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ORIGINAL ARTICLE

Clinical and laboratory practice for lupus anticoagulant testing: An International Society of Thrombosis and Haemostasis Scientific and Standardization Committee survey

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Abstract

Background: Current guidelines have contributed to more uniformity in the performance and interpretation of lupus anticoagulant (LA) testing. However, points to reconsider include testing for LA in patients on anticoagulation, cut-off values, and interpretation of results.

Objectives: The aim of this International Society of Thrombosis and Haemostasis Scientific and Standardization committee (ISTH SSC) questionnaire was to capture the spectrum of clinical and laboratory practice in LA detection, focusing on variability in practice, so that the responses could inform further ISTH SSC recommendations.

Methods: Members of the ISTH SSC on Lupus Anticoagulant/Antiphospholipid Antibodies and participants of the Lupus Anticoagulant/Antiphospholipid Antibodies Programme of the External quality Control of diagnostic Assays and Tests Foundation were invited to complete a questionnaire on LA testing that was placed on the ISTH website using RedCap, with data tallied using simple descriptive statistics.

Results: There was good agreement on several key recommendations in the ISTH and other guidelines on LA testing, such as sample processing, principles of testing, choice of tests, repeat testing to confirm persistent positivity and the use of interpretative reporting. However, the results highlight that there is less agreement on some other aspects, including the timing of testing in relation to thrombosis or pregnancy, testing in patients on anticoagulation, cut-off values, and calculation and interpretation of results.

Conclusions: Although some of the variability in practice in LA testing reflects the lack of substantive data to underpin evidence-based recommendations, a more uniform approach, based on further guidance, should reduce the inter-center variability of LA testing.

KEYWORDS

anticoagulation, antiphospholipid antibodies, confirmatory testing, cut-off values, lupus anticoagulant, pre-analytical

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1 | INTRODUCTION

Accurate diagnosis of antiphospholipid syndrome (APS) is essential to guide appropriate management with the aim of preventing the deleterious consequences of this acquired autoimmune disorder, characterized by thrombosis (arterial, venous, or microvascular) and/or obstetric morbidity in association with persistently positive antiphospholipid antibodies (aPL). The laboratory diagnostic criteria for aPL positivity comprise lupus anticoagulant (LA), IgG and/or IgM anticardiolipin (aCL), and/or anti-beta 2 glycoprotein I antibodies (aβ2GPI).[1] Identification of aPL positivity strengthens the decision for indefinite anticoagulation after a first unprovoked venous thromboembolism (VTE) or even after provoked VTE, particularly if the provoking factor for VTE appears to be disproportionately mild. It may also affect the type of oral anticoagulant that is prescribed.[2–5] In addition, it identifies women who require higher than standard prophylactic-dose anticoagulation with low molecular weight heparin (LMWH) during pregnancy,[6–8] and who also require low-dose aspirin and monitoring for placental insufficiency,[9] the latter to guide optimal timing of delivery, reducing the risk of perinatal morbidity and mortality. Approximately 50% of APS patients have LA alone,[10] with LA detection therefore critical for APS diagnosis in these patients. LA is thought to carry the highest risk for thrombosis among all aPL[11] and the occurrence of a thrombotic event may be associated with higher mortality in patients with LA.[12] LA has been reported to be the primary predictor of adverse pregnancy outcome in patients with aPL-associated pregnancies.[13] Detection of LA also enables diagnosis of triple-aPL-positive patients, who are perceived to be the APS patients at highest risk of thrombosis,[14,15] and thus, identification of LA enables risk stratification as well as appropriate management of APS patients.

External quality assessment studies on LA testing in Europe have shown considerable inter-laboratory variability, particularly in samples with "weak" LA, with false negative and false positive rates of 10%-20%.[16,17] North American studies have shown false-negative LA rates up to 28% and false-positive rates of around 11%, whereas Australasian studies reported false-negative rates up to 50% and false-positive LA rates of about 10%.[18,19] The discrepancies appear to be due to a variety of pre- and postanalytical factors as well as performance of the tests. There are many differences between laboratories in the selection of LA tests, source of reagents, methodological detail, and results.[18–24]

The 2009 International Society of Thrombosis and Haemostasis Scientific and Standardization Committee (ISTH SSC) guidelines on LA detection,[25] as well as the British Society for Haematology (BSH)[26] and Clinical and Laboratory Standards Institute (CLSI) guidelines,[27] have contributed to more uniformity in the performance and interpretation of LA testing. However, points to reconsider include testing for LA in patients on anticoagulation, cut-off values and interpretation of results. The aim of this ISTH SSC questionnaire was to capture the spectrum of clinical and laboratory practice in LA diagnosis, with particular focus on issues where there is variability in practice, so that the responses could help to inform the formulation of further ISTH SSC recommendations.

Essentials

- Current guidelines have contributed to more uniformity in lupus anticoagulant (LA) testing.
- An international survey of clinical and laboratory practice in LA testing has been performed.
- Uncertainty on testing in thrombosis, pregnancy, anticoagulation, and interpretation of results.
- A more uniform approach should reduce the inter-center variability of LA testing.

2 | METHODS

2.1 | Survey questionnaire

A survey questionnaire (Appendix 1) was formulated and respondents were requested to provide their opinions on LA testing. The questionnaire was placed on the ISTH website using RedCap and all members registered on the ISTH SSC for Lupus Anticoagulant/Antiphospholipid Antibodies on the ISTH website, who are workers in the field of aPL, were invited by email to participate (n = 479). Additionally, participants of the "Lupus Program" external quality exercises of the ECAT Foundation (n = 575) were asked to fill out the questionnaire.

