A Simplified Algorithm for the Laboratory Detection of Lupus Anticoagulants

Utilization of Two Automated Integrated Tests

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Abstract

Diagnosis of antiphospholipid antibody syndrome includes laboratory testing for lupus anticoagulants (LAs). Guidelines for testing have been published, but approaches vary, often incorporating multiple tests. We evaluated the performance of the activated partial thromboplastin time using 2 reagents, a dilute Russell viper venom time, and a hexagonal phospholipid assay for detecting LAs in 105 adults. Of the patients, 26 were taking anticoagulants at the time of testing. Based on findings, an algorithm was derived for optimal detection of LAs using 2 easily performed, automated, integrated test systems. Of 105 patient samples, 30 (28.6%) were positive for LAs, using the algorithm interpretive criteria. Of these 30 positive results, 10 were detected in the 26 patients taking anticoagulants. Analysis by \( \chi^2 \) showed no difference in performance of the integrated tests between samples from patients taking and not taking anticoagulants. The algorithm is offered as a means for standardization of laboratory testing for LAs.

Antiphospholipid antibody syndrome is an important clinical entity with a strong tendency for thrombosis accompanied by high morbidity and mortality. The diagnosis is made when arterial or venous thrombosis or recurrent pregnancy failure occurs in a patient with a laboratory test result persistently positive for an antiphospholipid antibody.1 Laboratory testing for antiphospholipid antibody syndrome includes enzyme-linked immunosorbent assay (ELISA), most commonly anticardiolipin and anti-\( \beta_2 \)-glycoprotein-1 antibodies, and coagulation assays for lupus anticoagulants (LAs). In general, LAs are considered more specific for the antiphospholipid antibody syndrome, whereas ELISAs are more sensitive.2 A persistently positive test result (>6 weeks) in either or both systems in association with appropriate clinical criteria constitutes a diagnosis of antiphospholipid antibody syndrome.

In 1991, the Scientific and Standardization Subcommittee for the Standardization of Lupus Anticoagulants recommended 4 criteria for detecting LAs by laboratory tests: (1) prolongation of a phospholipid-dependent clotting assay result; (2) an inhibitory effect demonstrated on normal pooled plasma; (3) evidence of phospholipid dependence, as shown by correction of the clotting time with excess phospholipids; and (4) ruling out specific inhibition of any one coagulation factor by clinical history (bleeding rather than clotting tendency) and by factor assays when indicated.4

In 1995, an update for the criteria to diagnose LAs recommended that at least 2 different types of laboratory assays, measuring different aspects of the coagulation pathway, be used for the detection of LAs. Screening tests were defined as clotting assays using only 1 dilution of phospholipids. The confirmatory study should correspond to the abnormal screening test, ie, it should measure the same aspect of the coagulation
Confirmatory tests introduce additional or modified phospholipids to a screening assay or are incorporated in integrated assay systems for the diagnosis of LAs. Integrated systems include a source of normal pooled plasma in the test sequence to correct for any factor deficiency and to compensate for the effects of oral anticoagulants. The integrated systems also include a heparin neutralizer because heparin will demonstrate an inhibitory effect when mixed with normal pooled plasma.

Integrated tests are becoming increasingly popular for the laboratory detection of LAs. Despite the published guidelines for testing for LAs and the availability of automated integrated assays, laboratory testing for LAs is nonstandard. A variety of tests are used to fulfill the guidelines, with differing interpretations as to what constitutes a positive result. Many approaches include performance of several tests in a stepwise manner to exclude the presence of LAs.

The present study was undertaken to devise a simplified method for detecting LAs, incorporating commonly used tests that could be automated and used with samples from patients taking anticoagulants. Because the activated partial thromboplastin time (aPTT) is a common screening test for LAs, we evaluated the performance of this test using 2 reagents sensitive to the presence of LAs. Integrated test systems for the dilute Russell viper venom time (dRVVT) and hexagonal phase phospholipid assay (StaclotLA, catalog No. 00594, Diagnostica Stago, Asnieres-sur-Seine, France) also were evaluated as screening and confirmatory tests for LAs. After review of results, an algorithm was derived for the detection of LAs, using only the integrated tests [Figure II].

