails to correct after the addition of even large amounts of plasma. In factor deficiency states, the KCT will correct with the addition of a small volume of plasma. Due to the inherent nature of this test, platelet contamination of the plasma sample greatly reduces its sensitivity. Another problem with the KCT, owing to the particulate nature of kaolin, is that it is unsuitable for some photo-optical devices, which makes full automation difficult. Although automation of the KCT has been proposed using a 0.5% kaolin suspension [89], strategies that use micronized silica instead of kaolin as an activator have been proposed for automatically detecting LA in photo-optical instruments [90,91].

Tissue thromboplastin inhibition test (TTI). The TTI is another test based on a modification of the PT assay. Thromboplastin, which is rich in PL, can be diluted so that its concentration becomes the rate-limiting step. Inhibition of prothrombinase by a LA will cause prolongation of the PT assay. Again, due to the various PL and its concentration in the reagent, the test varies in its sensitivity and specificity. New modifications of the test using recombinant human tissue factor (Innovin) have shown similar sensitivity to the DRVVT and high specificity that is dependent on the dilution of thromboplastin [92].

Mixing assays. After the identification of a prolonged clotting time by one of the previously mentioned tests, the inhibitory activity of the LA can be demonstrated by mixing the sample with
normal plasma. In a factor deficiency state, a 1:1 mixture with normal plasma will usually correct the clotting time. However, in the presence of a LA, the clotting time will not correct and may even be further prolonged—the so called lupus cofactor effect [93]. Because mixing studies dilute the LA antibody, false negative tests can occur.

**Confirmation of PL-dependence.** Normalization of the prolonged clotting time by adding PL to the plasma is the basis of demonstrating PL-dependence. The platelet neutralization procedure (PNP) is most commonly used. Washed normal platelets are activated with calcium ionophore or lysed by freezing-and-thawing, thus exposing PL. Adding these platelets to the plasma will correct the clotting time (aPTT, DRVVT, and the Taipan venom time) by bypassing the LA. However, platelet suspensions may also neutralize heparin or factor V inhibitors, causing false positive results.

Another neutralization technique, based on platelet-derived vesicles, has been described as an easily standardizable and reproducible procedure, which clearly separates lupus anticoagulants (LA) from other coagulation factor inhibitors [94]. Newer confirmation tests using hexagonal-phase phospholipids (Staclot-LA [95]) and high phospholipid confirmatory reagent (DVV Confirm) have also been developed to reliably and less expensively identify a LA. These tests have equal sensitivity and specificity compared to other PNP tests [96]. However, false positive results in patients with high titer factor VIII inhibitors have also been seen with these tests [81]. Thus, no clear evidence exists regarding the superiority of one test over another.

**Exclusion of specific factor inhibitors.** If a specific factor inhibitor is in question, the guidelines recommend performing specific factor assays [81]. As the plasma is diluted in the presence of LA, the factor activity will often increase, in contrast to what is observed with specific factor inhibitors. In the presence of strong LA, reductions in factors VIII and IX may be seen simultaneously [97].

**Practical aspects of the application of LA tests.** In general, functional assays play a major role in the identification of a LA. They are the only methods available to detect aCL antibody-negative cases and may show a higher predictive value for thrombotic complications in APS [98]. Despite the various tests available, no single test is sufficiently sensitive or specific to be used alone. Therefore, the guidelines recommend that at least 2 tests be used; in general aPTT-based assays are more sensitive to aPRO antibodies and RVVT-based tests are more sensitive to anti-β2GPI antibodies [99,100].

**Conclusions**

The diagnostic armamentarium with which the laboratory can assist in the diagnosis of APS is formidable. With such an elaborate selection of hematological and immunological methods, the precise choice of tests that most efficiently diagnose APS may seem complex. For example, using traditional measures of diagnostic utility, ie, sensitivity and specificity, one is faced with a plethora of conflicting data, as demonstrated by the brief compilation in Table 3 [5,101-103].

