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Background: Lupus anticoagulant (LA) screens are frequently ordered in the workup of thrombophilic patients or women with fetal loss. The sensitivity and specificity of LA detection vary depending on the choice of tests, cutoff values, and results interpretation. This variation is detrimental to patient management because persistent LA positivity in patients with a history of thrombosis is a requisite for long-term anticoagulation therapy. Numerous surveys have been performed to assess the state of the art for LA diagnosis. The control plasmas used in these surveys were from LA-positive or -negative patients or were normal plasmas with monoclonal antibodies against human \( \beta_2 \)-glycoprotein I (\( \beta_2 \)-GPI) added.

Methods: A large number of laboratories were asked to test a common set of lyophilized plasmas for LA, including three normal plasmas, to which increasing amounts of affinity-purified IgG from a patient positive for anti-\( \beta_2 \)-GPI had been added, and three LA-negative plasmas: one normal, one with a coagulation deficiency, and one with heparin.

Results: Overall, 69, 68, and 59 of 70 participants were able to detect LA in plasmas with high, intermediate, and low potency (sensitivity, 99%, 97%, and 84%). Conversely, 69, 50, and 53 of 70 were able to rule out LA in the normal, heparinized, and deficient plasma (specificity, 99%, 71%, and 76%).

Conclusions: Sensitivity for LA detection is satisfactory, whereas specificity could be improved. Surveys for LA detection should be carried out on a regular basis because they may help improve performance. Plasmas containing graded amounts of affinity-purified human anti-\( \beta_2 \)-GPI may be used as a convenient source of well-characterized naturally occurring LA material.

Lupus anticoagulant (LA) screens are frequently ordered in the workup of patients at increased risk to develop thrombosis or women with otherwise unexplained fetal loss (1–5). Because of the lack of specific tests, LA is searched for according to a set of diagnostic criteria issued by the Scientific and Standardization Committee of the International Society on Thrombosis and Hemostasis (6). Accordingly, LA is likely when (a) there is a prolongation of one (or more) phospholipid-dependent tests and the prolongation persists after mixing equal amounts of patient and normal plasma (screening procedure), and (b) the prolongation is reversed after the concentration of phospholipids is increased (confirmatory procedure) (6). The sensitivity and specificity of the above procedures vary depending on the choice of tests, cutoff values, and results interpretation. This variation is detrimental to patient management because persistent positivity for LA in patients with a documented history of thrombosis is one of the requisites for long-term anticoagulation therapy (7, 8). Numerous surveys involving various numbers of laboratories have been organized and carried out over

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Nonstandard abbreviations: LA, lupus anticoagulant; \( \beta_2 \)-GPI, \( \beta_2 \)-glycoprotein I; dRVVT, dilute Russell viper venom test; KCT, kaolin clotting time; ICA, index of circulating anticoagulant; and aPTT, activated partial thromboplastin time.
the years to assess the state of the art for LA diagnosis in clinical laboratories. The materials used in these surveys ranged from lyophilized plasmas from LA-positive or -negative patients (9–13) to normal plasmas to which increasing amounts of monoclonal antibodies against human β2-glycoprotein I (β2-GPI) had been added (14).

We report here on a survey carried out in Italy within the frame of activity of the Italian Federation of Anticoagulant Clinics and the Italian Committee for Standardization of Laboratory Methods. The survey involved the participation of a large number of clinical laboratories (n = 90), which were asked to test for LA a common set of lyophilized plasmas, including three normal plasmas to which increasing amounts of affinity purified IgG from a patient positive for anti-β2-GPI had been added and three LA-negative plasmas: one normal, one with a coagulation deficiency, and one with heparin.