Materials and Methods

Study Design

The study was performed January through July 2004 at University of Louisville Hospital, Louisville, KY, using samples from 105 adult patients with a variety of clinical diagnoses being evaluated for LAs [Table II]. A total of 26 were taking anticoagulants at the time of testing: unfractionated

Hexagonal phospholipid assay

Screen and confirm
Difference >8 s?

Both tests negative

No LA present

No

LA present

Yes

Repeat on 1:1 mix NPP

Normalized ratio >1.0?

Yes

Thrombosis?

Yes

No

Repeat at interval >6 wk for persistence

Platelet poor plasma

dRVVT S:C/NR

Prolonged?

Yes

No

No LA present

Repeat on 1:1 mix NPP

Normalized ratio >1.0?

Yes

Thrombosis?

Yes

No

Repeat at interval >6 wk for persistence

[Figure II] Screening and interpretation in laboratory testing for lupus anticoagulants (LAs). The testing approach begins with performance of a hexagonal phospholipid assay and a dilute Russell viper venom time (dRVVT) screen on platelet-poor plasma. In the hexagonal phospholipid assay, a shortening of the clotting time by >8 seconds after addition of hexagonal phase phospholipids is considered positive for LA. With the dRVVT, if screening result is prolonged, a normalized ratio (NR) is calculated; if the NR is >1.0, test plasma is mixed in equal parts with normal pooled plasma (NPP), and the calculation is repeated. Samples with an NR >1.0 with mixed plasma are positive for LA. Results are reported as negative for LA if both test systems are negative. S:C, screen/confirm ratio.
heparin, 14; warfarin, 9; and low-molecular-weight heparin (enoxaparin sodium), 3. The study population included 41 men and 64 women with an average age of 44.2 years.

All 105 samples were tested for LAs using 2 aPTT reagents designed to be sensitive to LAs and with the dRVVT and StaclotLA. However, 14 samples from patients taking unfractionated heparin were analyzed with the results of the dRVVT and StaclotLA only owing to the inhibitory effect of unfractionated heparin on the aPTT and mixing study. In 2 of the patient samples (both from patients taking unfractionated heparin), no clot was detected in the StaclotLA, and for these 2 samples only, a heparin level was determined.

**Interpretation**

If the aPTT result was prolonged (in either reagent system), the abnormal assay was repeated with a 1:1 mixture of the plasma with normal pooled plasma. If the test system did not show immediate correction of the result into the reference range, the test was considered to have demonstrated the presence of an inhibitor. The sample was considered positive for LAs only if results of further testing with the dRVVT and/or StaclotLA were positive. Abnormal results obtained by the dRVVT normalized ratio (NR) on neat plasma were tested further with a dRVVT mixing study. The dRVVT was considered positive for LAs only if the NR was more than 1.0 in the mixing study. All 30 of the samples positive for LAs by the dRVVT and/or StaclotLA were evaluated further by a factor VIII assay to eliminate the possibility of a specific factor VIII inhibitor. Clinical correlation was undertaken in patients with positive results for LAs to confirm a thrombotic tendency.

All tests were performed on the BCS automated coagulation analyzer (Dade Behring, Marburg, Germany). Reference ranges were established on samples from healthy volunteers (35 for the aPTT and 30 for the PTT-LA, dRVVT, and StaclotLA) at ±2 SD from the mean.

**Sample Preparation**

Blood was collected in tubes containing 3.2% sodium citrate as an anticoagulant in a 9:1 ratio of blood to sodium citrate solution, using 19- to 21-gauge needles and minimal stasis. Platelet-poor plasma (platelet count, <10 × 10³/µL [10 × 10⁹/L]) was harvested on receipt by centrifuging the specimen for 180 seconds at 8,500 rpm (StatSpin Express 2, Iris, Chatsworth, CA). Plasma was removed with a plastic pipette and spun again for 180 seconds at 8,500 rpm. A monthly quality assurance check of 4 centrifuged samples is performed to ensure that spun plasma has a platelet count of less than 10 × 10³/µL (<10 × 10⁹/L). The prepared platelet-poor plasma was tested within 4 hours or frozen at −20°C for up to 1 week and thawed once for testing. All assays were performed using platelet-poor plasma.

