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Preliminary Evaluation of a Novel Lupus Anticoagulant Activity Assay Unaffected by Anticoagulants

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ABSTRACT

Background: In Japan, clinical laboratories commonly assess lupus anticoagulant (LA) activity using phospholipid-dependent coagulation assays as part of the diagnostic evaluation of antiphospholipid syndrome (APS). However, LA detection involves complex procedures and may yield false-positive results in patients receiving anticoagulant therapy, such as direct oral anticoagulants (DOACs). This study aimed to clarify the effects of anticoagulants on guideline-based LA testing (diluted Russell's viper venom time (dRVVT) and/or LA-sensitive activated partial thromboplastin time (APTT)) and to evaluate whether they interfere with accurate LA assessment. We also explore a simple one-step method to minimize anticoagulant interference and improve diagnostic efficiency.

Methods: We developed a one-step method for assessing LA activity by calculating the LA-sensitivity ratio (LA-SR), defined as the ratio of APTT measured using high- and low-sensitivity APTT reagents. The clinical utility of the LA-SR assay was evaluated using plasma samples from 57 patients with systemic lupus erythematosus, 74 patients with prolonged APTT, and DOAC-spiked plasmas.

Results: The method showed agreement with the guideline-based LA testing. Of the 57 cases, 14 were LA-positive and 38 were LA-negative according to guideline-based testing. Among the 43 cases classified as LA-negative, five showed elevated LA-SR values despite negative guideline-based results. The LA-SR assay was minimally affected by DOACs, warfarin, heparin, or coagulation factor deficiencies.

Conclusion: This LA-SR-based method may serve as a useful screening tool for the evaluation of LA activity. A negative LA-SR result may help identify samples unlikely to have LA activity, while positive results should be interpreted cautiously and confirmed using guideline-based testing.

1 | Introduction

Antiphospholipid syndrome (APS) is an autoimmune disease characterized by arterial and venous thrombosis as well as pregnancy complications, associated with the presence of various antiphospholipid antibodies (aPLs) that target phospholipids and phospholipid-binding proteins in the patient's blood [1–3]. The laboratory diagnosis of APS is complex and requires the

Abbreviations: aCL, anti-cardiolipin antibodies; aPLs, antiphospholipid antibodies; APS, antiphospholipid syndrome; APTT, activated partial thromboplastin time; APTT-LA, activated partial thromboplastin time-based LA; aβ_2GPI, anti-β_2_glycoprotein I antibodies; DOACs, direct oral anticoagulants; dRVVT-LA, diluted Russell's viper venom time-based LA; ELISAs, enzyme-linked immunosorbent assays; FXa, activated factor X; ISTH, International Society on Thrombosis and Haemostasis; LA, lupus anticoagulant; LA-SR, LA-sensitivity ratio; NPP, normal pooled plasma; NVAF, nonvalvular atrial fibrillation; SD, standard deviation; SLE, systemic lupus erythematosus; SSC, Scientific and Standardization Committee.

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detection of at least one persistently positive aPL, including anti-cardiolipin antibodies (aCL) IgG/IgM and anti-β_2 glycoprotein I antibodies (aβ_2GPI) IgG/IgM using enzyme-linked immunosorbent assays (ELISAs) or the assessment of lupus anticoagulant (LA) activity through multiple coagulation assays, in accordance with established classification criteria [4, 5].

Clinical studies have demonstrated that LA activity is the most significant risk factor for thrombotic events in APS [6, 7], highlighting the clinical importance of its accurate detection. Although LA activity can be assessed via coagulation tests [8, 9], it should be noted that international guidelines recommend the use of both the diluted Russell's viper venom time-based assay (dRVVT-LA) and the activated partial thromboplastin time-based assay (APTT-LA) for comprehensive LA detection [10]. Additionally, both APTT- and dRVVT-based assay require a multi-step process, including screening, mixing, and confirmation steps [10]. dRVVT-based assays may be operationally more straightforward in routine laboratory practice, as dRVVT-LA kits allow screening and confirmatory steps to be performed within the same assay system [10]. Reliance on a single assay without appropriate confirmatory procedures carries the risk of missed diagnoses. This complexity places a considerable burden on clinical laboratories, and the need for simplification remains a challenge. However, in routine clinical practice, LA testing using coagulation-based assays is often emphasized as part of the diagnostic evaluation APS. This reflects practical considerations in laboratory workflows and assay availability, rather than strict adherence to classification criteria. Previous reports have described differences between Japanese laboratory practices and international recommendations for LA detection, including variations in testing algorithms and interpretation [11].

