**SPECIAL ARTICLE**

**International consensus statement on an update of the classification criteria for definite antiphospholipid syndrome (APS)**


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**Summary.** New clinical, laboratory and experimental insights, since the 1999 publication of the Sapporo preliminary classification criteria for antiphospholipid syndrome (APS), had been addressed at a workshop in Sydney, Australia, before the Eleventh International Congress on antiphospholipid antibodies. In this document, we appraise the existing evidence on clinical and laboratory features of APS addressed during the forum. Based on this, we propose amendments to the Sapporo criteria. We also provide definitions on features of APS that were not included in the updated criteria.

**Keywords:** anticardiolipin, antiphospholipid syndrome, β2 glycoprotein-I, classification criteria, lupus anticoagulant, thrombosis.

**Introduction**

Since the formulation of the international preliminary classification (Sapporo) criteria for antiphospholipid syndrome (APS) [1], a significant body of work in basic research and studies on laboratory and clinical manifestations of APS has appeared. A preconference workshop, preceding the Eleventh International Congress on antiphospholipid antibodies (aPL), considered revisions to the international classification criteria. Studies validating the Sapporo criteria [2,3] are few. Tested against patients with systemic lupus erythematosus (SLE) and lupus-like disease (LLD), the criteria have high sensitivity and specificity [2], but the high frequency of aPL in older populations and of thromboembolic disease in hospitalized patients suggests that the Sapporo criteria would perform poorly in these populations. The association of aging and of common risk factors for cardiovascular disease with thrombosis may cause classification bias (Evidence Level I) [4]. No published data provides a valid estimation of an age boundary for diagnosing APS. Standard definitions of premature cardiovascular disease [5] and conditions conferring risk for thrombosis (listed in Table 2) [6,7] should be taken into account (Evidence Level I). Thrombosis may be more frequent when multiple risk factors coexist. Strict exclusion criteria

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provide the commonly used clinical definitions for placental insufficiency (Table 2), and we suggest that the criterion for APS classification be any of these conditions associated with the decision of a clinical patient to deliver a morphologically normal fetus prior to 34 weeks' gestation.

Both lupus anticoagulant (LA) and anticardiolipin (aCL) immunoglobulin isotype G (IgG) and M (IgM) are maintained as laboratory APS criteria, and IgG and IgM anti-
beta-glycoprotein-I (anti-\(\beta\)-GPI) assays are added in the revised criteria (Table 2).

Medium and high titers of IgG and IgM aCL antibodies associate with clinical manifestations of APS, and were selected as criteria in Sapporo. However, the threshold used to distinguish moderate–high levels from low levels has no standard [13], and definition of the level that best corresponds to the risk of clinical manifestations is difficult [14]. Based on the best available evidence (Evidence Level II) [15–19], and until an international consensus is reached, the committee introduces a clear statement on threshold for positive: >40 GPL or MPL units, or >99th percentile (Table 2).

The revised criteria introduce a concept of subclassification of APS patients into four different categories of aPL assay positivity, specified in Table 2. Certain issues of specificity and predictive value of laboratory assays remain unresolved, whereas evidence suggests that multiple aPL positivity is associated with a more severe course of the disease, increasing significantly the rate of thrombosis (Evidence Level II) [20–23]. Investigators encouraged to subclassify patients with positive laboratory assays that fulfill the criteria for APS in clinical studies, according to the guidelines in Table 2.

Antiphospholipid syndrome requires the combination of at least one clinical and one laboratory criterion. A remote test avoids false results from interference with the event; however, in extreme cases, a positive test separated many years from a clinical manifestation also risks misclassification, as a causative relationship between event and test would then be in doubt. The Sapporo statement encouraged investigators to provide applicable information, but relevant existing data are rather poor. The stability of the laboratory testing over time is reassuring [24], yet spontaneous variation of aPL in individual patients occurs in up to a quarter of cases (Evidence Level II). Whether disease activity and treatment contribute to assay variability is unknown [25–27]. The committee suggests that researchers should not classify APS if more than 5 years separate the clinical event and the positive laboratory test, and that an allowance of at least 12 weeks between symptom and test will assist assessment of the relationship between clinical manifestations and aPL (Table 2). These time limits are valid independently of which feature of APS (clinical or laboratory) occurs first.

Persistent positivity of laboratory tests is important; the Sapporo criteria suggested an interval of at least 6 weeks between the two positive tests. In fact, there are no data to validate this interval. There are concerns that transient presence of epiphennomenal aPL – not infrequent in clinical practice – could risk misclassification (Evidence Level II) [28]. This committee proposes that increasing the interval to 12 weeks is
Table 2 Revised classification criteria for the antiphospholipid syndrome

Antiphospholipid antibody syndrome (APS) is present if at least one of the clinical criteria and one of the laboratory criteria that follow are met*.

Clinical criteria
1. Vascular thrombosis
   One or more clinical episodes of arterial, venous, or small vessel thrombosis, in any tissue or organ. Thrombosis must be confirmed by objective validated criteria (i.e. unequivocal findings of appropriate imaging studies or histopathology). For histopathologic confirmation, thrombosis should be present without significant evidence of inflammation in the vessel wall.
2. Pregnancy morbidity
   (a) One or more unexplained deaths of a morphologically normal fetus at or beyond the 10th week of gestation, with normal fetal morphology documented by ultrasound or by direct examination of the fetus, or
   (b) One or more premature births of a morphologically normal neonate before the 34th week of gestation because of: (i) eclampsia or severe pre-eclampsia defined according to standard definitions [11], or (ii) recognized features of placental insufficiency, or
   (c) Three or more unexplained consecutive spontaneous abortions before the 10th week of gestation, with maternal anatomic or hormonal abnormalities and paternal and maternal chromosomal causes excluded.