**Table 1**

<table>
<thead>
<tr>
<th>Clinical Diagnosis</th>
<th>No. of Patients Positive for LA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stroke (n = 44)</td>
<td>10</td>
</tr>
<tr>
<td>Deep venous thrombosis/ pulmonary embolus (n = 12)</td>
<td>7</td>
</tr>
<tr>
<td>Transient ischemic attack or syncope (n = 9)</td>
<td>2</td>
</tr>
<tr>
<td>Traumatic injury (n = 4)</td>
<td>2</td>
</tr>
<tr>
<td>Obstetric problem or recurrent abortion (n = 14)</td>
<td>2</td>
</tr>
<tr>
<td>Upper extremity thrombosis (n = 2)</td>
<td>1</td>
</tr>
<tr>
<td>Migraine (n = 2)</td>
<td>1</td>
</tr>
<tr>
<td>Acute renal failure (n = 1)</td>
<td>1</td>
</tr>
<tr>
<td>Malignant neoplasms (n = 2)</td>
<td>1</td>
</tr>
<tr>
<td>Thrombocytopenia or pancytopenia (n = 2)</td>
<td>1</td>
</tr>
<tr>
<td>Antiphospholipid antibody syndrome (n = 1)*</td>
<td>1</td>
</tr>
<tr>
<td>Seizure disorder (n = 1)</td>
<td>1</td>
</tr>
<tr>
<td>Pneumonia (n = 2)</td>
<td>0</td>
</tr>
<tr>
<td>Colitis (n = 2)</td>
<td>0</td>
</tr>
<tr>
<td>Bruising or hemorrhage (n = 4)</td>
<td>0</td>
</tr>
<tr>
<td>Multiple sclerosis or myasthenia gravis (n = 2)</td>
<td>0</td>
</tr>
<tr>
<td>Subclavian steal syndrome (n = 1)</td>
<td>0</td>
</tr>
</tbody>
</table>

LA, lupus anticoagulant.

* Patient with recurrent transient ischemic attacks and repeated positive testing for anticardiolipin antibody, IgG type, and LA (dilute Russell viper venom time and/or StaclotLA; for proprietary information, see the test.)

**Reagents**

The PTT-FSL reagent (Dade Actin FSL aPTT, catalog No. B4219-2, Dade Behring) contains purified soy and rabbit brain phosphatides in 1 × 10⁻⁴ èllagic acid with buffer. The reagent is designed for use in performing the aPTT as a global screening procedure to detect coagulation abnormalities in the intrinsic pathway and to monitor heparin therapy. The reagent is considered to have increased sensitivity to lupus-like inhibitors, but a confirmatory test to demonstrate phospholipid dependency is needed to establish the inhibitor as an LA. The laboratory reference range for the assay was 25.0 to 33.4 seconds.

The kit for PTT-LA (lupus anticoagulant–sensitive aPTT reagent, catalog No. 00599, Diagnostica Stago) is intended for determination of the aPTT by adding a particulate activator (silica) together with cephalin prepared from rabbit cerebral tissues, which is sensitive for detecting LAs. The presence of circulating anticoagulants of the antiprothrombinase type (LA) leads to prolongation of the aPTT, and this prolongation is longer with the PTT-LA reagent than with other regular aPTT reagents that are not sensitized to the LA. As with other aPTT reagents, the test result will be prolonged secondary to factor deficiencies, specific coagulation inhibitors, and the presence of heparin, as well as the presence of LAs, and further coagulation tests are needed to define the cause of the prolongation. The laboratory reference range was 26.3 to 34.3 seconds.

**Normal Pooled Plasma**

George King Normal Pooled Plasma (catalog No. 0010-1, George King Biomedical, Overland Park, KS) is composed
of fresh frozen citrated human plasma from healthy donors and is intended for use in coagulation assays.