Materials and Methods

IgG preparation
After informed consent, venous blood was collected from a patient testing positive for anti-β2-GPI antibodies and was anticoagulated with trisodium citrate (129 mmol/L). Total IgG was isolated from plasma by means of protein A-Sepharose affinity chromatography and dialyzed against Tris buffer, pH 7.4. The concentration of total IgG (1.4 g/L) was calculated based on the absorbance at 280 nm (A₂₈₀nm), using an absorptivity of 13.8. Bovine serum albumin (10 g/L; Sigma Chemical Co.) was then added as stabilizer. After dialysis against distilled water, the material (360 mL) was diluted 1:1, 1:2, and 1:4 with distilled water and coded as samples A, B, and C. One milliliter of each preparation was dispensed into conical plastic tubes (A, B, and C) were reconstituted with 1.1 mL of each of the above solutions was then used to reconstitute the corresponding vial of lyophilized normal plasma (A, B, and C). Likewise, 1 mL each of fluids D₁–F₁ was used to reconstitute the corresponding vial of lyophilized normal plasma (A, B, and C). Likewise, 1 mL each of fluids D₁–F₁ was used to reconstitute the corresponding vial of lyophilized normal plasma (A, B, and C). Likewise, 1 mL each of fluids D₁–F₁ was used to reconstitute the corresponding vial of lyophilized normal plasma (A, B, and C).

Data analyses were based on the interpretation (either LA-positive or -negative) by participants and on raw data provided by participants. In the latter, the likelihood for the presence of a circulating anticoagulant (screening) for all methods was based on two criteria. The first was based on whether the clotting time recorded for the mixture was higher than the upper limit of the reference interval declared by the participant. The second was based on the index of circulating anticoagulant (ICA). This had been calculated as follows:

\[
ICA = \frac{R_{\text{Mixture}} - R_{\text{Normal}}}{R_{\text{Patient}}} \times 100
\]

where R is the ratio calculated by dividing the relevant clotting time by the normal clotting time.

We assumed arbitrarily that an ICA value >15% is suggestive of LA positivity. The criterion for confirmation of LA with the dRVVT methods was based on whether the ratio (dRVVTtest/dRVVTC) was >1.2. The criterion for confirmation of LA with the activated partial thromboplastin time (aPTT) with hexagonal phospholipids was based on whether the difference between the clotting times without and with hexagonal phospholipids
was greater than the cutoff value declared by the participants.

Subgroup analyses according to methods were performed whenever the numbers of participants were appropriate.

Results
When the A, B, and C preparations were reconstituted in house as described and tested for LA activity (see IgG preparation above), the ratio values were 1.73, 1.39, and 1.23, respectively, with dRVVT and 1.74, 1.43 and 1.28, respectively, with KCT.

Of the 90 participants who received sets of plasmas, ~80% returned results. aPTT and dRVVT of any kind were used in most of the laboratories. KCT was used in approximately one-half of the laboratories. Confirmatory procedures based on dRVVT with increasing concentrations of phospholipids were used by 66 laboratories. Screening and confirmatory procedures based on tests with hexagonal phospholipids were used in 13 laboratories, whereas platelet neutralization or silica clotting times at different phospholipid concentrations were used in 5 laboratories.

The numbers of different screening procedure used by the laboratories ranged from one (6%) to five (3%) with the majority using two (21%), three (30%) or more than three (44%). The numbers of different confirmatory procedures ranged from one in the majority of laboratories (71%) to two (23%) or three (6%).

Analyses Based on Results Interpretation by Participants
Sensitivity and specificity were calculated on the basis of the diagnosis provided by the each laboratory. Overall, 69, 68, and 59 of 70 participants were able to detect LA in plasmas A (high potency), B (intermediate potency), and C (low potency), respectively, which corresponds to sensitivities of 99%, 97%, and 84%. Conversely, 69, 50, and 53 of 70 were able to rule out LA in plasmas D (normal), E (heparinized), and F (depleted), respectively, which corresponds to specificities of 99%, 71%, and 76%. When we considered the sensitivity and specificity results stratified according to the numbers of testing procedures used by the laboratories, sensitivity did not increase very much with the numbers of procedures for plasmas A (high potency) and B (intermediate potency), whereas it increased from 80% (one procedure) to 95% (two or more procedures) for plasma C (low potency). Specificity was not affected by the numbers of procedures for all plasmas.