In studies of populations of patients who have more than one type of pregnancy morbidity, investigators are strongly encouraged to stratify groups of subjects according to a, b, or c above.

Laboratory criteria**
1. Lupus anticoagulant (LA) present in plasma, on two or more occasions at least 12 weeks apart, detected according to the guidelines of the International Society on Thrombosis and Haemostasis (Scientific Subcommittee on LAs/phospholipid-dependent antibodies) [82,83].
2. Anticardiolipin (aCL) antibody of IgG and/or IgM isotype in serum or plasma, present in medium or high titer (i.e. >40 GPL or MPL, or > the 99th percentile), on two or more occasions, measured by a standardized ELISA [100,129,130].
3. Anti-β2-glycoprotein-I antibody of IgG and/or IgM isotype in serum or plasma (in titer > the 99th percentile), present on two or more occasions, at least 12 weeks apart, measured by a standardized ELISA, according to recommended procedures [112].

*Classification of APS should be avoided if less than 12 weeks or more than 5 years separate the positive aPL test and the clinical manifestation.

Coexisting inherited or acquired factors for thrombosis are not reasons for excluding patients from APS trials. However, two subgroups of APS patients should be recognized, according to: (a) the presence, and (b) the absence of additional risk factors for thrombosis. Indicative (but not exhaustive) such cases include: age (>55 in men, and >65 in women), and the presence of any of the established risk factors for cardiovascular disease (hypertension, diabetes mellitus, elevated LDL or low HDL cholesterol, cigarette smoking, family history of premature cardiovascular disease, body mass index ≥30 kg m⁻², microalbuminuria, estimated GFR < 60 mL min⁻¹), inherited thrombophilias, oral contraceptives, nephrotic syndrome, malignancy, immobilization, and surgery. Thus, patients who fulfil criteria should be stratified according to contributing causes of thrombosis. ¹A thrombotic episode in the past could be considered as a clinical criterion, provided that thrombosis is proved by appropriate diagnostic means and that no alternative diagnosis or cause of thrombosis is found. ²Superficial venous thrombosis is not included in the clinical criteria. ³Generally accepted features of placental insufficiency include: (i) abnormal or non-reassuring fetal surveillance test(s), e.g. a non-reactive non-stress test, suggestive of fetal hypoxemia, (ii) abnormal Doppler flow velocimetry waveform analysis suggestive of fetal hypoxemia, e.g. absent end-diastolic flow in the umbilical artery, (iii) oligohydramnios, e.g. an amniotic fluid index of 5 cm or less, or (iv) a postnatal birth weight less than the 10th percentile for the gestational age. **Investigators are strongly advised to classify APS patients in studies into one of the following categories: I. more than one laboratory criteria present (any combination); IIa, LA present alone; IIb, aCL antibody present alone; IIc, anti-β2-glycoprotein-I antibody present alone.

Features associated with APS, but not included in the revised criteria

This panel also discussed clinical and laboratory features not included in the revised classification criteria for APS. These include: (i) heart valve disease, (ii) livedo reticularis (LR), (iii) thrombocytopenia, (iv) nephropathy, (v) neurological manifestations, (vi) IgA aCL, (vii) IgA anti-β2-GPI, (viii) antiphosphatidylserine antibodies (aPS), (ix) antiphosphatidylethanolamine (aPE) antibodies, (x) antibodies against prothrombin alone (aPT-A), and (xi) antibodies to the phosphatidylserine–prothrombin (aPS/PT) complex. Some of the features above are undoubtedly frequent but not specific in patients with APS. The committee considered that adoption of these features as independent criteria for definite APS may decrease diagnostic specificity, even though their association with APS is recognized.

Another issue is how to classify (i) cases with aPL and non-criteria clinical manifestations of APS, and (ii) the infrequent cases that fulfil the clinical criteria, but test positive only for non-criteria aPL. Some members of the committee proposed the term ‘probable APS’. This concept was not
Mild regurgitation is very common in the general population. Regurgitation as criterion for aPL-associated cardiac valve disease, as recommended, because the features listed above cannot be used as alternative criteria for APS. With these limitations in mind, we believe it would be reasonable to use these features, which were not selected for diagnosis of individual patients as ‘probable APS’, ‘features associated with APS’ or ’non-criteria features of APS’. For clinical studies, patients falling into any of these categories should be classified separately from those that fulfill the revised classification criteria for APS. This policy may help clarify unsettled issues (specificity, associations of aPL with clinical manifestations, and differences in outcome and impact of treatment) between those features and definite APS. Thus, this committee encourages the separate recognition of non-criteria features of APS, and proposes a terminology (Tables 3–6). The evidence that precludes adoption as criteria is summarized in the section on Specific issues.

Specific issues

Cardiac manifestations

Heart valve lesions (vegetations, valve thickening and dysfunction) are frequent in APS, independent of SLE [33], but data are contradictory because of differences in echocardiography technique and descriptions for findings, inconsistent associations with aPL, and population heterogeneity1 (Evidence Level II) [36,37]; confounding factors associated with cardiac valve disease include age, hypertension and obesity (Evidence Level I) [38]. The committee proposes a minimal consensus regarding the valve dysfunction and provides relevant definitions of heart valve lesions in APS (Table 3), but recommends against adoption as criteria. Determination of aPL in patients coming to medical attention because of valve disease should be individualized rather than routine.