**Dilute Russell Viper Venom Time**

The kit containing LA-1 (screening reagent) and LA-2 (confirmatory reagent) (code No. OQGP, Dade Behring) is a simplified 1-stage dRVVT for the specific detection of LAs by using Russell viper venom to directly activate factor X and to confirm the phospholipid-dependent nature of an inhibitor. The LA-1 screening reagent contains Russell viper venom, phospholipids, antiheparin agents, calcium, buffers, and stabilizers. The LA-2 confirmation reagent is a phospholipid-rich reagent. The antiheparin agents in both reagents can neutralize up to 1.0 U/mL of heparin in the sample, according to the manufacturer. The LA-1 screening test and the LA-2 confirmatory test may be repeated after mixing the test plasma with an equal amount of normal pooled plasma to correct prolongation secondary to factor deficiency rather than LA. The laboratory reference range for LA-1 was 32.7 to 47.2 seconds and for LA-2, 28.1 to 34.3 seconds.

With the LA-1 screening test, if the result of the dRVVT is within the normal range, the screening test is considered negative for LA. Samples showing a prolongation of the dRVVT with the LA-1 reagent were studied further by repeating the dRVVT using the LA-2 reagent. The BCS instrument automatically calculated a screen/confirm ratio from the 2 results, and an NR was calculated manually. The NR is recommended to correct for differences in instrument-reagent combinations and to improve discrimination between normal and low-positive LA samples. The NR is calculated by introducing the laboratory’s mean normal time for the dRVVT for each reagent used, according to the following formula:

\[
NR = \frac{\text{LA-1 Patient Result/LA-1 Mean Normal Time}}{\text{LA-2 Patient Result/LA-2 Mean Normal Time}}
\]

The laboratory reference range for an NR was established as 1.0 or less by using samples from 30 healthy volunteers.

If the initial NR was greater than 1.0, the LA-1 screening and LA-2 confirmatory tests were repeated after mixing the test plasma with an equal part of normal pooled plasma, and the NR was recalculated. Only ratios more than 1.0 after mixing test plasma with normal plasma were considered positive for LAs by the test system.

**StaclotLA for Detection of Lupus Anticoagulants**

Testing was performed according to the manufacturer’s directions. The test kit is designed for the qualitative detection of LA in plasma by the use of hexagonal HII phase phospholipid molecules. Testing involves 2 steps. The test plasma is allowed to incubate at 37°C with (tube 2) and without (tube 1) hexagonal phase phosphatidylethanolamine, then an aPTT is performed using an LA-sensitive reagent. If a prolonged screening result is obtained (results for tube 1 >8.0 seconds longer than results for tube 2), the test is considered positive for LAs. The procedure included a reagent in each of the tubes that contains normal plasma and a heparin neutralizer, which makes the test system insensitive to a heparin level up to 1.0 U/mL, according to the manufacturer.

**Factor VIII Assay**

For the factor VIII assay, Standard Human Plasma (catalog No. ORKL13, Dade Behring), Factor VIII–Deficient Plasma (catalog No. OTXW17, Dade Behring), and Actin FSL aPTT reagent (Dade Behring) were used. Three dilutions (1:10, 1:20, and 1:40) were prepared using factor VIII–deficient plasma, and an aPTT was performed on each dilution. The aPTT result was used to determine the factor activity using a reference curve obtained with dilutions of standard human plasma. Activity considered adequate for hemostasis is 30% to 40%.

**Heparin Level**

To measure the heparin level, the Berichrom Heparin Assay Kit (catalog No. OWLD11, Dade Behring) was used. The amount of heparin was determined from the anti–factor X activity expressed by the antithrombin-heparin complex formed in plasma. Dade Behring Standard Human Plasma was used to construct a calibration curve using unfractionated heparin. If results were more than 1.0 IU/mL, the test was repeated after making a 1:2 dilution using George King Normal Pooled Plasma.

**Results**

A summary of test results for the positive samples is given in Table 2. The aPTT result using the Dade FSL performed on 91 patients (the 14 patients taking unfractionated heparin were not included) was prolonged and showed an immediate inhibitory effect with the mixing study (listed as positive in Table 2) in 4 samples (4.4%). The PTT-LA was prolonged with an immediate inhibitory effect in 5 samples (5.5%).

