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ORIGINAL ARTICLE

Comparison of different algorithms for lupus anticoagulant detection: a single-center experience

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Abstract

Background: The laboratory tests for lupus anticoagulant (LA) detection comprise complex and multistep coagulation testing procedures. There is no established gold standard assay or direct comparison of algorithms as recommended by different guidelines.

Objectives: This study aimed to evaluate and compare the LA detection performance of different laboratory algorithms suggested by the existing guidelines.

Methods: The routine LA test data of 1801 plasma samples, including 188 LA-positive and 1613 LA-negative samples, were re-evaluated by applying the algorithms recommended by existing guidelines and were interpreted using various methods. Diagnostic performance indices for each LA detection algorithm were compared with those of the other algorithms. The efficacies of the different interpretation methods were analyzed to determine a suitable interpretation methodology for each assay.

Results: The diagnostic performance for detecting LA varied by the algorithm and method of interpretation used. All laboratory algorithms displayed exceptional diagnostic performance with all diagnostic parameters of >90.0%. Nearly perfect agreement was observed in all algorithms when compared to the Clinical and Laboratory Standards Institute 2014 guideline interpreted by normalized screen-to-confirm ratio (NSCR) and mixing test–specific cutoff (MTC), as a reference assay (Cohen's kappa coefficient, >0.90 [range, 0.94-1.00]). A combination of the index of circulating anticoagulant and NSCR was optimal for interpreting the activated partial thromboplastin time–based test, whereas a combination of the MTC and NSCR was suitable for the diluted Russell's viper venom time–based test.

Conclusion: All laboratory algorithms showed equivalent diagnostic performance. Establishing the best method of interpretation for each assay is recommended to improve LA detection performance.

KEYWORDS
activated partial thromboplastin time, algorithms, circulating anticoagulant, diluted Russell's viper venom time, lupus anticoagulant

Essentials

- There are no direct comparisons of algorithms recommended by different guidelines.
- Various algorithms recommended by different guidelines were applied to the data of 1801 samples.
- All laboratory algorithms exhibited equivalent diagnostic performance.
- Determining the most suitable method of interpretation for each assay is recommended.

1 | INTRODUCTION

Lupus anticoagulant (LA) is part of a heterogeneous group of antibodies directed against phospholipids and phospholipid-binding proteins [1,2] and is 1 of the 3 laboratory criteria, including LA, anticardiolipin (aCL), and anti–β2-glycoprotein I (aβ2-GPI), for classifying antiphospholipid syndrome (APS), an autoimmune disorder defined clinically by vascular thrombosis and/or pregnancy morbidity [1,2]. In addition, the presence of LA is a well-known risk factor for clinical manifestations of APS patients [3–5]. Therefore, accurate and reliable detection of LA is essential for the diagnosis and management of patients with APS. While aCL and aβ2-GPI are detected by calibrated semiquantitative solid-phase immunoassays, LA is detected based on its interference behavior in several phospholipid-dependent coagulation assays [6]. Standardization of the assays for LA detection has been challenged by the heterogeneity of LA antibodies, interference from various pathological conditions and anticoagulant medications, variations in reagents and algorithms, and differences in data manipulation and interpretation methods [6–9]. As a result, there are currently no established gold standard assays or reference plasmas for LA detection [6–10].

Laboratory tests for LA detection comprise complex and multistep coagulation testing procedures, including screening, confirmatory, and mixing tests [11–13], and heterogeneity in these is an important cause of interlaboratory variation. The screening test is performed using reagents with a low quantity of phospholipid, while the confirmatory test is performed using the same coagulation assays previously run in the screening test with a high quantity of phospholipid added [11–13]. Because there is no single assay sensitive to all LA antibodies, at least 2 coagulation tests based on different assay principles are recommended for LA detection [11–13]. The diluted Russell's viper venom time (dRVVT) assay is the first recommended test for detecting LA due to its high sensitivity and specificity [14], while the LA-sensitive activated partial thromboplastin time (aPTT) assay can be used as a secondary test [11–14]. Various interpretation indices, including percentage of correction (% Correct), screen-to-confirm ratio (SCR), and normalized SCR (NSCR), are suggested to determine the presence of LA in these phospholipid-dependent assays [11–13]. Mixing tests are performed to improve the specificity of LA detection and rule out other causes of prolonged clotting time (CT), including coagulation factor deficiency, anticoagulant use, and coagulation factor inhibitors [15,16]. In addition, the mixing test–specific cutoff (MTC) and index of circulating anticoagulant (ICA) can be calculated and employed as interpretation indices for the mixing tests [15–19].