 Coronary artery disease (CAD) fulfills the thrombosis criterion for APS; we recommend that patients be stratified according to thrombosis risk stratification guidelines (Table 2). The workshop advises against routine performance of aPL tests in patients with CAD unless the patient’s young age and lack of identifiable risk factors suggest a rare etiology.

 Few data exist concerning the incidence of ventricular dysfunction in APS (Evidence Level IV). The committee advises that the rare cases with biopsy-proven myocardial microthrombosis, or with intracardiac thrombi be recognized as meeting the thrombosis criterion for APS (Evidence Level IV). Detection of cardiac microthrombosis or intracardiac thrombi without apparent explanation warrants aPL testing.

Neurological manifestations

Transient cerebral ischemia and stroke fall within the spectrum of thrombosis; thus, pertinent stratification recommendations apply. A consensus report on these manifestations has been published [39].

Antiphospholipid antibodies correlate with physical disability in the elderly [40] (Evidence Level II). In one small study of APS patients without SLE [41], long-term presence of LA is a risk factor for dementia (Evidence Level II). In SLE patients, persistent elevation of aPL is associated with cognitive dysfunction (Evidence Level I) [42,43]. Prospective studies deny an association between migraine and aPL (Evidence Level I) [44,45]. In patients with multiple sclerosis (MS), an association between aPL and clinical course cannot be supported (Evidence Level I) [46]. Patients with concomitant MS and SLE may be an exception, but studies are contradictory (Evidence Level II) [47,48]. Transverse myelopathy (TM) is a rare entity within APS [33]. Limited data suggest that in the 1% of SLE patients who manifest TM, the latter is associated with aPL (Evidence Level IV) [49]. Contradictory data exist on the relationship between aPL and seizures in SLE (Evidence Level I) [50,51] and in epilepsy patients (Evidence Level II) [52,53]. In unselected APS patients, epilepsy has been retrospectively associated with SLE, CNS ischemic events, thrombocytopenia, and LR (Evidence Level II) [54]. It is uncertain whether aPL can influence the clinical course of epilepsy, as relevant prospective data are missing. This committee considers that

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1For instance, although mitral valve thickness > 3 mm, measured with TEE, correlated significantly with aCL > 40 GPL in one study [34], the average mitral valve thickness in the control population of another study was 3.2 mm with Doppler echocardiography [35].

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Thrombocytopenia

Antiphospholipid antibodies correlate with lesions of renal small-artery vasculopathy and chronic renal ischemia (Evidence Level III) [65–68]. The committee recommends the term \textit{aPL-associated nephropathy} (APLN) to describe this entity (Table 5). Renal lesions are identical in SLE–APS and non-SLE–APS patients; and have been associated with extra-renal vascular thrombosis and pregnancy complications in SLE patients (Evidence Level II) [65,66,69,70]. They are independent of lupus nephritis and do not correlate with the rate of loss of renal function or end-stage renal disease [65,66]. Apart from thrombotic microangiopathy, which represents an acute event, other lesions of APLN reflect chronic vascular damage, are more frequent [66,68], and may be non-specific. In almost all reported cases, the diagnosis of APLN derived from multiple findings. Histologic criteria for APLN have not been validated. Patients with histologically proven APLN satisfy the thrombosis criterion for APS, provided that other conditions resulting in similar renal lesions are excluded. This committee does not suggest routine performance of renal biopsy in APS; this decision should be guided by conventional clinical indications.

Thrombocytopenia

Antiphospholipid antibodies are frequently found in patients initially diagnosed with idiopathic thrombocytopenic purpura (ITP), prospectively associated with thrombosis (Evidence Level I) [71,72]. This may suggest that aPL confers a high risk of thrombosis in patients with ITP, or that ITP is a first

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<th>Table 4</th>
<th>Definition of aPL-associated livedo reticularis (LR)</th>
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<td>aPL-associated LR is the coexistence of aPL (Laboratory Criteria for APS) and LR. Livedo reticularis is the persistent, not reversible with rewarming, violaceous, red or blue, reticular or mottled, pattern of the skin of trunk, arms or legs. It may consist of regular unbroken circles (regular LR) or irregular-broken circles (livedo racemosa). The width of the branching pattern can be ≥10 mm (large LR) or &lt;10 mm (fine LR). Four variants may be recognized: fine livedo racemosa, large livedo racemosa, fine regular LR, and large regular LR. Pathologic changes confirmative, but not required, for LR classification and diagnosis include partial or complete occlusion of the lumen of small- to medium-sized arteries and/or arterioles at the dermis-subcutis border with no evidence of perivascular inflammatory infiltrate and negative direct immunofluorescence examination [62]. Patients who fulfill Clinical Criteria for APS are excluded from the definition above.</td>
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<th>Table 5</th>
<th>Definition of aPL-associated nephropathy (APLN)</th>
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<td>aPL-associated nephropathy [66,68] is the coexistence of aPL (Laboratory Criteria for APS) along with the histopathologic detection of: Thrombotic microangiopathy involving both arterioles and glomerular capillaries and/or One or more of: Fibrous intimal hyperplasia involving organized thrombi with or without recanalization Fibrous and/or fibrocellular occlusions of arteries and arterioles Focal cortical atrophy Tubular thyroidization (large zones of atrophic tubules containing eosinophilic casts) Vasculitis, thrombotic thrombocytopenic purpura, hemolytic uremic syndrome, malignant hypertension, and other reasons for chronic renal ischemia are exclusions. Patients who fulfill Clinical Criteria for APS are excluded from the definition above. If SLE is also present, the above lesions should be distinguished from those associated with lupus nephropathy.</td>
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<th>Table 6</th>
<th>Definition of aPL-associated thrombocytopenia</th>
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<td>aPL-associated thrombocytopenia is the coexistence of aPL (Laboratory Criteria for APS) along with the following: Thrombocytopenia (&lt;100 × 10⁹ L⁻¹), confirmed at least twice 12 weeks apart. Exclusion of patients with thrombotic thrombocytopenic purpura, disseminated intravascular coagulation, pseudo-thrombocytopenia, and heparin-induced thrombocytopenia [135,136]. Thrombocytopenia is further characterized as moderate (platelet count 50–100 × 10⁹ L⁻¹) or severe (&lt;50 × 10⁹ L⁻¹). Subclassification of patients according to the presence or absence of SLE is advantageous. Patients who fulfill Clinical Criteria for APS are excluded from the definition above.</td>
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there is insufficient evidence to include cognitive dysfunction, headache or migraine, MS, TM, and epilepsy in the revised APS classification criteria; however, the data concerning cognitive dysfunction are suggestive and warrant further study.