The aPTT without an immediate mixing study also was evaluated as a screening test for the StaclotLA from patients taking and not taking anticoagulants. Of 20 samples showing a prolonged aPTT with the Dade FSL reagent, 9 (45%) were positive with the StaclotLA. Of 29 samples showing prolongation with the PTT-LA, 13 (44.8%) were positive with the StaclotLA. Conversely, only 13 (46.4%) of 28 samples testing positive for LAs by the StaclotLA showed a prolongation by the PTT-LA test result. From the findings, the aPTT was considered not to be an optimal screening test for LAs in the algorithm.
Results of the dRVVT and StaclotLA assays showed 30 (28.6%) of 105 patient samples to be positive for LAs. All of these had a factor VIII activity within the reference range or more. The most frequently positive test was the StaclotLA, being positive in 28 cases (26.7%). One additional positive StaclotLA result was determined to be a false-positive result owing to an acquired factor VIII inhibitor and, thus, was excluded from the study results. Only 3 (2.9%) of 105 dRVVT results were positive; however, in 2 cases, the dRVVT was the only positive test result. Analysis with \( \chi^2 \) showed the dRVVT added significantly to the identification of LAs, compared with zero (\( P = .03 \)).

Twelve patients had a dRVVT NR of more than 1.0 calculated from the initial screen/confirm ratio, with 9 subsequently having an NR of 1.0 or less with a dRVVT mixing study. Of these 9 samples, 7 were positive for LAs by the StaclotLA, indicating loss of sensitivity in the dRVVT system with routine calculation of an NR on the mixing study. Of 9 patients with samples testing positive for LAs by the initial dRVVT NR, 4 were taking oral anticoagulants, and the initial elevated ratio was attributed to factor deficiency, although 3 of the patients had positive results for LAs with the StaclotLA. Similarly, 4 patients in the nonanticoagulated group showed an initially positive NR of more than 1.0, but recalculation of the NR after mixing with normal pooled plasma was 1.0 or less. All 4 of these patients had positive results with the StaclotLA. One patient was taking unfractionated heparin, and a mixing study corrected the LA-1 and the LA-2 into the reference intervals, with the StaclotLA negative for LAs.

**Results From Anticoagulated Patients**

Samples from 2 patients taking unfractionated heparin showed “no clot detected” by the StaclotLA, with and without

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**Table 3**

Patterns of Positivity for LAs in Patients Taking or Not Taking Anticoagulants*

<table>
<thead>
<tr>
<th>Pattern of Positivity</th>
<th>PTT-FSL</th>
<th>PTT-LA</th>
<th>dRVVT</th>
<th>StaclotLA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Taking anticoagulant (n = 10)†</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Warfarin</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>Negative</td>
<td>Negative</td>
<td>Negative</td>
<td>Positive</td>
</tr>
<tr>
<td>2</td>
<td>Negative</td>
<td>Positive</td>
<td>Negative</td>
<td>Positive</td>
</tr>
<tr>
<td>1</td>
<td>Negative</td>
<td>Negative</td>
<td>Positive</td>
<td>Positive</td>
</tr>
<tr>
<td>Enoxaparin sodium</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>Negative</td>
<td>Negative</td>
<td>Negative</td>
<td>Positive</td>
</tr>
<tr>
<td>Unfractionated heparin</td>
<td>—</td>
<td>—</td>
<td>Negative</td>
<td>Positive</td>
</tr>
<tr>
<td>3</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Not taking anticoagulant (n = 20)‡</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>Negative</td>
<td>Negative</td>
<td>Negative</td>
<td>Positive</td>
</tr>
<tr>
<td>2</td>
<td>Positive</td>
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<tr>
<td>1</td>
<td>Positive</td>
<td>Negative</td>
<td>Positive</td>
<td>Positive</td>
</tr>
</tbody>
</table>

dRVVT, dilute Russell viper venom time; LAs, lupus anticoagulants; PTT, partial thromboplastin time.

* Analysis by \( \chi^2 \) showed no significant difference in the performance of the dRVVT between patients treated with anticoagulants and those not taking anticoagulants (\( P = 1.0 \)). Similarly, no difference was seen in the performance of the StaclotLA between the groups (\( P = .61 \)). A \( P \) value <.05 was considered significant. For proprietary information, see the text.