2.2 | Data analysis

The specific details of returned information were entered onto an Excel spreadsheet that included all records and fields, and data tallied (after the survey deadline) using simple descriptive statistics.

3 | RESULTS

3.1 | General information

One-hundred and eighty-five responses to the survey were received. The majority (58%) were from laboratory scientists, with hematologists making up 22%. Their views likely represented their laboratories' policies. Almost three-quarters (73%) of respondents worked in hospital laboratories, approximately 50% of whom were in university hospital laboratories. As regards the volume of samples tested, 59.1% of laboratories undertake between 500 to 4000 LA tests annually, with 5% of laboratories undertaking more than 6000 and 2.8% more than 10 000 LA tests annually.

3.2 | Pre-analytical factors

Timing of LA testing in relation to thrombotic events: the responses to the questionnaire showed little agreement on the timing of testing in relation to a thrombotic event. The most frequent responses were to test any time after a thrombotic event (37.6%; but 79%-54

of these 68 respondents-were laboratory based and probably not in a position to refuse to test), whereas 33.7% stated that the timing depended on the clinical situation, with 13.8% stating that they did not know or were uncertain.

Timing of LA testing in relation to pregnancy: the questionnaire asked for views on the timing of LA testing in relation to pregnancy (excluding considerations in relation to the effect of anticoagulation on LA detection, which are covered below). The majority (60%) stated that LA testing could be done at any time in relation to pregnancy, with 20% indicating that LA testing should be deferred for at least 6 weeks after pregnancy. Here, 16.7% stated that they did not know or were uncertain.

Sample processing: 86.8% agreed that samples for LA should be collected and processed in line with the 2009 guidelines (i.e., blood samples collected into 0.105-0.109 mol/L sodium citrate 9:1, should be double centrifuged at 2000 *g* for 15 minutes at room temperature to achieve a residual platelet count of $<10^9$/L.)[25] 51.1% indicated plasma for LA testing should ideally be frozen within 4 hours, although 30.8% thought that the plasma should ideally be frozen within 2 hours of collection.

Restriction of LA testing because of sample issues: 53.9% stated that they would restrict testing if the sample is hemolyzed, with 29.1% and 18% stating that they would restrict LA testing if the sample is lipemic or icteric, respectively. Of the former, 37% would reject any sample with visible hemolysis, but 33% set limits based on plasma hemoglobin concentration, analyser hemolysis/icterus/lipemia (HIL) flags or subjective scores. Some stated that they would restrict photometric based analyzer testing but perform mechanical end-point clotting methods in the case of lipemia or icterus, whereas 47% and 67% (for lipemia and icterus respectively) stated that they would use analyser HIL flags or subjective scoring in decision making.

3.3 | Testing for LA

Coagulation screen: 83.5% would do coagulation screening tests (prothrombin time [PT], activated partial thromboplastin time [APTT], thrombin time [TT], and/or fibrinogen assay) to provide background information about unexpected coagulopathies and undocumented anticoagulation.

LA testing: the overwhelming majority (94.5%) agreed that LA testing should include two phospholipid-dependent clotting tests, based on different principles, with LA considered positive if one of the two tests gives a positive result. The dilute Russell's viper venom time (DRVVT) (98.9%) and APTT using a reagent with proven LA sensitivity (79.7%) were used for LA detection by the majority of respondents.

LA mixing test and interpretation: 84.1% agreed that a mixing test should be performed, using pooled normal plasma (PNP) at a patient:PNP ratio of 1:1. Options suggested for the ideal PNP were: a commercial PNP that has been platelet depleted at collection and is suitable for LA testing (47.5%), prepared in-house PNP (13.8%), or that either commercial or PNP are suitable (32.0%).

Confirmatory test for LA and order of testing: there were various views on when a confirmatory test for LA should be performed, with 54.9% stating that a confirmatory test should be done only when the LA screening test is prolonged; and other views that confirmatory testing should be undertaken on all samples being tested for LA (17.6%) or only when the screening and mixing tests are prolonged (25.3%) (Figure 1A). With regard to the order of testing, 69.1% agreed that the components of LA tests should be performed in a specific order, but there was less agreement as to what the order should be, with the majority (56.5%) stating the order should be Screen, Mix,