The potencies of the three LA-positive samples was interpreted correctly by approximately one-half of the participants. Namely, 56%, 53%, and 47% of the participants identified A, B, and C as high-, intermediate-, and low-potency plasma samples, respectively. Uncertain assignments (6%) were mainly restricted to plasma C (low potency; Table 1).

Table 1. Identification of the relative potency of LA-positive plasmas.

<table>
<thead>
<tr>
<th>Assignment</th>
<th>A</th>
<th>B</th>
<th>C</th>
</tr>
</thead>
<tbody>
<tr>
<td>High potency</td>
<td>55.7</td>
<td>20.0</td>
<td>0</td>
</tr>
<tr>
<td>Intermediate potency</td>
<td>27.1</td>
<td>52.9</td>
<td>37.1</td>
</tr>
<tr>
<td>Low potency</td>
<td>15.7</td>
<td>24.3</td>
<td>47.1</td>
</tr>
<tr>
<td>Negative</td>
<td>1.4</td>
<td>1.4</td>
<td>10.0</td>
</tr>
<tr>
<td>Uncertain</td>
<td>0</td>
<td>1.4</td>
<td>5.7</td>
</tr>
</tbody>
</table>

* Distribution given by participants for plasmas A (high potency), B (intermediate potency), and C (low potency).

Further Analyses Based on Raw Data Provided by Participants
Subgroup analysis according to methods. Table 2 shows results stratified according to the testing procedures used by the laboratories. Three different commercial dRVVT reagents [dRVVT1, DVVtest/DVVconfirm (American Diagnostica); dRVVT2, LACscreen/LACconfirm (Instrumentation Laboratory); dRVVT3, LA1/LA2 (Dade Behring)] were used by a reasonable number of participants to make subgroup analyses. The appropriateness of the LA status for each plasma and participant was judged with regard to the cutoff values (for screening and confirmation) as reported (see data analyses).

dRVVT as screening test. When dRVVT was used as the screening test, the overall percentages of LA-positive identification were 91%, 86%, and 65% for plasmas A (high potency), B (intermediate potency), and C (low potency), respectively, with little difference among the three reagents except for plasma C (low potency), for which dRVVT1 and dRVVT3 showed the lowest (44%) and the highest (87%) percentages of LA-positive identification. The overall percentages of LA-positive identification for the other plasmas were 0% for plasmas D (normal) and E (heparinized) and 4% for plasma F (depleted), with no difference among reagents except for plasma F, for which dRVVT2 and dRVVT3 displayed the highest percentages of identification (5% and 7%; Table 2).

dRVVT as confirmatory test. When dRVVT was used as a confirmatory test, the overall percentages of LA-positive identification were 93% for plasmas A (high potency) and B (intermediate potency) and 84% for plasma C (low potency), with little difference among the three reagents except for plasma C (low potency), for which dRVVT1 and dRVVT3 showed the lowest (67%) and the highest (100%) percentages of LA-positive identification. The percentages of LA-positive identification for the other plasmas were 9% for plasmas D (normal) and E (heparinized) and 47% for F (depleted). The relatively poor overall specificity was attributable to dRVVT2 (55%) and dRVVT3 (87%; Table 2).
When dRVVT was used as both the screening and confirmatory test, the overall percentages of LA-positive identification were 84%, 81%, and 63% for plasmas A (high potency), B (intermediate potency), and C (low potency), with differences among the three reagents for plasmas B (intermediate potency) and C (low potency), for which dRVVT1 and dRVVT2 showed the lowest percentages of LA-positive identification. The overall percentage of LA-positive identification for plasmas D (normal) and E (heparinized) was 0%, whereas for plasma F (depleted) it was 2%. For this plasma, the highest percentage (7%) was recorded with dRVVT3.

**dRVVT as screening and confirmatory test.** When dRVVT was used as both the screening and confirmatory test, the overall percentages of LA-positive identification were 84%, 81%, and 63% for plasmas A (high potency), B (intermediate potency), and C (low potency), with differences among the three reagents for plasmas B (intermediate potency) and C (low potency), for which dRVVT1 and dRVVT2 showed the lowest percentages of LA-positive identification. The overall percentage of LA-positive identification for plasmas D (normal) and E (heparinized) was 0%, whereas for plasma F (depleted) it was 2%. For this plasma, the highest percentage (7%) was recorded with dRVVT3.