Skin manifestations

Livedo reticularis is more prevalent among APS patients with SLE, and in females (Evidence Level II) [33,55]. Studies of associations with specific aCL isotypes or LA are contradictory (Evidence Level II) [56–60]. In unselected APS patients, LR has been retrospectively correlated with aCL and arterial thrombosis, but not with anti-β2-GPI or LA, venous thrombosis, or pregnancy morbidity (Evidence Level II) [61]. There are no prospective studies on the ability of LR to predict thrombosis, with the exception of the rare Sneddon’s syndrome (Evidence Level II) [62]. The LR lesions can lead to ischemia and tissue infarction, called livedo vasculitis (purpuric macules, cutaneous nodules, and/or painful ulcerations) [63]; stating its presence is advisable. Inclusion of LR as an independent clinical criterion for APS would not serve to classify homogeneous patient groups, and definition is required (Table 4). The committee advises subclassification of LR variants for clinical studies. Although histologic findings sometimes may be helpful in most LR cases, there are no pathognomonic findings [62]; performing a biopsy is not routinely indicated or encouraged by this committee.

Other skin manifestations of APS include skin ulcerations, pseudo-vasculitic lesions, digital gangrene, superficial phlebitis, malignant atrophic papulosis-like lesions, subungal splinter hemorrhages [63], and anetoderma (a circumscribed area of loss of dermal elastic tissue) (Evidence Level IV) [64]. They are rare, and none merits inclusion as a criterion.

Renal manifestations

Antiphospholipid antibodies correlate with lesions of renal small-artery vasculopathy and chronic renal ischemia (Evidence Level III) [65–68]. The committee recommends the term ‘aPL-associated nephropathy’ (APLN) to describe this entity (Table 5). Renal lesions are identical in SLE–APS and non-SLE–APS patients, and have been associated with extra-renal vascular thrombosis and pregnancy complications in SLE patients (Evidence Level II) [65,66,69,70]. They are independent of lupus nephritis and do not correlate with the rate of loss of renal function or end-stage renal disease [65,66]. Apart from thrombotic microangiopathy, which represents an acute event, other lesions of APLN reflect chronic vascular damage, are more frequent [66,68], and may be non-specific. In almost all reported cases, the diagnosis of APLN derived from multiple findings. Histologic criteria for APLN have not been validated. Patients with histologically proven APLN satisfy the thrombosis criterion for APS, provided that other conditions resulting in similar renal lesions are excluded. This committee does not suggest routine performance of renal biopsy in APS; this decision should be guided by conventional clinical indications.
symptom of APS, as it may be of SLE. Thrombocytopenia is more common in patients with APS and SLE than in patients with APS alone (Evidence Level II) [33]. Antibodies directed against platelet glycoproteins are associated with thrombocytopenia (but not with thrombosis) in patients with aPL [73], and also in patients with APS, comparable with ITP patients [74] (Evidence Level III). The committee consented that thrombocytopenia occurring in patients with persistent aPL, in the absence of clinical manifestations of APS, should be considered to be different from ITP: such patients have an increased thrombotic risk and require closer follow-up. On the other hand, inclusion of thrombocytopenia as an independent clinical criterion for APS would likely add little to sensitivity with a potential cost in specificity; a clear distinction from thrombocytopenia because of SLE and ITP are required, and relevant data from prospective studies are inadequate. We propose the term ‘aPL-associated thrombocytopenia’ (Table 6) to stratify patients for clinical studies. We propose a platelet count $< 100 \times 10^9 \text{L}^{-1}$ as the upper cut-off limit for thrombocytopenia in APS (Evidence Level II) [33]. This relatively stringent limit (vs. $150 \times 10^9 \text{L}^{-1}$) could serve the criteria target of maximum specificity, while including the severe and moderate cases.

**Lupus anticoagulant**

Lupus anticoagulant better correlates with thrombosis (Evidence Level I) [75], pregnancy morbidity (Evidence Level II) [76], and thrombosis in SLE patients (Evidence Level I) [77] than does aCL. Inter-laboratory agreement is relatively poor for the large number of LA assays on the market [78,79]. The present committee recommends that laboratories performing LA comply with existing rules to improve inter-laboratory concordance (Evidence Level II) [78,80–83].