Furthermore, direct oral anticoagulants (DOACs), widely used worldwide for the prevention and treatment of thromboembolic diseases such as deep vein thrombosis, pulmonary embolism, and stroke in atrial fibrillation [12, 13], have been shown to interfere with guideline-based LA testing (dRVVT and/or LA-sensitive APTT), complicating the accurate assessment of LA activity during anticoagulation therapy [14–17].

In this study, we aimed to clarify the influence of four DOACs (dabigatran, rivaroxaban, edoxaban, and apixaban) on guideline-based LA testing (dRVVT and LA-sensitive APTT). Additionally, we sought to establish a novel LA assay method that minimizes DOAC interference and simplifies the testing process compared to guideline-based LA testing.

2 | Materials and Methods

2.1 | Study Participants

2.1.1 | Plasma From Healthy Volunteers for Determination of Cutoff Value

Any volunteers showing signs or a history of hemostatic disorders were excluded. Plasma used to determine the cut-off value was isolated from blood samples collected from 41 healthy volunteers (13 males, aged 22–60, mean age 31.5; and 28 females, aged 21–59, mean age 33.6) using sterile Benoject II vacuum blood collection tubes (Terumo Corporation, Japan) containing 3.2% (0.109 mol/L) trisodium citrate as an anticoagulant. Platelet-poor plasma (PPP) was prepared by double centrifugation at 1500×*g* for 15 min at room temperature to ensure adequate removal of platelets.

Healthy volunteers were defined as individuals who did not meet any of the following exclusion criteria: (i) Those who were regularly visiting the hospital due to chronic diseases; (ii)Those who had hospitalized for acute illness or surgery within the past month; (iii) Those who had taken any medications within the past 3 days; (iv) Those who consumed excessive amounts of alcohol the day before blood collection; (v) Those who consumed a meal within 3 h prior to blood collection; (vi) Those who had awareness of being unwell at the time of blood collection.

2.1.2 | Plasma From Systemic Lupus Erythematosus Patients

We used plasma samples from 57 patients with systemic lupus erythematosus (SLE), a population in which antiphospholipid antibodies are relatively common, allowing efficient identification of LA-positive samples. In this study, the LA-positive or LA-negative status was determined in accordance with current guidelines using guideline-based LA testing (dRVVT and LA-sensitive APTT assays), performed according to the recommended three-step procedure (screening, mixing, and confirmatory tests) as described in the current international guidelines of the International Society on Thrombosis and Haemostasis (ISTH)—Scientific and Standardization Committee (SSC) [10].

Briefly, samples showing prolonged clotting time in the screening assay were further evaluated using a mixing study and a phospholipid-dependent confirmatory test. APTT-LA confirmation testing was performed using the Staclot LA assay (Diagnostica Stago), based on the hexagonal-phase phospholipid neutralization principle. A correction value (CT1−CT2) ≥ 8 s was interpreted as LA-positive according to the manufacturer's instructions.

For the dRVVT-based assay, LA was interpreted according to the manufacturer's instructions and current ISTH-SSC recommendations. Briefly, clotting times were measured using both screening and confirmatory reagents, and the ratio of screen to confirm was calculated.

Based on the combined results of these assays, samples were categorized as LA-positive or LA-negative in accordance with guideline recommendations. The study group included 6 males (31–78 years, mean age 47) and 51 females (8–81 years, mean age 45.6).

