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COAGULATION WHITE PAPER | November 2023*

Guidelines from the Scientific and Standardization Committee of the ISTH for the detection of lupus anticoagulant in anticoagulated patients [1]

Introducing antiphospholipid syndrome

Antiphospholipid syndrome (APS) is an acquired prothrombotic autoimmune disorder defined by arterial or venous thrombosis and/or pregnancy morbidity in patients who exhibit a persistent presence of antiphospholipid antibodies (aPL) – mostly IgG and IgM subtypes, but more rarely also IgA [2]. According to the Sydney revised Sapporo criteria, APS is diagnosed based on clinical and laboratory criteria summarised in Table 1. Definite APS is considered present if at least one of the clinical and one of the laboratory criteria are met.

The syndrome can either be associated with an existing autoimmune disease, in which case it is called 'secondary APS' or, if there is no evidence of an existing underlying disease, it is called 'primary APS'.

Up to 5–10 % of the healthy population carry antiphospholipid antibodies in their blood without exhibiting a clinical sign of an APS.

Table 1 Summary of classification criteria for APS according to Sydney revised Sapporo criteria classification.

Clinical criteria	Vascular thrombosis	One or more episode of arterial, venous or small vessel thrombosis in any tissue or organ (confirmed by imaging or histopathology).
	Pregnancy complications	Recurrent pregnancy loss (after > 10 weeks' gestation) or one or more premature births due to pregnancy complications.
Laboratory criteria	Detection of lupus anticoagulant	Lupus anticoagulant (LA) in plasma on 2 occasions at least 12 weeks apart.
	Detection of anticardiolipin antibodies	Anticardiolipin/antiphospholipid antibodies (ACA/APA) of IgG and/or IgM isotype on 2 occasions at least 12 weeks apart.
	Detection of anti-β2 glycoprotein 1 antibodies	Anti-β2 glycoprotein 1 antibodies of IgG and/or IgM isotype on 2 occasions at least 12 weeks apart.

* Revision of the original article published in April 2023.

Central updates to the guidelines

The most frequently used tests for the determination of lupus anticoagulant (LA) in the patient's blood are the activated partial thromboplastin time (APTT) and the diluted Russell Viper venom test (dRVVT). Other but less frequently used tests are the Taipan venom coagulation time or Textarin, the diluted pro-thrombin time, the kaolin coagulation time (KCT) or the neutralization of LA activity with hexagonal phase phospholipids. However, these tests are not frequently performed in laboratories. Certainly, also because the current guidelines give a clear recommendation for the use of two different coagulation assays that are sensitive to LA and that have been designed according to different principles.

In 2020, the updated guideline from the Scientific and Standardization Committee of the ISTH for lupus anti-coagulant/antiphospholipid antibodies detection and interpretation recommended, the dRVVT, (carried out at a low and a high phospholipid concentration), and an LA-sensitive APTT (also carried out at a low or a high phospholipid concentration) be utilised as first and second line tests. The results are reported as the ratio of the clotting times measured with the low (screening, sensitive to LA) and high (confirmation, insensitive to the presence of LA) phospholipid concentration. Positive LA plasmas have Screen/Confirm ratios > 1.20. However, since there are differences from reagent to reagent and from batch to batch for the normal range of the Screen / Confirm ratio, a nor-malised ratio is used instead or the percentage correction [(screen – confirmation) / screen × 100] of the direct ratio. LA is likely when the screen / confirmation (LA) ratio or percent correction is above the 99th centile.

Measures to prevent recurrence of clinical presentations of an APS include the administration of anticoagulants such as heparin, vitamin K antagonists (VKA) or direct oral anti-coagulants (DOACs). This may lead to some difficulties for the diagnosis of APS, especially when determining LA in the patient's blood.

dRVVT assays are usually insensitive to heparin because they contain a heparin neutralizing agent. However, in the presence of VKAs and DOACs, their sensitivity will vary and therefore false results are possible. On the other hand, APTT tests are usually highly sensitive to heparin and VKAs, which can lead to a prolonged clotting time.

Against this background, assessing the presence of LA in patients on anticoagulant therapy is not recommended. In clinical reality, however, this is not always possible, since a successful therapy goes hand in hand with knowledge of the underlying disease, among other things. The guide-lines also recommend that the laboratory diagnosis of APS must be confirmed again twelve weeks after the initial diagnosis to rule out any temporary antibodies and thus the APS. This requires the patient to be tested even though they are receiving anticoagulant therapy.

The ISTH SSC have tried to support laboratory operators, scientists and clinicians in their daily challenges and have issued guidance for the diagnosis of LA in anticoagulated patients.

Different approaches to LA determination in anticoagulated patients are examined in this white paper.