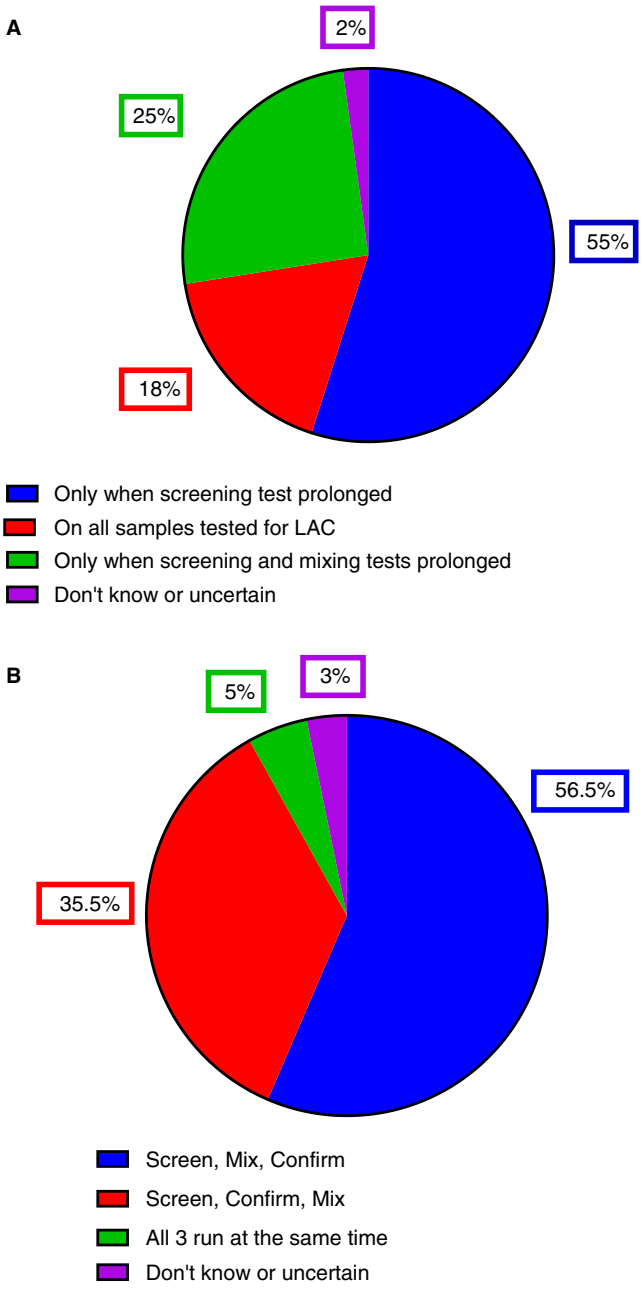

FIGURE 1 Responses to questions about performance of lupus anticoagulant (LA) tests. (A) When should a confirmatory test for LA be performed? (182 respondents). (B) If you think that it is important to perform the components of the LA test in a specific order, what should it be? (124 respondents)

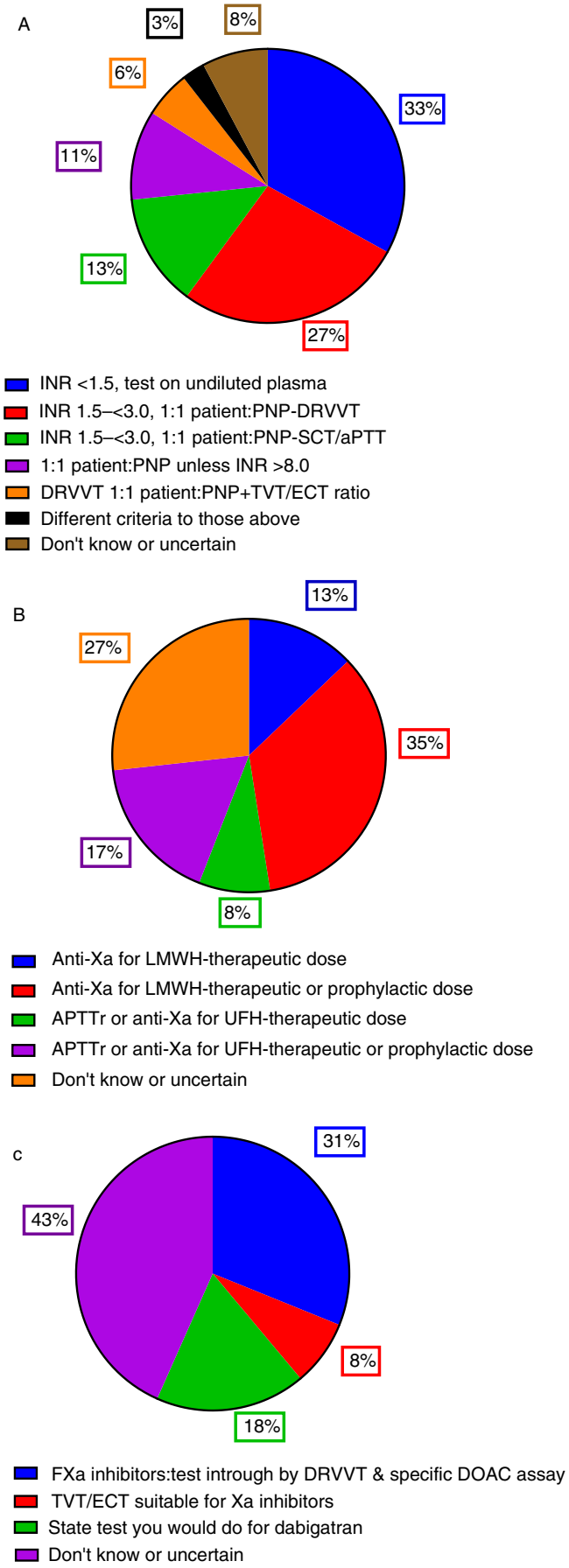

FIGURE 2 Responses to questions about performance of lupus anticoagulant (LA) tests in patients on anticoagulants. (A) If doing LA tests in patients on VKAs (218 respondents). (B) If doing LA tests in patients on LMWH or UFH (202 respondents). (C) If doing tests in patients on DOACs (180 respondents)

Confirm, and 35.5% stating that it should be Screen, Confirm, Mix (Figure 1B).

Interpretative report on LA result: there was almost universal agreement (97.3%) that an interpretative report should be provided on the LA result.

3.4 | LA testing in patients on anticoagulants

LA testing in patients on vitamin K antagonists (VKAs): only 41.7% indicated that it would be appropriate to do LA testing in patients on VKAs, with 52.8% stating that it would not be appropriate. Among the former group, 36.5% stated that blood samples for LA testing should be taken before starting the VKA, 9.5% that they would wait for at least 7 days after stopping the VKA and 50% applied other criteria (which mostly included using tests unaffected by VKA; testing depending on the International Normalized Ratio (INR) value; using a mixture of patient plasma and PNP; and one participant suggested using adapted cut-off values).