Several guidelines and recommendations for LA detection have been established by various expert groups, including the International Society on Thrombosis and Haemostasis (ISTH) Scientific and Standardization Committee (SSC) guidelines in 2009 [11] and their latest updated guidance in 2020 [20], the British Committee for Standards in Haematology (BCSH) guidelines in 2012 [12], and the Clinical and Laboratory Standards Institute (CLSI) guidelines in 2014 [13]. Several issues in LA testing procedures, such as sample preparation, choice of the test, generating reference intervals (RIs) and cutoff values, and testing priority, vary across guidelines. The ISTH SSC 2009 and 2020 guidelines advise using 99.0th percentile cutoffs, whereas the BCSH 2012 and the CLSI 2014 guidelines advise using 97.5th percentile cutoffs [11–13,20]. While the ISTH SSC 2009 and the BCSH 2012 guidelines suggest that the screening test should be followed by the mixing study and then the confirmatory test (screen-mix-confirm), the CLSI 2014 guideline reprioritizes the test order to screen-confirm-mix to reduce false negatives by mixing tests [11–13,20]. In contrast, the ISTH SSC 2020 guideline recommends performing the mixing and confirmatory tests simultaneously if the screening test shows the presence of LA [20]. Commonalities and contrasts among the different guidelines are summarized in Supplementary Table S1 [11–13,20]. Although these guidelines and recommendations have contributed to more consistency in the protocols and interpretations of LA tests [6,8], many issues, including calculating and interpreting the results, are problematic [6,8,9,21]. Furthermore, no study has compared the testing algorithms recommended by each of the abovementioned guidelines in terms of their LA diagnostic performance.

In this study, a single-center retrospective study was performed to assess and compare the diagnostic performance of the laboratory algorithms recommended by different guidelines for LA detection using common diagnostic data from nonanticoagulated patients. In addition, the efficacies of applying different methods of interpretation were also analyzed to determine the most suitable interpretation methodology for each LA detection assay.

2 | METHODS

2.1 | Patients and samples

This study included 1938 patient samples enrolled from January 2020 to December 2022. All samples were obtained from Thai patients who were considered clinically appropriate patients being investigated for APS at the Laboratory of Special Hematology, Division of Hematology,

Department of Medicine, Phramongkutklao Hospital, Bangkok, Thailand. All LA results of the patients were obtained from routine tests following the algorithm recommended by the CLSI 2014 guidelines [13]. Additional baseline screening coagulograms and fibrinogen levels were performed in all patient samples to exclude factor deficiencies and undisclosed anticoagulation. Anti–factor Xa (FXa) activities, direct oral anticoagulant levels, and medication history in the medical records were evaluated to exclude the patients who receive anticoagulant drugs, including vitamin K agonists, unfractionated and low-molecular-weight heparins, or direct oral anticoagulants. Other antiphospholipid antibodies, including aβ2-GPI and aCL, were also investigated. Clinical data, including age, sex, and patient diagnosis, were obtained by retrospective medical record review. The study protocol was approved as an exempt review study by the Institutional Review Board of the Royal Thai Army Medical Department (project number S050b/66_Xmp). It was conducted in accordance with the principles of the Declaration of Helsinki. The requirement for informed consent was waived by the Institutional Review Board of the Royal Thai Army Medical Department because this was a retrospective study of deidentified data retrieved from medical records.

2.2 | Plasma sample preparation

Blood samples were collected into 3.2% trisodium citrate tubes (Vacuette, Greiner Bio-One Ltd) at a 9:1 ratio. Platelet-poor plasma was obtained by single centrifugation at 2000 × *g* for 15 minutes. Supernatants were collected, and a second centrifugation was performed at 2500 × *g* for 15 minutes. Plasma samples were immediately tested, aliquoted, and stored frozen at −20 °C for up to 1 week if a repeat test was necessary.