Table 2 Comparison of the LA test option with the guideline recommendation.

Conditions	Guideline recommendation
■ Dilution of patient plasma with PNP	Not recommended, due to false positive or negative results likely.
■ Integrated assays for LA	Not recommended in patients under DOAC treatment. It is recommended to evaluate the responsiveness to LMWH of the local LA assay and to measure the anti-FXa activity of the patient under unfractionated heparin (UFH) and LMWH treatment. Laboratories should assess the insensitivities of the LA reagents to UFH and LMWH. Unexpected results should be considered as influenced by anticoagulant treatment.
■ Taipan venom or Ecarin clotting time	Recommendation pending upon the provision of independent evidence from collaborative studies with standardised kits.
■ Use of antidotes like idarucizumab or andexanet-alfa	Scant information currently available, and the cost incurred for the antidote should be considered. Further investigations are needed.
■ Use of neutralisers (DOAC-Stop or DOAC-Remove)	Useful for DOAC treated patients, but further investigations needed. Not recommended in patients under heparin treatment.
■ Discontinuation of anticoagulant therapy	Recommended only, whenever LA-detection is deemed of special interest for decision-making in individual patients. Whenever possible, blood collection for LA testing should be made before anticoagulant therapy starts. Information on current patient clinical and pharmacological history should be provided in the laboratory request form. Perform prothrombin time (PT), APTT and thrombin time (TT) assay in patients with unknown pharmacological or clinical history.

The basic recommendation is that LA testing in patients on anticoagulation should be undertaken with the awareness that anticoagulants may prolong the clotting time of the tests used for LA detection and that this effect may give rise to false-positive or false-negative LA. Therefore, whenever possible, samples for LA detection should be collected before initiation of anticoagulation.

If necessary, comments should be made on the final conclusion including any interference (e.g., haemolysis, etc.) or evidence of anticoagulants. This information should be used in interpreting the results. Close interaction between the laboratory and clinician is essential.

HYPHEN BioMed HEMOCLOT™ LA-S and LA-C reagents in the light of this ISTH guideline

The dRVVT reagents HEMOCLOT™ LA-S and LA-C manufactured by HYPHEN BioMed overcome many of the limitations of existing and commercially available reagents by using synthetic and optimised phospholipids as well as highly purified RVV.

High-risk patients (e. g. diabetics), patients with severe atherosclerosis or with increased platelet turnover might benefit from different antiplatelet regimens [8, 10–12]. An increased immature platelet count was identified as a key factor associated with insufficient platelet inhibition in response to aspirin, clopidogrel and prasugrel treatment [7, 13–16].

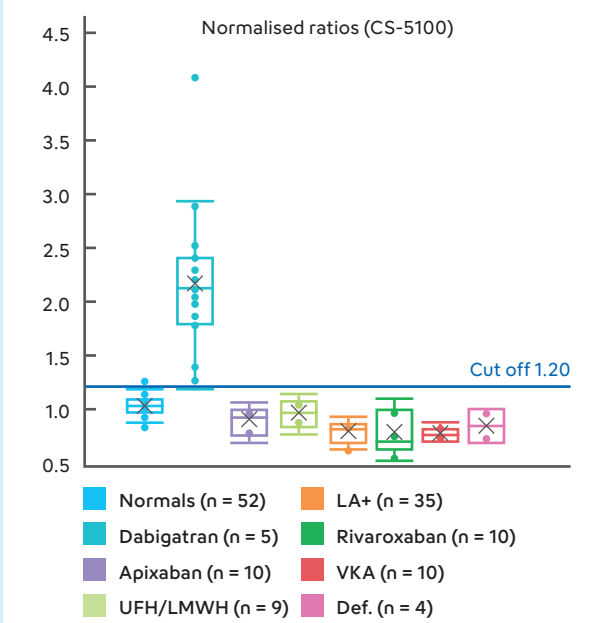

Fig. 1 Limited false positive results on DOACs, VKA, Heparinised samples with HEMOCLOT™ LA-S and HEMOCLOT™ LA-C [3].