There were also various opinions about selecting samples based on the INR range, the commonest responses being that if the INR was <1.5, LA could be tested on undiluted plasma (41.9%); if the INR was 1.5-3.0, a DRVVT (34.3%) or silica clotting time/APTT (16.9%) could be used on a 1:1 patient:PNP mixture. Some respondents (13.4%) would test on equal volume mixtures of plasma regardless of INR up to an INR of 8.0 (Figure 2A). Alternative tests such as Taipan/Ecarin Venom time (TVT/ECT) are not commonly used (7%).

LA testing in patients on LMWH or unfractionated heparin (UFH): there were a variety of opinions about whether and when to test in such patients: not to test patients on LMWH/UFH (33.5%); test for LA during the trough period (i.e., at least 18 hours after the last dose) on therapeutic LMWH (32.4%) or prophylactic LMWH (27.5%); or to test on prophylactic, but not therapeutic LMWH or UFH (25.8%). Approximately 10% did not know or were uncertain as to whether or when to test for LA in individuals on LMWH or UFH.

There were also differences in opinion about verification of the plasma heparin level in relation to the dose, to ensure that the LA method is unaffected by anticoagulation: 42.2% stated that an anti-Xa assay should be performed for LMWH regardless of whether the patient received therapeutic or prophylactic dose, whereas 15.7% would test for therapeutic dosing only. There was less confidence about dealing with UFH: 21.1% would perform an APTT or anti-factor Xa assay regardless of type of dose and 10.2% would test for therapeutic dosed patients only; 33% stated that they did not know or were uncertain about the appropriate action in patients receiving LMWH or UFH (Figure 2B).

LA testing in patients on direct oral anticoagulants (DOACs): 70.3% stated that LA testing should not be undertaken in patients

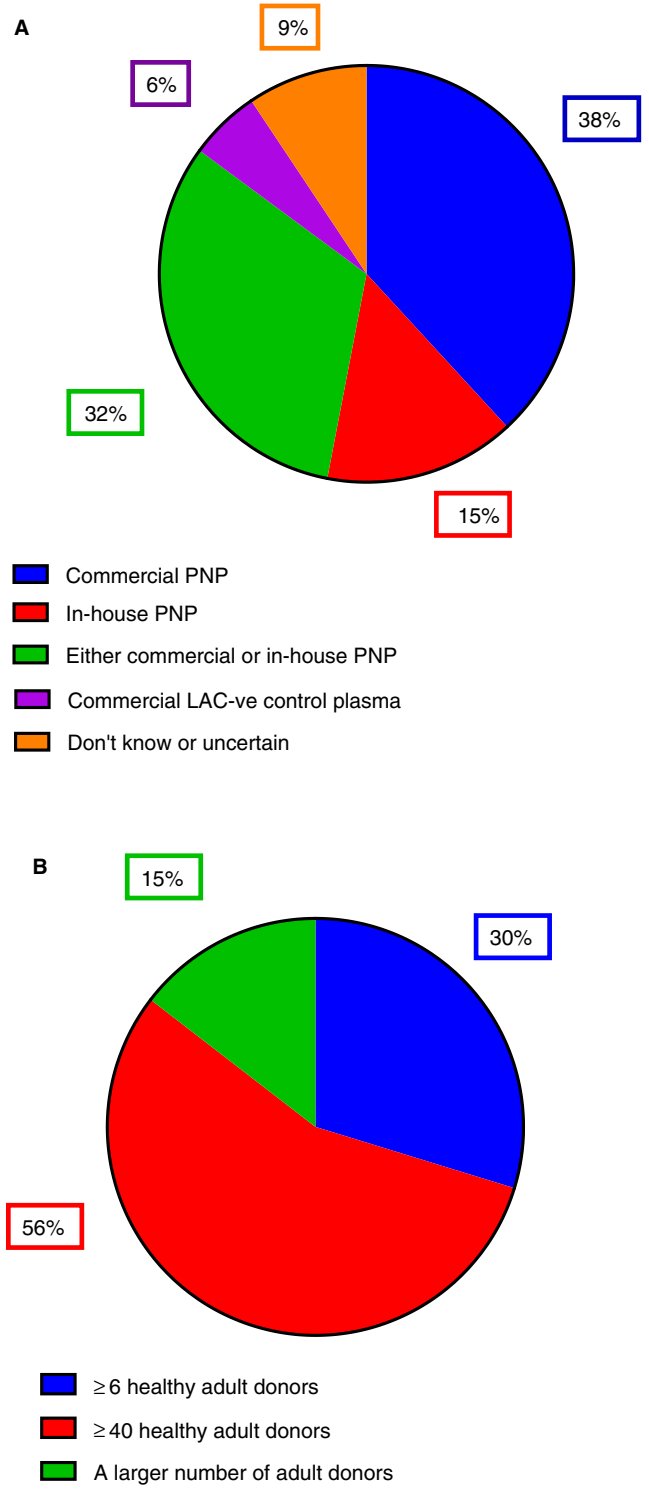

FIGURE 3 Responses to questions about normalized ratios. (A) Which type of plasma should be used for normalization? (191 respondents). (B) If in-house PNP is used for normalization, how many donors should be used for the pool? (158 respondents)

on DOACs. There were various suggestions about pre-analytical strategies such as testing during the trough period (17%) or after pre-treatment of the sample with commercial adsorbent or antidote preparations (11%). A small proportion (2.7%) stated that LA testing may be undertaken in some circumstances in patients on DOACs during the peak period.

There were also various suggestions about which tests to do, both for factor Xa (FXa) inhibitors and dabigatran. For patients receiving FXa inhibitors, 35% would use the DRVVT during the trough period and undertake a specific DOAC assay. However, almost half the respondents (49.4%) stated they did not know or were uncertain about how to test for LA in patients on DOACs (Figure 2C).