2.1.3 | Plasma From APTT-Prolonged Patients

Plasma samples from 74 patients with prolonged APTT were randomly selected from specimens submitted for routine

coagulation testing at Osaka University Hospital and analyzed (39 males [33–81 years, mean age 62.2]; 35 females [14–93 years, mean age 72.9]). The underlying causes of APTT prolongation included coagulation factor deficiency ($n = 33$), anticoagulant therapy with warfarin ($n = 10$) or heparin ($n = 15$), and cases of unknown etiology ($n = 16$).

2.1.4 | Plasma From Healthy Volunteers for Pooled Plasma

Plasma was separated from blood collected from eight healthy volunteers (1 male [59 years old] and 7 females [21–23 years old, mean age 22.3]) using sterile Benoject II vacuum blood collection tubes. Equal volumes of plasma from each individual were mixed to prepare pooled plasma from healthy volunteers.

2.1.5 | Plasma From LA-Positive Patients

Plasma samples were collected from four LA-positive patients (1 male [79 years old] and three females [71–73 years old, mean age 72.0]). All patients were classified as LA-positive based on the ISTH-SSC guideline-recommended three-step APTT-based assay [10], comprising screening, mixing, and confirmatory tests. Persistent LA positivity was confirmed on at least two occasions at least 12 weeks apart. These samples were used to evaluate the potential influence of direct oral anticoagulants (DOACs) on the newly established LA activity assessment method. In contrast, plasma samples from 57 patients with systemic lupus erythematosus (SLE) were used to evaluate the sensitivity and specificity of the assay.

2.1.6 | DOAC-Treated Model Plasma

Four DOACs—dabigatran, rivaroxaban, edoxaban, and apixaban (ChemScene, USA)—were added at both low and high concentrations to pooled plasma from healthy volunteers and to plasma from LA-positive patients to create two model types: DOAC-treated normal plasma and DOAC-treated LA-positive plasma.

The low and high concentrations of each DOAC were determined based on the concentrations of a commercial DOAC Plasma Calibrator (HYPHEN BioMed, France) (Table 1), and DOAC solutions from ChemScene (USA) were used for spiking. The concentrations listed in Table 1 represent the final concentrations in the spiked plasma samples. The high concentration corresponded to a value above the upper peak plasma level in patients undergoing DOAC therapy, while the low concentration reflected a value between the upper trough and lower peak levels [18, 19]. The volume ratio of DOAC solution to plasma was adjusted to maintain plasma integrity and minimize dilution effects, typically less than 5% of total volume.

TABLE 1 | Final concentration of DOAC used in the preparation of model plasma.

DOAC	High concentration (ng/mL)	Low concentration (ng/mL)
Dabigatran	516	274
Rivaroxaban	526	262
Apixaban	632	317
Edoxaban	489	242

Abbreviation: DOAC, direct oral anticoagulant.

2.2 | Reagents for APTT Measurement

In this study, we used the following two types of reagents to measure APTT.

2.2.1 | PTT-LA Reagent "FR"

PTT-LA Reagent "FR" (Diagnostica Stago, France) [20] uses cephalin as the phospholipid source and silica as the activator of the contact factor system. It is recommended by the Scientific and Standardization Committee of the Japanese Society on Thrombosis and Hemostasis (JSTH-SSC) as a highly sensitive reagent for LA detection [21].

2.2.2 | Datafy APTT

Datafy APTT (Sysmex Corporation) [22] uses cephalin as the phospholipid source and ellagic acid as the activator of the contact factor system. It is used for general APTT measurements and screening for coagulation abnormalities and is particularly suitable for evaluating the activity of endogenous coagulation factors in routine clinical practice in Japan.

2.3 | LA-Sensitivity Ratio (LA-SR)

In this study, the LA-sensitivity ratio (LA-SR) was calculated to establish a one-step method for determining LA activity, using PTT-LA Reagent "FR" as the high LA-sensitivity reagent (first reagent) and Datafy APTT as the low LA-sensitivity reagent (second reagent).