No definite recommendation can be given on the assays of choice for LA testing. Both activated partial thromboplastin time (APTT)-based assays and dilute Russell’s viper venom time (dRVVT) are suitable for LA (Evidence Level II) [79,80], provided that the APTT used for LA testing is LA sensitive. One positive test suffices for LA positivity; as no single test is 100% sensitive for LA, it is advised to use two or more tests with different assay principles before the presence of LA is excluded.

Unless one uses an LA test system that includes a heparin neutralizer (most of the commercial dRVVT-assays), the thrombin time should always be measured to exclude unforeseen presence of unfractioned heparin. If the patient is on oral anticoagulants, measurement of LA is better postponed (Evidence Level III) [84], or patient samples be diluted 1 : 2 with normal plasma before the test is performed, provided that international normalized ratio (INR) is $< 3.5$. When INR is $> 3.5$, the LA testing is unworkable (Evidence Level IV). Several phospholipids (rabbit brain extract, hexagonal phase phospholipids, defined phospholipid vesicles, washed-activated platelets, frozen-thawed platelets and lyophilized platelet extracts) have been used successfully in LA confirmation assays; no evidence exists for superiority of any particular one.

Little objective information and no relevant guidelines exist to define a positive-screening test [82]. The use of fixed-time cut-off limits in different laboratories with different instruments was discouraged by this panel. For dRVTT and other LA assays, better precision can be achieved when individual laboratories determine their own cut-off levels for positive results (Evidence Level II) [78,80,85]. Use of normalization ratios (test sample : control sample) is the best way to compensate for inter- and intra-assay variation. Clotting time ratios (test/control) of $> 1.1$ for the dRVTT and $> 1.2$ for KCT are applied in many laboratories, and indicate LA according to earlier guidelines [86,87]. Ratios have high specificity but may have low sensitivity (Evidence Level II) [80]. An appropriate way to establish cut-off levels is to measure LA in 40 healthy controls and determine the geometric mean $\pm$ 2SD.

The interpretation of the confirmation procedure – the so-called lupus ratio (mathematically identical with the screen/confirm ratio), or the confirm/normal ratio – that offers the highest risk for thromboembolic complications remains uncertain. Integration of screening and confirmation into a single assay makes LA testing less time consuming, and may increase diagnostic accuracy and inter-laboratory agreement (Evidence Level III) [88]. Nevertheless, the result of the LA test that best correlates with clinical events of APS cannot be concluded. Until further evidence is provided, the committee advises considering positive every sample outside the normal range (Evidence Level IV).

The use of widely available pooled patient plasmas for positive controls (instead of the traditional plasma samples of local LA-positive patients) is currently advised [83]. Plasma spiked with monoclonal antibodies will soon be available; its use and standardization through participation in multicenter studies (e.g. ECAT and Scientific and Standardization Committee [SSC]) are encouraged.

Two new methods enable discrimination between $\beta_2$-GPI and prothrombin antibodies causing LA. The first uses cardiolipin vesicles and can only be used in an APTT-based assay [89]. The second is based on changes in the final calcium concentration in the assay, but does not work when a mixture of both anti-$\beta_2$-GPI and antiprothrombin antibodies is present [90]. In patients with autoimmune diseases, $\beta_2$-GPI-dependent LA strongly correlates with a history of thrombosis, in contrast to the $\beta_2$-GPI-independent assay (Level of Evidence II) [91]. An international multicenter trial to confirm this assertion will begin shortly.

**Anticardiolipin assay**

Interlaboratory agreement on aCL measurement remains marginal with both home-based and commercial assays (Evidence Level I) [14,92,93]. Discrepancies are mainly because of cut-off, calibration, and other methodological issues. Expression of aCL assays in ranges of positivity achieves better interlaboratory and inter-run agreement than do quantitative readouts [94]. We note that IgM aCL tends to give false-positive results, particularly in the low-positive

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range, especially in the presence of rheumatoid factor or cryoglobulins [95,96].

The ISTH-SSC recommended in 2002 that the aCL test should be replaced by anti-β2-GPI and the LA tests [97]. However, the best available evidence indicates that anti-β2-GPI cannot yet be considered a substitute for aCL (Evidence Level II) [21,22,98]; this committee recommends that aCL continue to be a laboratory criterion for APS.

To optimize standardization, new reference samples (monoclonal antibodies, named HCAL and EY2C9) [99] will be distributed from the Center for Disease Control and Prevention to investigators free of charge. They will have to be validated against existing calibrators, and their specificity, avidity and stability over time should be monitored. Because these preparations cannot mirror the heterogeneity present in patient samples [100], firm recommendations cannot be given at this time.

IgA aCL

The IgA aCL are usually detected together with either IgG and/or IgM isotypes in patients with APS (Evidence Level II) [101–103], and agreement among patients grouped according to aCL titers for IgA seems lower than those for the other isotypes [104]. Specificity and standardization considerations for the other aCL isotypes apply also to the IgA aCL assay. In patients with collagen disease, IgA aCL associates with thrombocytopenia, skin ulcers and vasculitis, indicating a patient subgroup at risk for specific clinical manifestations (Evidence Level I) [106]. Hence, this isotype appears to identify patient subgroups rather than adding diagnostic power. The committee consents that IgA aCL cannot be considered as a laboratory criterion for APS.