† Of the cases tested, 7 were negative by all 4 tests.

‡ Of the cases tested, 59 were negative by all 4 tests.

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**Table 4**

Number of Prolonged Results for dRVVT at Each Testing Step: Pre-dRVVT and Post-dRVVT Mixing Studies*

<table>
<thead>
<tr>
<th>First NR, &gt;1.0</th>
<th>Second NR, &gt;1.0</th>
<th>Interpreted as Positive for LA</th>
</tr>
</thead>
<tbody>
<tr>
<td>LA-1</td>
<td>LA-2 Mix</td>
<td></td>
</tr>
<tr>
<td>With warfarin (n = 5)</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>Without warfarin (n = 6)</td>
<td>6</td>
<td>6</td>
</tr>
<tr>
<td>With other anticoagulants (n = 1 [heparin])</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Total (n = 12)</td>
<td>12</td>
<td>12</td>
</tr>
</tbody>
</table>

dRVVT, dilute Russell viper venom time; LA, lupus anticoagulant; NR, normalized ratio; PTT, partial thromboplastin time.

* LA-1 constitutes initial screening dRVVT, and LA-2, the confirmatory reagent with increased phospholipids. LA-1/LA-2 mix were performed on plasma mixed with an equal amount of normal pooled plasma. Initial dRVVT screening and confirmatory results indicated 10 samples to be positive; only 3 remained positive after mixing study and recalculation of the NR (final NR, 1.94, 1.11, and 1.07). For proprietary information, see the text.

† One patient’s result was positive for LA by dRVVT and StaclotLA.
phospholipids, and heparin levels were 1.28 U/mL and 0.87 U/mL. These 2 samples were negative by the dRVVT and were considered negative for LAs, although the StaclotLA results were not interpretable.

Although the number of positive samples determined by the dRVVT was small, \( \chi^2 \) analysis showed no significant difference in the dRVVT performance in patients treated with anticoagulants (all anticoagulants) and those not taking anticoagulants (\( P = 1.0 \)). Similarly, no difference was seen in the performance of the StaclotLA between samples from patients treated with anticoagulants (all anticoagulants) and those not taking anticoagulants (\( P = .61 \)). Statistical testing was based on contingency tables using the likelihood ratio, performed using JMP statistical software (SAS Institute, Cary, NC), with 2-tailed test (\( P < .05 \) considered significant).

**Discussion**

LAs are antibodies directed against a spectrum of phospholipid-binding proteins. The heterogeneity of these antibodies effects different responses among assays to detect LAs.\(^7\)

This was documented in the present study by the marked differences in results among 4 commonly used laboratory tests for LAs. Furthermore, the study confirmed that no single assay is positive for all LAs, necessitating testing by 2 or more assays before reporting a patient sample as negative for LAs. For this reason, the algorithm uses the dRVVT and the StaclotLA for routine testing for LAs.

The study found 28.6% of the patient samples to be positive for LAs by the dRVVT, StaclotLA, or both. The incidence of LAs is difficult to determine through review of literature because a variety of laboratory tests are used to detect the antibodies. In healthy subjects, LAs may be found in 1% to 5%\(^8\). The percentage is higher, however, when LA testing is performed in selected patient populations. The percentage of LAs in patients with deep vein thrombosis or pulmonary embolism was reported as 16.0% in a study by Eschwège et al\(^9\) (using the aPTT and StaclotLA) and 8.5% in a study involving deep vein thrombosis by Simioni et al\(^10\) (using the PTT-LA and dRVVT as screening tests, with a platelet neutralization procedure and StaclotLA as confirmatory tests). A study by Leebeek et al\(^11\) found LAs in 12.1% of patients with upper-extremity deep vein thrombosis (using aPTT and dilute prothrombin time mixing studies). The percentage of LAs in patients with systemic lupus erythematosus may range from 34% to 50% or, possibly, greater, depending on the laboratory test used to detect the antibodies.\(^7,8\)