For each test, 50 μL of test plasma was dispensed into two separate sample cups. Subsequently, 50 μL of the high-sensitivity reagent was added to cup 1, and 50 μL of the low-sensitivity reagent to cup 2. After incubation at 37°C for 3 min, 50 μL of 0.025 M $CaCl_2$ (Sysmex Corporation) was added to each cup. APTT was measured using the KC4 Delta coagulation analyzer (Tcoag, Ireland).

LA-SR was calculated using the following formula:

$$\text{LA} - \text{SR} = \frac{\text{APTT measured with a high sensitivity reagent [s]}}{\text{APTT measured with a low sensitivity reagent [s]}}$$

LA-SR takes advantage of the property that LA activity in test plasma causes more pronounced APTT prolongation when measured with highly sensitive reagents.

2.4 | Cutoff Value Setting (LA-SR)

The cutoff value for the LA-SR was determined using plasma samples from 41 healthy volunteers. LA-SR was calculated for each sample, and the mean and the cutoff value were determined using a nonparametric method based on the 99th percentile, as recommended to optimize specificity. This approach aligns with current international recommendations stating that cutoff values should be calculated in-house and tailored to each reagent and instrument combination [10].

3 | Results

3.1 | LA-SR Cut Off Value Using PTT-LA Reagent "FR" and Datafy APTT

LA-SR was calculated using plasma samples from 41 healthy volunteers. Based on the calculated 99th percentile of healthy volunteers, the cutoff value was set at 1.46.

3.2 | Comparison Between Guideline-Based LA Testing (dRVVT and LA-Sensitive APTT) and the New LA Activity Determination Method

The sensitivity and specificity of the new method were evaluated using 57 plasma samples from patients with SLE, whose LA status had been confirmed as positive or negative using the guideline-based LA testing (dRVVT and/or APTT-LA) [10]. LA positivity was defined as persistent positivity confirmed on at least two occasions at least 12 weeks apart. Among these samples, 14 were classified as LA-positive and 38 as LA-negative by guideline-based LA testing, indicating concordant results (Table 2). However, five samples showed LA-SR values above the cutoff despite being negative according to the guideline-based LA testing (dRVVT and/or APTT LA), with negativity confirmed on at least two occasions at least 12 weeks apart. Based on these findings, the sensitivity and specificity of the LA-SR method were 100% and 88.45%, respectively. The agreement between the two methods was further evaluated using Cohen's kappa coefficient, which was 0.79 (95% confidence interval, 0.53–1.00; $p < 0.001$), indicating substantial agreement.

TABLE 2 | Comparison between LA guideline-recommended method and the new LA activity determination method.

		LA guideline recommended method [10]		
		(+)	(−)	Total
New LA activity determination method	(+)	14	5	19
	(−)	0	38	38
Total		14	43	57

Abbreviation: LA, lupus anticoagulant.

3.3 | Evaluation of the Clinical Usefulness of the New Method for Determining LA Activity

To assess the clinical utility of the new LA activity determination method, we analyzed 74 randomly selected cases with prolonged APTT using blinded patient information. LA positivity or negativity was determined using the LA-SR method. After testing, the results were compared with patient background information. All patients with coagulation factor deficiencies or undergoing heparin treatment were negative for LA. In contrast, 2 out of 10 patients receiving warfarin therapy tested positive for LA. Notably, 10 out of 16 patients with unexplained APTT prolongation were identified as LA-positive (Table 3).

However, detailed information regarding the underlying causes of APTT prolongation was not available for all cases. For instance, the specific coagulation factor(s) involved, their activity levels, and whether the deficiencies were congenital or acquired were not evaluated. Additionally, INR values for patients on warfarin and anti-Xa activity or heparin type for patients on heparin were not assessed.

Therefore, we measured the guideline-based LA tests (dRVVT and LA-sensitive APTT) in 2 warfarin-treated patients and 10 patients with unexplained APTT prolongation who tested positive using the rapid LA differentiation method. These results were then compared with antiphospholipid antibody titers measured by ELISA (Table 4).