**aPTT with hexagonal phospholipids.** Among the 13 laboratories that used the aPTT with hexagonal phospholipids (Staclot LA; Stago), the percentages of LA-positive identification were 86% for plasmas A (high potency), B (intermediate potency), and C (low potency), whereas they were 0% for plasmas D (normal) and 7% for plasmas E (heparinized) and F (depleted).

**Responsiveness**
The relative responsiveness of aPTT, dRVVT, and KCT for screening of LA-positive plasmas is shown in Fig. 1. In general, ratios (patient-to-normal) decreased linearly from plasma A (high potency) to plasma C (low potency) for all tests. There was a more marked difference in responsiveness among different aPTT reagents than among different dRVVT or KCT reagents (Fig. 1).

**MIXING STUDIES**
Further analyses were concerned with mixing studies (patient and normal plasma) performed by participants grouped for aPTT, dRVVT, and KCT. The percentages of LA identification obtained by participants who investigated the plasmas according to mixing studies performed with aPTT, dRVVT, or KCT are shown in Fig. 2. Overall, the ICA criterion for the aPTT was as effective as the reference interval criterion in identifying LA in plasmas A (high potency), B (intermediate potency), and C (low potency; Fig. 2). This is also valid for ruling out LA in plasma E (heparinized) or plasma F (depleted; Fig. 2). Patterns similar to the above were also observed for those participants who performed mixing studies based on dRVVT or KCT (Fig. 2).

**Discussion**
Searching for LA in patients with a past history of thrombosis is helpful for clinicians in determining the duration of anticoagulation therapy after the first episode of venous and/or arterial thrombosis. This is because persistent positivity for LA/anti-phospholipid antibodies is considered a major risk factor for recurrence (7, 8). In this respect, continuous assessment of the performance of clinical laboratories for LA detection seems appropriate. As part of the activity aimed at increasing awareness toward oral anticoagulation, the Italian Federation of Anticoagulation Clinics organized and carried out a sur-
vey on LA detection among clinical laboratories. At variance with other surveys of the same kind carried out recently, we elected to use control plasmas to which were added increasing amounts of affinity-purified IgG from a patient with strongly LA-positive antibodies against human β2-GPI. These plasmas mimic very closely the practical situation of referring an individual patient to many clinical laboratories for LA detection. Moreover, adding graded amounts of IgG to control plasmas gives the opportunity to assess the capacity of clinical laboratories and methods to estimate the degree of LA positivity. Our results show that the sensitivity of the procedures used in clinical laboratories can be considered as satisfactory, especially when the investigated plasmas present with strong LA potency.

Interestingly, the sensitivity is correlated with the amount of IgG added to the control plasmas (i.e., 99% at the highest potency and 84% at the lowest potency). Specificity was quite high (99%) for plasmas with normal clotting times, but lower for heparinized plasmas (71%) or for plasmas with a partial deficiency of coagulation fac-

Fig. 1. Responsiveness of aPTT, dRVVT, or KCT screening tests used by participants for three LA-positive plasmas and one normal LA-negative plasma.

Cotting time ratios (patient-to-normal) for plasmas A (high potency), B (intermediate potency), C (low potency), and D (normal) were calculated using the clotting time of the local normal plasma as denominator. Each point represents the mean value obtained by groups of participants using the specified commercial reagents. Groups smaller than five are not included.

Fig. 2. Percentage of LA identifications obtained by participants who investigated plasmas (see legend to Fig. 1) according to mixing studies performed with aPTT (n = 62), dRVVT (n = 58), or KCT (n = 31).

R.R., reference interval (see text for details).
tors (76%). Furthermore, specificity as assessed for the heparinized or the depleted plasma was not dependent on the numbers of testing procedures adopted by the laboratory. It is also tempting to speculate that not all participants performed the thrombin clotting time, which would have been helpful to identify heparin as the cause of the clotting time prolongation in the heparinized plasma. Although heparin-enriched plasmas do not necessarily behave as ex vivo heparinized plasmas in all LA tests, these results suggest that the two commonest causes of clotting time prolongation (heparin or coagulation factors deficiency) may give rise to false-positive LA in approximately one-fourth of cases. Thus, laboratory results cannot be used as the sole criterion to make a decision on the duration of the oral anticoagulant treatment.