3.5 | Cut-off values and calculations for LA tests

Plasma for normalization of clotting times: there was little agreement on the ideal plasma for the calculation of normalized ratios, as shown in Figure 3A.

Number of healthy adult donors for the preparation of in-house pooled PNP used for the calculation of normalized ratios: 29.7% stated that at least six healthy adult donors should be used, whereas 55.7% stated that the number of donors should be at least 40% and 14.6%, that a larger number of donors should be used (Figure 3B).

Derivation of normalization of clotting times: 65.7% of respondents stated that the denominator to derive normalization of clotting times should be PNP analyzed in the same run and 19.4% that the denominator should be the mean of the reference interval; 11.4% stated that they did not know or were uncertain.

Cut-offs for screen, mixing, and confirmatory tests based on testing on plasmas from healthy donors: 50% stated that the cut-off should be the value above the 99th centile of the distribution, 33.9% stated that the cut-off value should be above the 97.5th centile, and 10.6% did not know or were uncertain (Figure 4A).

In-house cut-off values were calculated by 78.9% of respondents' laboratories. More than one-half (58.1%) stated that cut-off values could be based on 60-120 healthy donors, with the remainder of views on the number of donors for cut-off values ranging between <20 and 120, with 14.0% stating that they did not know or were uncertain (Figure 4B). Among those who indicated to use the 99th centile, only 12% indicated to use >120 healthy donors to do so, 13% indicated to use 60-120 healthy volunteers and the majority (56%) indicated to use 20-60 healthy donors. Reasons given to not calculate in-house cut-off values were that it is too laborious, the high cost and lack of availability of healthy donors.

Confirmation of manufacturer cut-off values for LA positivity by local validation: 81.2% agreed that this should be undertaken, whereas 8.8% did not agree, and 9.9% did not know or were uncertain.

Cut-off for the percentage correction (if used) based on testing on plasma from healthy donors mixed with the PNP at 1:1 proportion: there were divided views as to whether the percentage correction should be above the 99th or 97.5th centile, with 39.5% stating that this should be the value above the 99th centile of the distribution, 31.4% above the 97.5th centile, and 24.4% stating that they did not know or were uncertain (Figure 5A).

Interpretation of the mixing test: approximately one-half of the respondents (45.8%) used a normalized clotting time, with 17.5% using

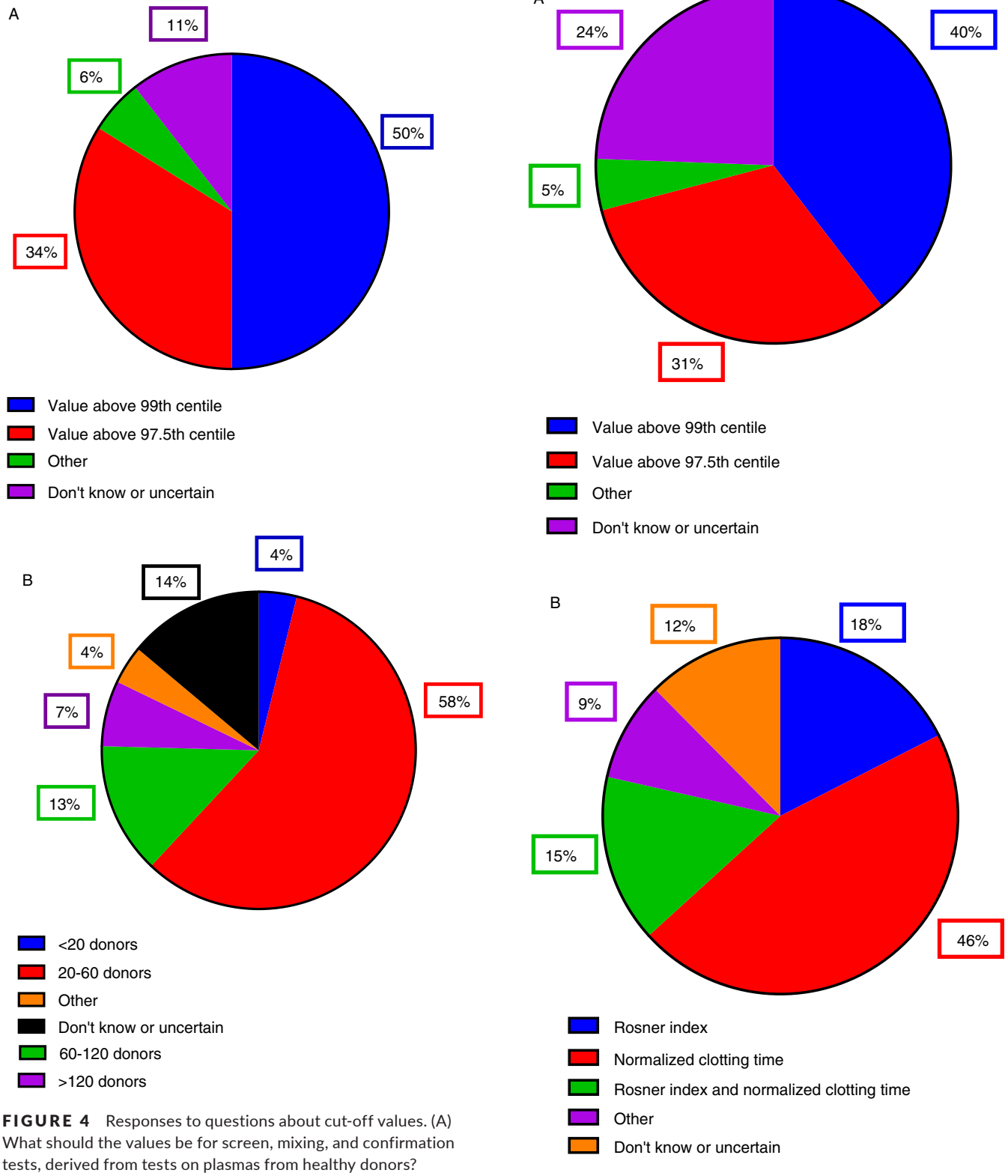

FIGURE 4 Responses to questions about cut-off values. (A) What should the values be for screen, mixing, and confirmation tests, derived from tests on plasmas from healthy donors? (180 respondents). (B) In-house cut-off values (centiles) should be calculated using how many healthy donor plasmas? (179 respondents)