Notably, both patients taking warfarin showed positive results in the LA confirmation test and were also positive for antiphospholipid antibodies by ELISA, indicating the presence of antiphospholipid antibodies.

However, a definitive diagnosis of APS requires repeat testing at least 12 weeks apart, and this was not performed in the current study. Therefore, APS cannot be conclusively diagnosed based on these results alone.

TABLE 3 | Clinical usefulness of the new method for determining LA activity.

		Coagulation factor deficiency	Warfarin	Heparin	Unknown	Total
LA-SR	(+)	0	2	0	10	12
	(−)	33	8	15	6	62
Total		33	10	15	16	74

Abbreviations: LA, lupus anticoagulant; LA-SR, LA-sensitivity ratio.

TABLE 4 | Breakdown of positive cases detected by the new LA activity determination method.

No.	Cause of APTT prolongation	LA confirmation test	aCL IgG (U/mL)	aCL IgM (U/mL)	$a\beta_2$GPI IgG (U/mL)	$a\beta_2$GPI IgM (U/mL)	aPS/PT IgG (U/mL)	Test result
			Reference range					
			Positive: >20.8	Positive: >12.3	Positive: >20.0			
1	Warfarin	Positive	21.5	1.6	11.0	2.8	4.5	LA positive
2	Warfarin	Positive	550.0	6.5	2611.0	1.2	26.2	LA positive
3	Unknown	Positive	10.4	31.6	14.3	46.5	3.0	LA positive
4	Unknown	Positive	<2.6	37.2	13.1	58.2	2.8	LA positive
5	Unknown	Positive	<2.6	23.5	<6.4	11.4	11.3	LA positive
6	Unknown	Positive	6.3	3.5	<6.4	<1.1	1.0	LA positive
7	Unknown	Positive	<2.6	3.3	<6.4	3.0	2.3	LA positive
8	Unknown	Negative	7.4	5.4	<6.4	1.6	2.6	Delayed inhibitor
9	Unknown	Negative	<2.6	4.4	<6.4	1.3	1.0	Delayed inhibitor
10	Unknown	Negative	<2.6	6.4	<6.4	2.5	1.0	LA negative
11	Unknown	Negative	<2.6	<1.0	<6.4	<1.1	3.8	LA negative
12	Unknown	Negative	<2.6	<1.0	<6.4	<1.1	0.6	LA negative

Abbreviations: $a\beta_2$GPI, anti-β_2–glycoprotein I antibodies; aCL, anticardiolipin antibodies; APS, Antiphospholipid syndrome; aPS/PT, anti-phosphatidylserine/prothrombin; APTT, activated partial thromboplastin time; LA, lupus anticoagulant.

Among the 10 patients with unexplained APTT prolongation, 5 tested positive in the LA confirmation test, and 3 of them were also positive for antiphospholipid antibodies by ELISA.

In the remaining five cases, discrepancies were observed between the results of the newly established rapid LA differential test and the LA confirmation test. Therefore, a cross-mixing test was performed after 2 h of incubation at 37°C using these five discrepant samples. As a result, two cases were positive for delayed-type inhibitors. In the remaining three cases, LA was considered negative based on guideline-based testing, and the prolonged APTT was classified as being of undetermined cause.

3.4 | Investigation of LA-SR in DOAC Therapy Model Plasma

Our laboratory previously conducted an evaluation of dRVVT-LA in DOAC-containing, LA-negative model plasma using the "Gradipore" LA test [16]. In that study, all samples resulted in false-positive dRVVT-LA results except for the plasma containing low concentration of apixaban. In this study, a novel LA activity assay was performed using LA-negative model plasma derived from DOAC therapy (i.e., DOAC-containing pooled plasma from healthy volunteers). The LA-SR ranged from 0.83 to 1.23 for both low and high concentrations across all four types of DOAC-containing plasma. All samples were correctly identified as LA-negative (Table 5).