Concerning the type of testing to be used in the detection of LA, dRVVT has long been considered the test of choice to detect LA (15, 16). Recently this notion was further supported by the finding that LA-positive patients are more likely to develop thrombosis as a consequence of LA when they present with an in vitro clotting time abnormality detected by the dRVVT compared with KCT (17). Although these findings need to be confirmed by further clinical studies, the specificity of dRVVT for the identification of patients more susceptible to develop thrombosis as a consequence of LA has also been supported by the findings that a small group of patients with a past history of thrombosis and antibodies against human β2-GPI are more likely to be identified with dRVVT than with other coagulation tests, such as KCT or the tissue thromboplastin inhibition test (18). However, the above conclusions have been drawn from studies using only one formulation of dRVVT reagent. Therefore, in view of the considerable variation that can be expected among the commercial reagents, generalization is not possible. A typical example emerged from our survey, in which three different commercial dRVVT reagents were used by a reasonable number of participants to make meaningful subgroup analyses. When dRVVT was used only as the screening test, the percentages of LA identification obtained with the three brands of reagents were different, especially for the weak LA-positive plasma (44%, 60%, and 87%). The percentages of LA identification obtained with the three brands of reagents for the LA-negative plasmas were 0% for one reagent and 5–7% for the other two (see Table 2). When dRVVT was used only as the confirmatory test, there were again between-reagent differences in terms of sensitivity but also in terms of specificity. In fact, the percentages of identification obtained with the three dRVVT reagents for the LA-negative plasmas were different, especially for the depleted plasma, for which two reagents displayed a considerable high rate of false-positive (55% and 87%) LA detection (see Table 2). However, it should be noted that this poor specificity was completely reversed or reduced when the algorithm used to assess results took into account dRVVT as both the screening and confirmatory tests (see Table 2). This indicates that to maximize diagnostic efficacy, the algorithm to interpret dRVVT should include results from screening and confirmatory procedures and that the two should be used in combination.

Another drawback with the laboratory procedure for LA detection is the interpretation of results stemming from mixing studies. Current guidelines issued by the Scientific and Standardization Committee of the International Society on Thrombosis and Hemostasis and other standardization authorities suggest performing such studies but do not specify how to interpret the results. Rosner et al. (19) had suggested the ICA as a reliable index to interpret mixing studies. These authors indicated that an ICA >15% may be suggestive of a circulating anticoagulant. Alternatively, results of mixing studies can be interpreted by comparing the clotting time obtained for the mixture with the clotting time corresponding to the upper end of the reference interval established for the relevant test in the laboratory. LA is likely when the former clotting time is greater than the latter. According to our results, there was little to choose between the two criteria for aPTT, dRVVT, and KCT in all but the depleted plasma, for which the ICA was more effective in ruling out LA (see Fig. 2). In this evaluation we used cutoff values for ICA corresponding to 15% regardless of the laboratories and methods (19). Presumably, the above results would change with different cutoff values. Hence, the optimal ICA value should be established in each laboratory.

In conclusion, the survey shows that the sensitivity of clinical laboratories in LA detection may be considered satisfactory, especially for plasmas with high or intermediate potency, whereas the specificity could be improved. Proficiency testing programs should be carried out on a regular basis because these may help in improving performance, especially when they are implemented in combination with the dissemination of guidelines on laboratory procedures (13). In this respect, plasmas enriched with graded amounts of affinity-purified human anti-β2-GPI antibodies or murine anti-β2-GPI monoclonal antibodies (14) may be used as sources of LA material. The former, being naturally occurring LA, would be considered more representative of the real situation than the latter. Furthermore, the relationship observed between the IgG concentration and the degree of abnormality recorded for many coagulation tests makes these plasmas potentially suitable as reference materials for the quantification of LA activity.

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References