Anti-β2-GPI

By majority2, the committee agreed that IgG and IgM anti-β2-GPI should be included as part of the modified Sapporo criteria. Anti-β2-GPI antibodies are an independent risk factor for thrombosis (Evidence Level II) [107,108] and pregnancy complications (Evidence Level I) [109,110], though some studies deny these associations mainly because of methodological differences and lack of standardization [107,108]. Interlaboratory variation of anti-β2-GPI is better than that found with the aCL assay for both home-made [111] and commercial kits [112] (Evidence Level II). The anti-β2-GPI assay shows higher specificity than aCL for APS diagnosis (Evidence Level II) [21,22,113–115]. In 3–10% of APS patients, anti-β2-GPI may be the only test positive (Evidence Level I) [23,98,116]. The association of anti-β2-GPI with pre-eclampsia and/or eclampsia in unselected pregnant women who tested negative for aCL (Evidence Level I) [109] implies that the inclusion of anti-β2-GPI may also help clarify this pregnancy morbidity.

Methodology and standardization limitations expressed for aCL also apply for anti-β2-GPI [111,112]. Laboratories measuring anti-β2-GPI are encouraged to standardize the types of plates; purity, concentration and source of β2-GPI; and calibrators and units of measurement [18,112]. Validation of monoclonal anti-β2-GPI [99] antibodies and comparison with the existing standards is encouraged. High titers of anti-β2-GPI antibodies are associated with high risk of thrombosis, but it is difficult to define boundaries for medium and high titers at this stage. Until an international consensus is reached, this committee proposes a threshold for positive anti-β2-GPI antibodies > 99th percentile of controls. The possible interference of cryoglobulins and rheumatoid factors should be considered in the interpretation of IgM anti-β2-GPI. Outside the context of clinical studies, testing for anti-β2-GPI can be helpful for APS diagnosis, particularly when aCL and LA are negative and APS is strongly suspected.

IgA anti-β2-GPI and other ELISAs for aPL detection

Data are inadequate for establishing IgA anti-β2-GPI as an independent risk factor for APS in the absence of other anti-β2-GPI isotypes (Evidence Level III) [117]. IgA anti-β2-GPI are the most frequently detected antibodies in patients in specific ethnic groups (Evidence Level II) [118,119]. A significant proportion of IgA anti-β2-GPI-positive tests have no apparent association with any clinical manifestation of APS (Evidence Level IV). Although a few Evidence Level II studies report association of aPE antibodies with thrombosis and fetal loss [120,121], experience with these antibodies is inadequate. Uniform guidelines how to perform the test, units of measurement and control materials do not exist. The committee concludes that it is premature to recommend that tests for an aPL other than IgG and IgM anti-β2-GPI be included in the revised-Sapporo criteria.

Antiprothrombin antibodies

Antiprothrombin antibodies detected by ELISA are a heterogeneous population including antibodies against prothrombin alone (aPT-A) and antibodies to the phosphatidylserine–prothrombin complex (aPS/PT). Data on the clinical associations of aPT-A are contradictory, and they imply low specificity of these antibodies for APS diagnosis (Evidence Level II) [122–127]. A systematic review on antiprothrombin antibodies and risk of thrombosis in APS failed to reveal an association, irrespective of isotype, site and type of event, or presence of SLE [107]. Both the sensitivity and specificity of aPS/PT are higher than those for aPT-A, whereas 95% of patients with aPS/PT are also LA positive, suggesting that aPS/PT can also serve as a confirmatory assay for LA (Evidence Level II); these results, however, only come from one study [128], and concerns regarding aPS/PT arise from multivalent antibody binding; the possibility of measuring antibodies against non-complexed

2Consensus was not reached regarding this issue. Two members of the committee considered that existing evidence does not justify inclusion of anti-β2-GPI as a criterion.
phospholipids present in the sample needs to be excluded. Prospective studies examining the association of aPT-A or aPS/PT with APS clinical features are still missing. This committee considers that the inclusion of antiprothrombin antibodies in the classification criteria for APS is premature.

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Conflict of interest statement
No conflicts of interest are declared for this document.

Addendum
Contribution of each author to this manuscript was as follows:
SM: grading of the evidence, synthesis, and writing of the manuscript
MDL: grading of the evidence, manuscript editing
SAK: grading of the evidence, manuscript editing, and authors coordination

The following authors contributed to parts of the manuscript as listed:
TA: thrombocytopenia, antiprothrombin antibodies
DWB: obstetric manifestations
RLB: neurological manifestations
RC: cardiac manifestations
RHWM: lupus anticoagulant
PGdG: lupus anticoagulant
TK: thrombocytopenia, antiprothrombin antibodies
PLM: renal manifestations
GR: anticardiolipin, anti-ß-GPI
YS: skin manifestations
AT: anticardiolipin, anti-ß-GPI
PGV: renal manifestations.

Appendix: Members Of The Workshop Panel
In addition to the authors, the workshop panel comprised the following individuals:

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This workshop was chaired by Drs. Ronald Derksen, Steven Krilis, Michael Lockshin, and Spiros Miyakis.

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Update of the guidelines for lupus anticoagulant detection

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Summary. One of the conclusions of the subcommittee meeting on Lupus Anticoagulant/Phospholipid dependent antibodies, held in Geneva on 2007, was the need to update the guidelines on Lupus Anticoagulant (LA) detection. Particular emphasis was given to several aspects discussed in this official communication. A new paragraph is dedicated to the patient selection, and aims to minimize inappropriate requests for LA testing. Modalities for blood collection and processing are fully delineated and the choice of tests is limited to dRVVT and a sensitive aPTT. Calculation of cut-off values for each diagnostic step are clearly stated. A final paragraph reports the interpretation of the results in general and in particular situations.

Keywords: anticoagulants, antiphospholipid syndrome, diagnosis, lupus anticoagulant, thrombosis.