In contrast, when testing LA-positive model plasma derived from DOAC therapy (i.e., DOAC-containing LA-positive patient plasma (1) to (4)), the LA-SR values ranged from 1.45 to 2.87 at low concentrations and from 1.36 to 2.24 at high concentrations. While most reagent–DOAC combinations showed higher LA-SR values at higher DOAC concentrations, one specific combination showed a higher LA-SR value at the lower DOAC concentration. Although the apixaban high-concentration sample and edoxaban low-concentration sample showed LA-SR values slightly below the cutoff value, the majority of the samples still exceeded the cutoff and were identified as LA-SR positive (Table 6).

4 | Discussion

The presence of aPLs is known to cause arterial and venous thrombosis and habitual abortions specific to APS. The risk of complications is particularly high when multiple high-titer antibodies are present. The recurrence rate of thrombosis is especially elevated in patients who are triple-positive for aCL, $a\beta_2$GPI, and LA activity [23].

However, the detection of aCL and $a\beta_2$GPI requires standardized assay reagents and specialized or expensive equipment. As a result, these tests are limited to a few facilities, such as university hospitals and specialized laboratories. In contrast, LA testing is commonly performed in routine coagulation testing in many laboratories. However, the standard procedures

TABLE 5 | Lupus anticoagulant-sensitivity ratio (LA-SR) in LA-negative model plasma with direct oral anticoagulant therapy.

	LA-SR (cutoff: 1.46)		dRVVT ratio (cutoff: 1.2)	
DOAC	**High concentration**	**Low concentration**	**High concentration**	**Low concentration**
Dabigatran	1.18	1.23	1.3	1.2
Rivaroxaban	0.84	0.83	1.6	1.4
Edoxaban	1.14	1.12	1.3	1.3
Apixaban	1.14	1.14	1.4	1.2

Abbreviations: DOAC, direct oral anticoagulant; dRVVT, dilute Russell's viper venom time; LA, lupus anticoagulant; LA-SR, LA-sensitivity ratio.

TABLE 6 | Lupus anticoagulant-sensitivity ratio (LA-SR) in LA-positive model plasma with direct oral anticoagulant therapy.

	Plasma 1		Plasma 2		Plasma 3		Plasma 4	
DOAC	**High**	**Low**	**High**	**Low**	**High**	**Low**	**High**	**Low**
Dabigatran	2.03	2.86	2.11	2.68	1.77	1.62	1.93	2.25
Rivaroxaban	1.78	1.92	2.01	2.12	2.09	1.71	1.61	1.89
Edoxaban	2.00	1.88	2.13	2.87	1.50	1.45	1.93	1.68
Apixaban	1.88	1.50	2.24	2.26	1.94	1.70	1.36	1.89

Abbreviations: DOAC, direct oral anticoagulant; LA, lupus anticoagulant; LA-SR, LA-sensitivity ratio.

[24] recommended by the Working Group on Standardization of Specimen Handling in Coagulation Testing of the Japanese Society for Laboratory Hematology, including centrifugation conditions, anticoagulant concentration, and residual platelet count, are not sufficiently widespread. Therefore, variations in LA testing results have occurred.

Furthermore, LA determination includes multiple and complex testing steps, which represent a significant burden for clinical laboratory technologists and may affect the reproducibility and accuracy of testing. Against this background, this study aimed to establish a new method for determining LA activity using a one-step procedure. Additionally, this approach provides a simpler and faster alternative for preliminary assessment compared to guideline-based LA testing (dRVVT and LA-sensitive APTT) and can be implemented in a wide range of laboratories while complying with sample processing guidelines established by the Japanese Society for Laboratory Hematology.

The new LA activity determination method established in this study focuses on the difference in APTT measured using LA high-sensitivity and low-sensitivity reagents. Based on the principle that a larger difference in APTT between the two reagents corresponds to a higher LA-SR value, this method allows for the differentiation between APTT prolongation due to LA activity and that caused by other factors. In other words, in cases of coagulation factor deficiency, antithrombotic therapy such as heparin or DOAC use, or the presence of immediate inhibitors of coagulation factors, APTT is equally prolonged with both reagents, and therefore LA-SR is unlikely to increase.