FIGURE 5 Responses to questions about percentage correction and interpretation. (A) What should be the cut-off for percentage correction, when testing plasmas from healthy donors mixed 1:1 with PNP? (172 respondents). (B) How do you interpret the mixing test? (177 respondents)

the Rosner index (index of circulating anticoagulant), both Rosner index and normalized clotting time (15.3%) and 12.4% stating that they did not know or were uncertain (Figure 5B).

Confirmation of persistent LA positivity: 88.8% stated that a first LA should be confirmed to be persistently positive on a second sample after 12 weeks.

4 | DISCUSSION

The results of this ISTH SSC survey are encouraging as they show good agreement on several key recommendations in the current ISTH and other guidelines on LA testing,[25–27] such as sample processing, principles of testing, choice of tests, repeat testing to confirm persistent positivity, and the use of interpretative reporting. However, they highlight that there is less agreement on some other aspects of LA testing, including the timing of testing in relation to thrombosis or pregnancy, testing in patients on anticoagulation, cut-off values, and calculation and interpretation of results. Although some of the variability in practice reflects the lack of substantive data to underpin evidence-based recommendations, a more uniform approach in many aspects of LA testing should be feasible and would reduce the inter-center variability in LA test results.

Notably, the responses to the survey showed little agreement on the timing of testing in relation to a thrombotic event. The 2009 ISTH guideline advises caution in interpretation of LA results close to a thromboembolic event because patients may be treated with full doses of heparin and/or VKA and furthermore, acute-phase reactants such as FVIII may be increased during acute events.[25] aPL may fluctuate during pregnancy, and LA test results may not be representative during all three trimesters.[28–30] LA testing may be required during pregnancy, particularly when patients with pregnancy morbidity have not been previously investigated for aPL. In this situation, LA testing should be undertaken with the cognizance that negative aPL during pregnancy does not exclude a diagnosis of APS and that testing should be undertaken post-delivery to establish true aPL status.

The rejection of samples because of hemolysis appeared to be common, but lower numbers of respondents rejected samples because of lipemia or icterus. Local policies are likely to vary depending on the type of analyser used, its end-point detection system and the ability to objectively assess the level of the interfering substance.

There is not uniform agreement on LA testing in patients on anticoagulation with regard to whether to test or not and which methods to use, and this is reflected in the variable approaches suggested by respondents to the survey. Only 42% indicated that it would be appropriate to do LA testing in patients on VKAs, with various opinions on criteria for timing of blood sampling. Opinions also varied about testing at different INR ranges, whether one should do the test on mixed plasmas and which tests to do. Although LA testing in patients on VKAs is challenging, definition of LA status in patients on VKAs could identify APS patients with single aPL positivity for LA. The TVT/ECT test for LA may be useful in patients on VKA as, unlike Russell Viper venom, Taipan venom directly activates prothrombin and is not affected by VKA.[31–33] The TVT/ECT test is currently being validated in an ISTH SSC project in APS patients on VKAs,[34] but appears to have good specificity, although (in nonanticoagulated patients) it is less sensitive than the DRVVT.[35] In APS patients on DOAC FXa inhibitors, APTT-based tests are problematic and false-positive results have been reported with the DRVVT, even at trough rivaroxaban levels.[36] The TVT/ECT has been shown to be unaffected by rivaroxaban.[37,38] The use of adsorbent reagents to remove DOAC and allow LA testing in the normal way are being explored and preliminary results are encouraging.[39–41]

There were various views on when a confirmatory test for LA should be performed, with 55% of respondents stating that confirmatory testing should only be undertaken when the screening test is prolonged. The majority of respondents (69%) agreed that the components of lupus anticoagulant tests should be performed in a specific order, but there was less agreement as to what the order should be. The range of views probably reflects the variability between individual laboratories with regard to how they are set up in terms of analyzers, degree of automation, computer systems, and logistics, and these factors should be taken into account when making recommendations on LA testing.

There was considerable lack of agreement on the majority of aspects related to cut-off values and calculation and interpretation of results. Although 79% stated that they calculate their own in-house values, there were divided views on whether the cut-off should be the 99th or the 97.5th centile. It is important that any recommendation about this should have a valid statistical basis.[42] Laboratories need to consider whether they are calculating an in-house cut-off value (in which case at least 120 different healthy normal subjects are needed to calculate the 97.5th centile with 95% confidence) or verifying a manufacturer's cut-off (when 20-40 normal subjects may be used).[43–45] From a statistical point of view, the minimum sample size for a reliable estimation of the 99th centile is at least 300.[42,46] The poor agreement on the number of donors needed to calculate the cut-off is probably determined by the local availability and costs rather than strong views about what should be done.