These guidelines are intended to update the criteria for the detection of the presence of lupus anticoagulants (LA) that were originally proposed by Brandt et al. in 1995 [1]. The subcommittee on Lupus Anticoagulant/Phospholipid-dependent Antibodies acknowledges that the present guidelines have been extremely useful during the past 13 years but that it is now appropriate to provide additional details and specifications in light of the knowledge and experience that has been accumulated since their publication.

Patient selection

Testing for LA should be limited to patients who have a significant probability of having the antiphospholipid syndrome (APS), or who have unexplained prolonged aPTT in the course of routine laboratory testing. Appropriate tests for LA can be performed in patients who have a high likelihood of LA. Low: venous (VTE) or arterial thromboembolism in elderly patients; Moderate: accidentally found prolonged aPTT in asymptomatic subjects, recurrent spontaneous early pregnancy loss, provoked VTE in young patients; and High: unprovoked VTE and (unexplained) arterial thrombosis in young patients (< 50 years of age), thrombosis at unusual sites, late pregnancy loss, any thrombosis or pregnancy morbidity in patients with autoimmune diseases (systemic lupus erythematosus, rheumatoid arthritis, autoimmune thrombocytopenia, autoimmune hemolytic anemia) [2]. Generalized searches performed on blood samples obtained from asymptomatic individuals or categories of patients other than described here are highly discouraged to avoid the risk of obtaining false-positive results that are relatively common on account of the poor specificity of the assays. Once a patient has been identified as positive for LA, it is imperative that testing be repeated on a second occasion > 12 weeks after the initial testing. It is preferable for samples to be obtained prior to, or in the absence of, anticoagulant therapy as this may interfere with the assay. Recommendations of the subcommittee are summarized in Table 1. How to determine and interpret cut-off values is described in Table 2.

Explanation and clarification of the recommendations summarized in Table 1

Numerous variables can affect assays used for LA detection. Among them, the content and type of phospholipids (PL) in the reagent mixture, activator, plasma preparation, expression of results and cut-off values greatly influence the results. Moreover, as the spectrum of antibodies and their epitope specificity may vary widely, reference material constitutes a major problem. As a consequence, a high variability in the performance of clinical laboratories with respect to sensitivity and specificity of LA tests has recently been reported [3–7]. The rates of false-positive and false-negative detections remain...
(A) Blood collection

1. Blood collection before the start of any anticoagulant drug or a sufficient period after its discontinuation
2. Fresh venous blood in 0.109 M sodium citrate 9:1
3. Double centrifugation
4. Quickly frozen plasma is required if LA detection is postponed
5. Frozen plasma must be thawed at 37 °C

(B) Choice of the test

1. Two tests based on different principles
2. dRVVT should be the first test considered
3. The second test should be a sensitive aPTT (low phospholipids and silica as activator)
4. LA should be considered as positive if one of the two tests gives a positive result
5. For interpretation see Table 2 (screening test)

(C) Mixing test

1. PNP for mixing studies should ideally be prepared in house. Adequate commercial lyophilized or frozen PNP can alternatively be used
2. A 1:1 proportion of patient : PNP shall be used, without preincubation within 30 min.
3. LA cannot be conclusively determined if the TT of the test plasma is significantly prolonged
4. For interpretation see Table 2 (mixing test)

(D) Confirmatory test

1. Confirmatory test(s) must be performed by increasing the concentration of PL of the screening test(s)
2. Bilayer or hexagonal (II) phase PL should be used to increase the concentration of PL.
3. For interpretation see Table 2 (confirmatory test)

(E) Expression of results

1. Results should be expressed as ratio of patient-to-PNP for all procedures (screening, mixing and confirm)

(F) Transmission of results

1. A report with an explanation of the results should be given

*(Further explanations and qualifications are reported in the text. PNP, pooled normal plasma; TT, thrombin time; PL, phospholipids relatively high. The former are of particular concern because they qualify the patients for long and unnecessary oral anticoagulant treatment [7].

(A) Blood collection

A3: Double centrifugation of the sample should be performed to ensure that the sample is platelet poor. This can be achieved by transferring the plasma after the initial centrifugation process (2000 g, 15 min, room temperature) to a non-activating plastic centrifuge tube using a plastic pipette, then recentrifuging the plasma for an additional 10 min at a higher speed (> 2500 g). When aliquoting to a secondary tube, take care to not include the residual platelets that may have collected at the bottom of the centrifuge tube [8]. Plasma filtration is not recommended as this introduces variables (type of filter, amount of plasma to be filtered, loss of von Willebrand factor and added costs [9]. Moreover, the problems of filters availability and adjunctive costs must be considered.

A4: Freezing of the samples must be performed as quickly as possible after venipuncture to prevent loss of coagulation factors. Freezing the plasma in a freezer at −70 °C or below is reasonable.

A5: Before analysis, frozen plasma must be thawed by total immersion of sample content in a water bath at 37 °C for 5 min to avoid formation of cryoprecipitate and then mixed thoroughly before testing.

(B) Choice of the test

B1: The risk of false-positive results is increased to an unacceptable level if more than two screening tests are performed.
B2: There is evidence that no single test is sensitive for all LA. The recommendation is to perform two different tests that represent different assay principles. Diluted Russell Viper
Venom time (dRVVT) is widely used in clinical laboratories and is believed to be specific for detecting LA in those patients at high risk of thrombosis [10]. An international External Quality Assessment Programme for laboratories working in the field of thrombosis showed that dRVVT is the most robust test in detecting LA [11].