Conversely, when LA activity is present, APTT prolongation is more marked with the LA high-sensitivity reagent and only mildly prolonged with the LA low-sensitivity reagent, resulting in a distinct difference in clotting times and a significant increase in LA-SR.

Unlike the conventional screen/confirm ratio used in guideline-based LA testing (dRVVT and LA-sensitive APTT), the LA-SR introduced in this study is calculated as the ratio of clotting times obtained using two APTT reagents with intentionally different intrinsic sensitivities to LA. Conventional APTT-based LA assays typically rely on differences in phospholipid concentration between screening and confirmatory reagents, whereas the LA-SR method is based on the differential response of reagents to LA sensitivity. The formula—"clotting time with a high-sensitivity reagent ÷ clotting time with a low-sensitivity reagent"—is designed to capture this difference in LA sensitivity rather than to correct baseline differences in clotting time. Therefore, normalization using healthy plasma may not be required, as the diagnostic value lies in the relative prolongation attributable to reagent sensitivity.

Unlike conventional dRVVT and APTT-based guideline-based LA tests (dRVVT and LA-sensitive APTT), this new method provides an indirect measure of phospholipid-dependent prolongation of clotting time associated with LA and may be less affected by exogenous factors such as DOACs.

International guidelines recommend the use of both APTT-based and dRVVT-based assays in parallel to minimize false negative results. If one assay yields a positive result, further testing with the other method may not be required, especially when clinical suspicion is high, as treatment decisions remain unaffected by the assay type. This supports a targeted and pragmatic approach rather than blanket multi-test screening. It is important to acknowledge, however, that this assay is based solely on

APTT and is not a comprehensive solution for LA detection. Importantly, the LA-SR method proposed in this study is not intended to replace guideline-recommended LA testing using dRVVT and LA-sensitive APTT. Rather, it should be regarded as a complementary or screening approach that may assist in the preliminary evaluation of LA activity and help guide subsequent confirmatory testing according to current international guidelines.

Commercial DOAC neutralization systems, such as DOAC-Stop and DOAC-Remove, have been developed to mitigate anticoagulant interference in LA testing. Although these approaches can reduce DOAC effects in many cases, several studies have reported that complete elimination of interference is not always achieved, and interpretation of LA results may still remain challenging in certain situations. We have previously reported that both the dRVVT method, the internationally preferred approach for LA testing, and the APTT cross-mixing test, widely used in Japan, carry the risk of false-positive LA results due to DOAC use. Furthermore, our findings demonstrated that even with the use of DOAC-Remove, the impact of DOACs on the APTT cross-mixing test could not be entirely eliminated, leading to cases where interpretation remained inconclusive [16, 17]. In contrast, ELISA-based aCL and aβ_2GPI assays are unaffected by DOACs because they do not rely on clotting time.

DOAC is the first-line anticoagulant therapy for nonvalvular atrial fibrillation (NVAF). In contrast, warfarin is considered the first-line drug for arterial and venous thrombosis caused by APS, as DOAC is considered inferior in preventing recurrence [25]. If a patient with NVAF on DOAC is accidentally diagnosed as LA-positive (i.e., suspected APS), they may be unnecessarily switched to warfarin, increasing the risk of stroke or intracranial bleeding. Since DOACs offer advantages such as no requirement for monitoring and fewer interactions with food and other drugs [19, 26], treatment errors stemming from LA misjudgment can directly impact patient prognosis. Therefore, rapid and accurate determination of LA activity is of great clinical significance.