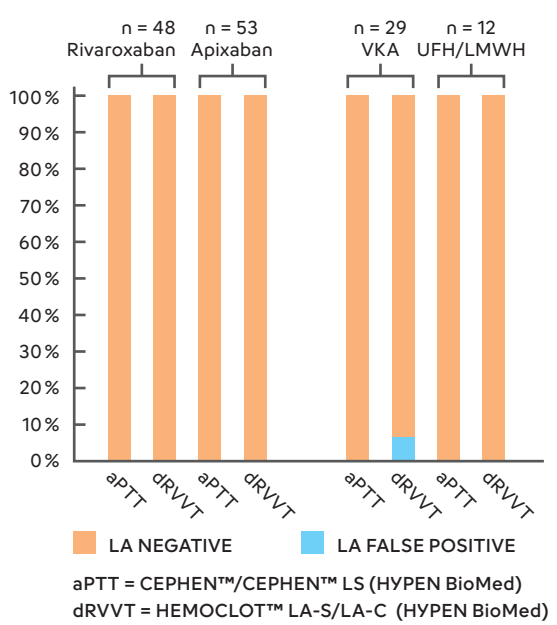

Fig. 2 Sensitivity of CEPHEN™/CEPHEN™ LS and HEMOCLOT™ LA-S/LA-C reagents towards direct anti-factor Xa and VKA drugs [4].

References

[1] Tripodi, A, Cohen, H, Devreese, KMJ. (2020): Lupus anticoagulant detection in anticoagulated patients. Guidance from the Scientific and Standardization Committee for lupus anticoagulant/antiphospholipid antibodies of the International Society on Thrombosis and Haemostasis. J Thromb Haemost. 2020; 18: 1569– 1575.

[2] Miyakis S, Lockshin MD, Atsumi T, Branch DW, Brey RL, Cervera R, Derksen RH, De Groot PG, Koike T, Meroni PL, Reber G, Shoenfeld Y, Tincani A, Vlachoyiannopoulos PG, Krilis SA. (2006): International consensus statement on an update of the classification criteria for definite antiphospholipid syndrome (APS). J Thromb Haemost 2006; 4: 295–306.

[3] Data from HYPHEN BioMed internal study on patient plasma. Neuville-sur-Oise, 2019.

[4] Frere C. (2018): Evaluation of the CEPHEN™/CEPHEN™ LS and HEMOCLOT™ LA-S/LA-C Reagents for Lupus Anticoagulant testing.

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Appendix Comparison of the LA test option.

Conditions	Pro	Cons
Dilution of patient plasma with PNP		
■ For patients under VKA ■ Not suitable for patients under DOACs ■ Certified (platelet free and factor concentration close to 100 % of norm (100 IU/mL)) pooled normal plasma (PNP) must be used	■ Plasma pools are easy to make in every laboratory	■ In-house manufacture PNP must be suitable to be used to detect LA in patient plasma and the laboratory must have sufficient facilities to prepare and store them ■ Dilution will affect LA potential too ■ Degree of correction is dependent on the reagent composition ■ Little evidence available for value of this method
Integrated assays for LA		
■ Two aliquots of the same sample at low (screen) and high (confirm) phospholipid concentrations, cut-off > 1.2 is indicative of LA	■ Well standardised ■ Easy to perform in every laboratory ■ Well characterised reagent performance	■ Screen and clotting times in the presence of DOAC's are not proportionally prolonged, with screen more prolonged than confirm ■ Incorrect positive LA results for DOACs and enoxaparin ■ Depending on their anti-factor Xa/factor IIa ratio, some brands of low molecular weight heparin (LMWH) can lead to a considerable prolongation of the APTT and APTT-like tests and therefore influence the LA detection
Taipan venom or Ecarin clotting time		
■ For patients under VKA therapy ■ For patients under DOACs therapy	■ Less affected by VKAs and anti-FXa DOACs	■ Insufficient data from LA positive patients and efficiency in patients on DOACs different from rivaroxaban ■ Lack of reagent standardisation
Use of antidotes		
■ Antidote for dabigatran (idarucizumab) to neutralise anticoagulant effect *in-vitro* ■ Andexanet-alfa same effect on rivaroxaban, apixaban and edoxaban treated patients	■ Minor effect on clotting time	■ Little supporting evidence available ■ Possible over-correction of screen/confirm ratio leading to incorrect negative results in weak LA patients
Use of neutralisers		
■ DOAC absorbents (DOAC-Stop and DOAC-Remove) inactivate DOACs *in-vitro*	■ Minor effect on clotting times ■ Neutralise any type of DOACs	■ Lack of data for LA positive patients under DOAC treatment ■ DOAC-Stop may remove anticoagulant proteins from patient plasma ■ False negative LA results in weak LA patients ■ DOAC absorbents to be used in DOAC treated patients, not in heparin or non-anticoagulated patients ■ After adding DOAC absorbents, complete reversal of the anti-FXa effect does not occur in every sample
Discontinuation of anticoagulant therapy		
■ Based on individual clinical evidence only	■ Patients on VKA may switch to LMWH if the LMWH does not affect LA test or anti-FXa activity is low ■ DOACs patients may have LA test 48 h after last dose, considering individual clinical condition and inhibitor levels	■ General risk of thrombosis as well as bleeding ■ Return to VKA requires intensive monitoring during stability phase