In conclusion, the good agreement on several key recommendations in the current ISTH and other guidelines on LA testing,[25–27] such as sample processing, principles of testing, choice of tests, repeat testing to confirm persistent positivity, and the use of interpretative reporting, suggests that that the recommendations on LA testing are associated with more uniformity in LA testing between different laboratories. The lack of agreement on other aspects of LA testing, including the timing of testing in relation to thrombosis or pregnancy, testing in patients on anticoagulation, cut-off values, and calculation and interpretation of results, at least in part, reflects the lack of substantive data to underpin evidence-based recommendations. However, a more uniform approach in these aspects of LA testing, based on further guidance that addresses these areas, should reduce the inter-center variability of LA testing. The plan for development of this guidance is to aim for the "best fit," based on a current review of literature led by the ISTH SSC, with the members of the ISTH SSC for Lupus Anticoagulant/Antiphospholipid Antibodies as a Sounding Board.

ADDENDUM

H. Cohen devised and analyzed the LA survey questionnaire, wrote the first draft of the manuscript, and undertook critical revision of

the manuscript. K. M. J. Devreese devised the questionnaire and undertook critical revision of the manuscript. I. J. Mackie provided critical review of the questionnaire and undertook critical revision of the manuscript.

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CONFLICT OF INTEREST

H. Cohen, I. J. Mackie, and K. M. J. Devreese have no relevant conflicts of interest to declare.

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APPENDIX

Questionnaire on lupus anticoagulant (LAC) testing

Resize font:

The update of the guidelines for lupus anticoagulant detection dates from 2009, and have been proven to be very useful to reach more uniformity in performance and interpretation of LAC testing.

Nevertheless, we feel that for some points these recommendations need another update.

LAC testing in samples from anticoagulated patients, cut-off values, interpretation of results, etc. are some points to reconsider.

We greatly value your opinion. Your responses will be very helpful for the subcommittee to formulate further recommendations.

Kind regards,
Katrien Devreese,
Hannah Cohen
and all Co-chairs of the SSC on LAC/aPL

GENERAL INFORMATION

Are you a:

* must provide value

- ○ Laboratory scientist
- ○ Haematologist
- ○ Rheumatologist
- ○ Other specialist clinician (specify below)
- ○ Other (specify below)

reset

Type of laboratory you are working in:

- ☐ Private lab
- ☐ Hospital lab
- ☐ University hospital lab
- ☐ University lab
- ☐ Other (specify below)
- ☐ Clinician with input into laboratory testing
- ☐ Clinician without input into laboratory testing

What is the number of LAC tests performed in your lab?

- ○ < 500/year

- 500-1000/year
- 1000-2000/year
- 2000-4000/year
- 4000-6000/year
- >6000/year
- >10 000/year (specify below)
- Not applicable

reset

PRE-ANALYTICAL FACTORS

Timing of LAC testing in relation to a thrombotic event:

- No restriction - i.e. test any time after a thrombotic event
- Defer testing for at least 12 weeks after a thrombotic event
- Defer testing for another time interval after a thrombotic event (specify below)
- Depending on the clinical situation: i.e. test immediately if may influence treatment (e.g. CAPS, stroke, MI) and preferably defer testing if VTE
- Don't know or uncertain

reset

Do you think that it is appropriate for LAC testing to be done in patients on vitamin K antagonists (VKAs)?

- Yes
- No
- Don't know or uncertain

reset

LAC testing on low molecular weight heparin (LMWH) or unfractionated heparin (UFH)

- LAC testing should not be undertaken in patients on LMWH or UFH
- LAC testing is suitable on prophylactic dose LMWH or UFH but not on therapeutic dose LMWH or UFH
- In patients on therapeutic dose LMWH, do LAC testing during the

	trough period, i.e. at least 18 hours after last dose of LMWH ☐ In patients on prophylactic dose LMWH, do LAC testing during the trough period, i.e. at least 18 hours after last dose of LMWH ☐ Don't know or uncertain
LAC testing in patients on direct oral anticoagulants (DOACs)	☐ LAC testing should not be undertaken in patients on DOACs ☐ LAC testing may be undertaken in patients on DOACs during the trough period ☐ LAC testing may be undertaken in some circumstances in patients on DOACS during the peak period ☐ LAC testing can be performed after prehandling the sample with adsorbant or antidote (specify below) ☐ LAC testing may be undertaken only with adapted method (no aPTT/dRVVT) ☐ Don't know or uncertain
Timing of LAC testing in relation to pregnancy to have representative results *(excluding considerations in relation to effect of anticoagulation on LAC detection, which are covered elsewhere in this questionnaire)*	○ No restriction, i.e. test any time during pregnancy or postpartum ○ Defer testing until at least 6 weeks after pregnancy ○ Defer testing for another time interval after pregnancy (specify below) ○ Don't know or uncertain reset
Blood samples, collected into 0.105 - 0.109 M sodium citrate 9:1, should be double centrifuged at 2000g for 15 min at 15-22ºC to achieve a residual platelet count of < 10^9/L	○ Agree ○ Disagree ○ Don't know or uncertain reset

Plasma for LAC testing should ideally be frozen:	○ Within 2 hours of collection ○ Within 2-4 hours of sample collection ○ Within 4-6 hours of sample collection ○ Other (specify below) ○ Don't know or uncertain reset
Would you restrict LAC testing if the blood sample is haemolysed?	○ Yes ○ No ○ Don't know or uncertain reset
Would you restrict LAC testing if the sample is lipaemic?	○ Yes ○ No ○ Don't know or uncertain reset
Would you restrict LAC testing if the sample is icteric?	○ Yes ○ No ○ Don't know or uncertain reset
TESTING FOR LAC	
A prothrombin time-International Normalised Ratio (PT-INR), activated partial thromboplastin time (APTT) and thrombin clotting time/Clauss fibrinogen should be performed, to provide background information about unexpected coagulopathies and undocumented anticoagulation.	○ Agree ○ Disagree ○ Don't know or uncertain reset
LAC testing should include two phospholipid-dependent clotting tests, based on different principles, with LAC considered positive if one of the two tests gives a positive result.	○ Agree ○ Disagree ○ Don't know or uncertain reset

Which two phospholipid-dependent clotting tests would you do to detect LAC?