While current guidelines do not recommend routine LA testing in patients undergoing anticoagulant therapy, emergency clinical settings sometimes necessitate prompt evaluation even during ongoing treatment. Waiting for therapy interruption is not always feasible, especially in critical cases where treatment decisions must be made urgently. This highlights the clinical value of assays that are minimally affected by anticoagulants and can provide meaningful information without therapy cessation. In addition, in two reagent–DOAC combinations, the LA-SR value was higher at the lower DOAC concentration than at the higher concentration. This observation may suggest that reduced anticoagulant interference at lower DOAC levels allows the phospholipid-dependent clotting prolongation associated with LA to become more apparent.

In addition to DOACs, other anticoagulant therapies such as warfarin and heparin have also been reported to influence LA testing to some extent [27–31]. However, the newly established method for determining LA activity is simpler than guideline-based LA testing (dRVVT and LA-sensitive APTT), is less susceptible to interference by anticoagulants (DOACs, warfarin, and heparin), and may help differentiate coagulation factor deficiency and assist in the preliminary assessment of LA activity. This method is expected to be highly useful in clinical practice, as it addresses the limitations of current LA testing in both accuracy and usability.

In addition, some cases showed prolonged APTT despite being negative by both the LA-SR method and guideline-based LA testing. These findings suggest that the APTT prolongation in such cases is unlikely to be attributable to LA. Possible causes include deficiencies in contact pathway factors, such as prekallikrein or high-molecular-weight kininogen, as well as other pre-analytical or analytical variables. These factors are known to prolong APTT without reflecting clinically significant bleeding risk or LA activity, which may explain the observed discrepancy [32, 33].

Nevertheless, we recognize that the findings of this study are preliminary and do not yet provide definitive evidence that this method will reduce diagnostic errors or improve patient prognosis. While simplification of laboratory workflows is a desirable goal, diagnostic accuracy must remain the highest priority. In cases where LA testing is complex or ambiguous, clinical laboratories should either develop the capability to manage such complexity or refer the samples to specialized facilities.

Since this method can be easily implemented in general hospital laboratories and mounted on general-purpose automated analyzers, it is expected to be developed as a "screening-type LA testing" that can be introduced in laboratories of small- and medium-sized hospitals. Therefore, in the context of clinical practice in Japan, the LA-SR method proposed in this study is positioned not as a confirmatory test for LA, but as an initial screening approach. A positive result would prompt subsequent guideline-based LA testing for definitive diagnosis. Conversely, a negative LA-SR result may help identify samples unlikely to have LA activity, highlighting its potential utility for excluding LA in routine laboratory screening.

5 | Limitations

One limitation of this study is the relatively small number of healthy donor samples used to determine the cutoff value. Current guidelines such as those from the ISTH and CLSI recommend using at least 120 healthy individuals to establish the 99th percentile cutoff. However, recruitment of a sufficient number of healthy donors was challenging, and the large sample volume required made it difficult to procure an adequate quantity of commercial normal pooled plasma (NPP).

In addition, all measurements in this study were performed using a single reagent lot. If normalization across reagent lot changes is not performed, recalculation of the cutoff value may be necessary when a new reagent lot is introduced.

Another limitation of this study is that the clinical samples were obtained primarily from patients with systemic lupus erythematosus (SLE), a population in which antiphospholipid antibodies are relatively common. Although this allowed efficient identification of LA-positive samples, the performance of the LA-SR method in other clinical settings—such as infectious diseases,

pregnancy, hemolysis, vaccination, or other autoimmune conditions that may influence LA testing [34]—was not evaluated in this study. Therefore, further investigations including a broader range of clinical conditions are necessary to confirm the generalizability of the present findings.

6 | Conclusion

The findings of this study may contribute to reducing the risk of missed or misclassified cases during the evaluation of LA, may support improved accuracy in LA testing, and may facilitate the development of laboratory workflows for APS diagnostic evaluation that are compatible with routine clinical practice.

Author Contributions

J.N., Y.M., and R.K. conceived and designed the study. A.H. and S.N. performed the measurements. A.H. and R.K. analyzed the data and drafted the original manuscript. R.K. contributed equally to this work. All authors reviewed the draft and approved the final version for submission.

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Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

The data that support the findings of this study are available from the corresponding author, upon reasonable request.

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