Although LAs often are noticed clinically by the unexplained prolongation of an aPTT result, the aPTT is not an optimal screening test for detecting LAs. The insensitivity of the aPTT was demonstrated in the present study because only about one half (46.4%) of the samples testing positive for LAs by the StaclotLA showed a prolongation of the aPTT result with either testing reagent. Approximately one half of the samples positive for LAs by the StaclotLA would have been regarded as negative for LAs if an aPTT test alone had been used. Finding a prolonged aPTT result and demonstrating an inhibitory effect on mixing with normal pooled plasma is not diagnostic of LAs. The aPTT is affected by the presence of heparin and inhibitors against specific coagulation factors, and the sample must be tested further for LAs using a confirmatory assay, such as the StaclotLA.

The dRVVT is a frequently used assay for the detection of LAs, and integrated systems that include a confirmatory step often are used. In the present study, the dRVVT result was positive in only 3 samples, 2 of which would not have been positive for LAs if only the aPTT and StaclotLA had been used. This demonstrates the need for use of 2 or more assays for the detection of LAs as stated in the testing guidelines because no single assay detects all LAs. The observation that the StaclotLA was positive in 7 of 9 samples negative by the dRVVT after mixing with normal pooled plasma suggests that false-negative results were occurring with the dRVVT. Routine performance of a dRVVT mixing study might have masked the effect of a weak inhibitor, giving a false-negative result, as described with aPTT mixing studies.\(^12\) Another explanation might be that the dRVVT screen result was prolonged by factor deficiencies or oral anticoagulants, and the confirmatory portion of the test resulted in a false-positive result, corrected by the addition of normal pooled plasma. Review of data indicates that routine calculation of the NR after the dRVVT mixing study decreased the number of positive test results (Table 3). The dRVVT result, similar to the aPTT result, may be prolonged by coagulation factor deficiencies, oral anticoagulants, and inhibitors to specific coagulation factors, including factor VIII or IX inhibitors or deficiencies.\(^13\)

For the study, the value considered positive for LAs after calculating the dRVVT NR was established as more than 1.0. The recommended NR suggested by the manufacturer for a positive test is more than 1.2. If the cutoff value from the manufacturer had been used, only 1 of the 105 patient samples in the study would have been positive for LAs by the dRVVT because the NRs for the 3 samples positive by the dRVVT were 1.94, 1.11, and 1.07. The importance of locally derived reference ranges for the dRVVT and standardized calculation of results was noted in a study by Gardiner et al,\(^14\) in which an arbitrary ratio of less than 1.1 as the cutoff value for normal was considered inappropriate. Mean test/confirm ratios for the dRVVT in healthy subjects ranged from 0.92 to 1.25. The study determined that the way in which the screen and confirmatory results were analyzed had a major impact on the interpretation of dRVVT results.

The StaclotLA is an integrated test system for LAs, but also may be used as a confirmatory test for aPTT-based
screening assays. The study demonstrated that the detection rate for LAs in an institution is dependent on the sequence in which assays are used. If the StaclotLA had been used only as a confirmatory test for the aPTT, the overall percentage of positive test results would have been reduced to 7%, even if both reagents had been used for screening. For this reason, the StaclotLA is used as a screening and confirmatory test in the algorithm.

Although the assay contains a heparin neutralizer, 2 of the samples from patients taking unfractionated heparin therapy failed to have a clot detected. In 1 sample, the heparin level of 1.28 U/mL exceeded the capacity of the neutralizer, which is stated by the company as being able to neutralize up to 1.0 U/mL of heparin. In the second sample, however, the heparin level was 0.87 U/mL, indicating a possible effect on the system by unfractionated heparin at levels less than 1.0 U/mL. The positive StaclotLA result found in 1 patient taking enoxaparin (Lovenox) was considered to be a true-positive, although the aPTT results using both reagents in the case were within reference ranges, and the dRVVT screen (LA-1) was within the reference range.

The effect of Lovenox and other new antithrombin pharmaceutical agents on the StaclotLA has not been well described, and further study of this area is needed. Storbeck et al studied the effect of low-molecular-weight heparins (reviparin and enoxaparin sodium) on the StaclotLA using in vivo and ex vivo experiments and found no false-positive results due to low-molecular-weight heparin. Similarly, our inhouse study using 26 samples from patients taking enoxaparin sodium showed no relationship between the results of the StaclotLA in seconds and the heparin level in units per milliliter (data not shown).