Another approach is represented by the numerous authors who have evaluated the usefulness of examining the odds ratios of various symptoms such as thrombosis or fetal loss, thus recognizing that APS is a syndrome of heterogeneous causes and manifestations.

<table>
<thead>
<tr>
<th>Test</th>
<th>Reference</th>
<th>Sensitivity (%)</th>
<th>Specificity (%)</th>
</tr>
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<tbody>
<tr>
<td>LA</td>
<td>*sa ISTH guidelines [101]</td>
<td>86</td>
<td>79</td>
</tr>
<tr>
<td></td>
<td>*sa ISTH guidelines [102]</td>
<td>100</td>
<td>73</td>
</tr>
<tr>
<td>aCL and anti-β2GPI</td>
<td>aCL/ anti-β2GPI [101]</td>
<td>56</td>
<td>86</td>
</tr>
<tr>
<td></td>
<td>anti-β2GPI       [103]</td>
<td>89</td>
<td>56</td>
</tr>
<tr>
<td></td>
<td>anti-β2GPI       [5]</td>
<td>na</td>
<td>98</td>
</tr>
<tr>
<td></td>
<td>anti-β2GPI       [102]</td>
<td>86</td>
<td>67</td>
</tr>
<tr>
<td></td>
<td>aCL              [5]</td>
<td>na</td>
<td>78</td>
</tr>
<tr>
<td></td>
<td>aCL              [102]</td>
<td>71</td>
<td>53</td>
</tr>
<tr>
<td>Anti-PRO</td>
<td>aPRO/PS          [101]</td>
<td>57</td>
<td>92</td>
</tr>
<tr>
<td></td>
<td>aPRO             [5]</td>
<td>na</td>
<td>98</td>
</tr>
<tr>
<td></td>
<td>aPRO             [102]</td>
<td>71</td>
<td>51</td>
</tr>
</tbody>
</table>

*sa: secundum artis, ie, following the method in ISTH guidelines, ref. [81].
Despite the promise shown by some of the more esoteric assays, properly-executed LA testing, as described above, and the application of anti-β2GPI and/or β2GPI-dependent aCL assays will suffice to diagnose APS in the presence of appropriate clinical findings. In addition to clinical criteria (which are not discussed here), the most recent consensus guidelines [104] recognize only LA, aCL, and anti-β2GPI as appropriate laboratory criteria for classification of APS, as follows:

(a) Presence of LA in plasma on 2 or more occasions at least 12 weeks apart, detected according to the guidelines of the International Society of Thrombosis and Haemostasis;

(b) Presence of IgG or IgM aCL at a titer >40 GPL or MPL (or >99th percentile) in serum or plasma on 2 or more occasions, 12 weeks apart, using a standardized assay; and

(c) Presence of anti-β2GPI of the IgG or IgM class at >99th percentile, using a standardized assay, on 2 occasions, 12 weeks apart.

The most recent consensus guidelines [104] further recommend that APS patients be classified into the categories shown in Table 4. Other laboratory tests, described herein, were considered for inclusion by the consensus group. However, since the tests were considered premature or lacking in evidentiary strength, they were not included in the guidelines. Further studies and technical refinements may lead to future consideration of these criteria.

Table 4. Updated laboratory data-based classification system for APS, as recommended by the 2006 International Consensus Guidelines (ref. [104]).

<table>
<thead>
<tr>
<th>Category</th>
<th>Criteria</th>
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<tbody>
<tr>
<td>I</td>
<td>More than 1 laboratory criterion present in any combination</td>
</tr>
<tr>
<td>IIa</td>
<td>LA present, only</td>
</tr>
<tr>
<td>IIb</td>
<td>aCL present, only</td>
</tr>
<tr>
<td>IIc</td>
<td>anti-β2GPI present, only</td>
</tr>
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References


Laboratory testing for the antiphospholipid syndrome