B3: Any aPTT test performed with silica as an activator and low PL content is the second test of choice because of its sensitivity [3,6] for LA. Kaolin as an activator is not recommended because of its problematic behaviour in automated coagulometers. Ellagic acid as an activator is not recommended as a result of its insensitivity for LA. The Subcommittee does not recommend the following tests: dilute prothrombin time (dPT) because of variability in thromboplastin reagents; assays based on snake venoms such as Ecarin and Textarin because there are no standardized commercial assays available based on this principle; Kaolin Clotting Time (KCT) as a result of its poorer reproducibility compared with the other tests available [11].

(C) Mixing test
C1: The pooled normal plasma (PNP) should be prepared ‘ad hoc’ (home-made) by double centrifugation to ensure that the PNP contains minimal residual platelets (< 10^7 mL^-1) and to ensure approximately 100% activity for all clotting factors. The material must be stored frozen (~70 °C) in small aliquots. Commercial lyophilized or frozen normal plasmas can be used if they fulfill the above specifications or have otherwise been validated for use in LA testing. When testing for LA during pregnancy, normal range(s) of clotting times defined for normal pregnant women should be considered (aPTT is, in general, shortened as a result of high factor VIII levels during pregnancy, but also dRVVT can change for unknown reasons). No reference plasma is available. Therefore, established positive and negative plasmas should be used as controls to validate the assay.

C3: The coagulation time of a mixing test could also be prolonged because of the presence of heparin or inhibitors to coagulation factors. The thrombin time (TT) performed on test plasma or the clinical history of bleeding will help to identify heparin or specific inhibitors to clotting factors, respectively. LA screening is not possible if the test plasma is unclottable or if the content of heparin in the test plasma exceeds the reagent neutralization capacity. There are commercial dRVVTs and APTTs containing neutralizers able to quench heparin up to 0.8 U mL^-1. Although limited experience is available on the effect of low-molecular-weight heparin (LMWH), screening for LA in patients treated with LMWH is possible. It should, however, be noted that the effect on LA assays may vary depending on the ratio between factor (F) Xa to FIIa activity of each LMWH preparation. The effect of direct thrombin or FXa inhibitors on LA assays is unknown. Whereas hydroxychloroquine may weakly interfere with LA testing directly affecting the formation of IgG-β2GPI complexes on phospholipid bilayers [12], aspirin and clopidogrel do not interfere.

(D) Confirmatory test
D2: Freeze/thawed platelets are not recommended as the source of PL for the confirmatory tests because of poor batch-to-batch consistency.

(F) Transmission of results
F1: LA test results should be reported with quantitative results, and an interpretative comment that indicates whether the findings are compatible with the presence/absence of LA. This is important as many clinicians may not be aware of the significance of all the complex testing procedures carried out by the laboratory. Comments such as borderline or dubious LA are highly discouraged and in these cases the comment should be limited to the following: ‘to be tested again in 1 week’.

Interpretation of results

Integrated tests

Integrated tests include screening and confirmation in a single procedure. Such tests consist of testing the plasmas under investigation twice by means of the dRVVT [13] or APTT [13,14] performed in parallel at low (screen) and high (confirm) phospholipid concentrations. In principle, these tests do not require performance of the mixing test and the results may be interpreted according to the specific cut-off values by calculating either the percentage correction [(screen – confirm)/screen × 100] [13] or the LA ratio (screen/confirm) [15]. Both the percentage correction and the LA ratio may benefit from normalization of results against a PNP run in parallel with the test plasmas [(screen/confirm) patient divided by (screen/confirm) PNP]. Some of the above tests are designed to measure the coagulation times on a mixture of patient and PNP [14].

LA detection during acute thromboembolic events

Caution should be exercised in interpretation of the results of tests performed close to a thromboembolic event as patients may be treated with full doses of unfractionated heparin and/or vitamin K antagonists (VKA). Furthermore, acute-phase reactants as FVIII may be increased during acute events.

LA detection in patients on long-term vitamin K antagonists (VKA)

1 The interpretation of results is difficult because of the prolonged basal clotting time. To avoid misinterpretation, it is recommended to perform laboratory procedures 1 to 2 weeks after discontinuation of treatment or when the international normalized ratio (INR) is less than 1.5. Bridging VKA discontinuation with LMWH is recommended with the last dose of LMWH administered more than 12 h before the blood is drawn for LA testing.

2 Alternatively, if the INR is between 1.5 and < 3.0, a 1:1 dilution of patient plasma and PNP can be considered. Note,
that the interpretation of results may still be difficult and that the LA titer will be diluted 2-fold.

3 Detection procedures by Textratin(Taipan)/Ecarin clotting times [17,18] or integrated tests (i.e. % correction for APTT, SCT and DRVVT at low and high phospholipid concentration) [13] are not currently recommended as they require further critical evaluation.

Antiphospholipid antibody profiles A LA result should always be considered in the context of a full laboratory aPL profile comprising anticardiolipin (aCL) and antiβ2glycoprotein I (aβ2GPI) antibody ELISAs. The presence of medium-high titer aCL and aβ2GPI of the same isotype (most often IgG) is in agreement with a positive LA and identifies patients at high risk for thrombosis. Less information is available for the correlations with fetal losses. Isolated LA positivity is significantly more frequent in subjects without clinical events [19] or may be false-positive especially if identified as ‘mild in potency’, if it is found in elderly patients or if it is diagnosed for the first time [7].

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The authors state that they have no conflict of interest.

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