The StaclotLA result may be positive in the presence of an acquired factor VIII inhibitor. During the study, a patient with a bleeding tendency was evaluated for LAs. The patient’s aPTT result was prolonged in both reagent systems and showed an inhibitory effect on normal pooled plasma, the dRVVT screen (LA-1) was within the reference range, and the StaclotLA result was positive. Because of the history, factor assays were performed with the finding of a very low factor VIII activity, and a factor VIII inhibitor was demonstrated in a Bethesda assay. This underscores the need to review the clinical history of patients being evaluated for LAs, so mixing studies, factor assays, and Bethesda assays may be performed in patients with history of bleeding or in patients whose clinical presentation is not definitive for a bleeding tendency or a tendency for thrombosis. Treatment for specific (neutralizing) coagulation inhibitors differs from that of LAs, making correct diagnosis essential. Because of this, review of patient history and performance of additional coagulation tests when LAs are detected by the dRVVT or StaclotLA is part of the algorithm.

Laboratory testing for LAs is not standardized, and various combinations of screening, confirmatory, and integrated test systems can be used. Some of the tests used traditionally to detect LAs, including the aPTT and dRVVT, may be insensitive to the presence of LAs, necessitating the use of multiple assays before reporting a patient sample as negative for LAs. Based on our experience with screening for LAs, an algorithm is presented that uses 2 test systems for maximum LA detection. Integrated test procedures allow screening of patients for LAs while they are taking anticoagulants because statistical analysis indicated no difference in performance of the tests between anticoagulated and nonanticoagulated patients.

In the algorithm, testing for LAs begins with the performance of the StaclotLA and the dRVVT. Samples showing initial prolongation of the dRVVT NR with the screening and confirmatory reagents are repeated after mixing the test plasma with an equal part of normal pooled plasma with recalculation of the NR. Samples showing an NR of more than 1.0 after mixing with normal pooled plasma are considered positive for LAs. Most of the positive results from the algorithm will be in the StaclotLA system, and the dRVVT as a second test will detect a few additional positive results. Because both of the methods are integrated assays, the first 3 criteria for laboratory detection of LAs are fulfilled. Reviewing patient history and clinical presentation will allow for detection of specific coagulation factor inhibitors by factor and inhibitor assays and fulfill the fourth criterion. Some LAs may be transient and associated with inflammatory events, and all positive cases should be repeated by the same testing approach after 6 weeks to detect persistence of the antibody, which is considered more clinically significant than the finding of LAs at one point in time. Because the algorithm uses readily available and automated tests, it offers an approach that could be standardized between laboratories testing for LAs. Individual laboratories would need to establish reference ranges and interpretive criteria for their particular hexagonal phase phospholipid and dRVVT methods.

Cost of Testing for LA

The aPTT is considered to be a readily available and inexpensive screening test for LAs, despite its limitations. Often, laboratory testing schemes for LAs incorporate several screening tests before reporting a negative result for LAs, leading to additional expense for LA testing. In our laboratory, the cost per test for the aPTT with the FSL reagent is $12.30 and for the PTT-LA, $21.83 (doing an immediate 1:1 mixing study adds $4.65). A 2-hour incubated mixing study incurs a cost of $57.05, which does not include the initial aPTT test. A thrombin time to check for the presence of heparin is $20.21 and is a routine part of the aPTT mixing study. Addition of a heparin neutralizer adds $5.90 to the procedure.
We have reduced the cost of using the algorithm for LA testing by batching the samples. In our institution, LA testing is performed once a week, with an average number of 8 samples per run. The cost to perform a dRVVT screen (LA-1 only) is $19.77, an NR is $33.48 (LA-1 and LA-2), and a StaclotLA is $14.25 (total = $34.02 for a negative result by both tests). The cost of a positive result in both test systems (dRVVT and StaclotLA) is $71.61. Time and money are conserved by using the algorithm because the tests are automated and interpretation is straightforward, with interpretative comment reported as part of results.

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