- [ ] A dilute Russell viper venom time test (DRVVT)
- [ ] APTT using a reagent with proven LA sensitivity
- [ ] Modified APTT
- [ ] Dilute prothrombin time
- [ ] Other (specify below)
- [ ] Don't know or uncertain

Should a mixing test for LAC using pooled normal plasma (PNP) be performed?

- ○ No
- ○ Yes - Patient:PNP ratio = 1:1
- ○ Yes - Other ratio of Patient:PNP (specify below)
- ○ Don't know or uncertain

reset

Should PNP for a mixing test ideally be:

- ○ A commercial PNP which has been platelet depleted at collection and is suitable for LAC testing
- ○ Prepared in-house
- ○ Either commercial or in-house PNP is suitable
- ○ Don't know or uncertain

reset

A confirmatory test for LAC should be performed:

- ○ Only when the screening test is prolonged
- ○ On all samples being tested for LAC
- ○ Only when screening and mixing test are prolonged
- ○ Don't know or uncertain

reset

Do you think that it is important to perform the components of the LAC tests in a specific order?

- ○ Yes
- ○ No
- ○ Don't know or uncertain

reset

An interpretative report should be provided on the LAC result.

- Agree
- Disagree

reset

CUT-OFF VALUES, CALCULATIONS AND QUALITY CONTROL FOR LAC TESTS

The plasma used for calculation of normalized ratios should ideally be:

- A commercial PNP which has been platelet depleted at collection and is suitable for LAC testing
- PNP prepared in-house
- Either commercial or in-house PNP is suitable
- A commercial LAC negative control plasma
- Don't know or uncertain

reset

If in-house pooled PNP is used for the calculation of normalized ratios, this should be prepared from:

- At least 6 healthy adult donors
- At least 40 healthy adult donors
- A larger number of adult donors (specify below)

reset

Normalization of clotting times should be derived using:

- Denominator = pooled normal plasma analysed in the same run
- Denominator = mean of reference interval
- Other (specify below)
- Don't know or uncertain

reset

The cut-off for screen, mixing and confirmation tests based on testing on plasmas from healthy donors should be:

- The value above the 99th centile of the distribution
- The value above the 97.5th centile of the distribution
- Other (specify below)
- Don't know or uncertain

reset

The manufacturer cut-off values for LA positivity should be locally validated.	○ Agree ○ Disagree ○ Don't know or uncertain reset
Do you calculate your in-house cut-off values?	○ Yes ○ No reset
In-house cut-off values (percentiles) should be calculated on how many plasmas from healthy donors?	○ <20 ○ 20-60 ○ 60-120 ○ >120 ○ Other (specify below) ○ Don't know or uncertain reset
The cut-off for the percentage correction (if used) based on testing on plasmas from healthy donors mixed with the PNP at 1:1 proportion should be:	○ The value above the 99th percentile of the distribution ○ The value above the 97.5th percentile of the distribution ○ Other (specify below) ○ Don't know or uncertain reset
How do you interpret the mixing test:	○ Rosner index (index of circulating anticoagulant) ○ Normalized clotting time ○ Both Rosner index and normalized clotting time ○ Other (specify below) ○ Don't know or uncertain reset
Should a first LAC positive result be confirmed	○ Yes, after twelve weeks

to be persistently positive on a second sample?	○ Yes, after 6 weeks ○ Yes, after (please specify below) weeks ○ No ○ Don't know or uncertain reset

LAC TESTING IN PATIENTS ON ANTICOAGULATION

If doing LAC testing in patients on VKAs:	☐ If INR < 1.5, test for LAC on undiluted plasma ☐ If INR 1.5 - < 3.0, use a 1:1 dilution of patient plasma and PNP in both screen and confirm parts of the DRVVT ☐ If INR 1.5 - < 3.0, use a 1:1 dilution of patient plasma and PNP in both screen and confirm parts of the SCT/aPTT ☐ Screen and confirm tests on 1:1 mixture with PNP regardless of INR (unless excessively raised, >8.0) ☐ Taipan Venom time test/Ecarin clotting time (TSVT/ECT) ratio ☐ DRVVT on 1:1 mixture with PNP as well as TSVT/ECT ☐ Different tests or criteria to those above (specify below) ☐ Don't know or uncertain
If doing LAC testing in patients on LMWH or UFH the plasma level of heparin should be verified, to ensure that the LAC method will be unaffected by anticoagulation, by:	☐ Anti-Xa assay for LMWH if patient on therapeutic but not on prophylactic dose LMWH ☐ Anti-Xa assay for LMWH regardless of whether patient on therapeutic or prophylactic dose ☐ APTT Ratio or anti-Xa assay for UFH if patient on therapeutic, but not on prophylactic dose UFH ☐ APTT Ratio or anti-Xa assay for UFH

	if patient on UFH regardless of whether patient on therapeutic or prophylactic dose ☐ Don't know or uncertain
If doing LAC testing in patients on DOACs:	☐ For patients on direct factor Xa inhibitors, undertake LAC testing during trough period using DRVVT together with assay for specific DOAC ☐ For patients on factor Xa inhibitors, TSVT/ECT ratio is suitable ☐ State what test you would do for LAC testing in patients on dabigatran (specify below) ☐ Don't know or uncertain
Please detail below any comments about LAC testing, either about the points covered above or any further issues.	Expand
Please fill in your email address:	

Submit