2.3 | Reagents and instruments

For aPTT tests, Dade Actin FSL and Dade Actin FS aPTT reagents (Siemens Healthineers) were used for aPTT screening and confirmatory tests, respectively. LA1 Screening and LA2 Confirmation reagents (Siemens Healthineers) were employed for dRVVT screening and confirmatory tests, respectively. Additional coagulation indices measured prior to LA testing, including prothrombin time, thrombin time, and fibrinogen level (Clauss method), were measured by the same analyzer using Thromborel S, Thromboclotin, and Dade Innovin thrombin reagents (Siemens Healthineers), respectively. In a 1:1 mixing test, a commercial standard human plasma (Siemens Healthineers) was used as the normal pooled plasma (NPP). All assays were performed on a Sysmex CS−2500 Coagulation analyzer (Sysmex).

2.4 | LA screening and confirmatory tests

According to the requisition policy for LA testing in our laboratory, LA screening, LA confirmatory, and mixing tests must be requested concurrently. In practice, the LA screening and confirmatory tests were performed in all cases, while the mixing tests were performed in all screening-positive cases. However, the mixing test results were reported in only cases of positive confirmatory test results and interpreted following the CLSI 2014 guideline.

In this study, LA screening and confirmatory tests were applied to all samples. A screening test CT higher than the upper limit of the RI, either aPTT or dRVVT, was considered a positive result of the LA screen. To interpret the phospholipid-dependent LA confirmatory test results, various interpretation parameters, including %Correct, SCR, and NSCR, were calculated as follows: %Correct = $([CT_{Screen} - CT_{Confirm}] / CT_{Screen}) \times 100$, SCR = $CT_{Screen} / CT_{Confirm}$, and NSCR = ($CT_{Screen}$ / CT_{Screen} of NPP) / ($CT_{Confirm}$ / $CT_{Confirm}$ of NPP). For the confirmatory test, the result was defined as consistent with the presence of LA when the %Correct, SCR, or NSCR was greater than the respective cutoff value.

2.5 | LA mixing test and interpretation

The LA mixing test was performed in all screening-positive cases by mixing the patient's plasma with the NPP to prepare a 1:1 mixture. The mixture was used to immediately perform the screening tests without incubation. To interpret the mixing test results, the mix ratio was calculated by using the following formula: Mix ratio = CT_{Screen} of 1:1 mix / mean of the RI (RI_m) of normal 1:1 mix. In addition, the ICA (or the Rosner index) was calculated using the following formula: ICA = (CT_{Screen} of 1:1 mix − CT_{Screen} of NPP) / CT_{Screen} of the patient. The mixing test was considered positive when the mix ratio was greater than the MTC or the ICA was greater than the ICA cutoff value.

2.6 | Normal RIs and cutoff values

Additional plasma samples from 120 healthy subjects with normal coagulation test results were used to perform LA testing. The protocol for sample preparation and LA testing of normal plasmas was similar to the protocol described in the previous subsection. The normal RI range for each assay was $RI_m \pm 2.3$ SDs (1.0th–99.0th percentile) under the ISTH SSC 2009 [11] and 2020 [20] guidelines and $RI_m \pm 2$ SDs (2.5th–97.5th percentile) under the BCSH 2012 [12] and CLSI 2014 [13] guidelines [11–13,20]. Cutoff values were determined from the upper limits of the RI in each assay. In addition, %Correct, SCR, and NSCR of the normal plasmas were calculated, and their cutoff values were determined by the 99.0th or 97.5th percentile, depending on the guideline used. To establish the MTC, all 120 normal plasma samples were mixed with commercial standard human plasma (Siemens Healthineers), which was used as the NPP at a ratio of 1:1. The mixtures were subjected to the aPTT and dRVVT screening tests, and the RI_m of the 1:1 mix for each assay was determined. The normal mix ratio was calculated, and the 99.0th or 97.5th percentile of these ratios was calculated as the MTC.

2.7 | Comparison of LA detection algorithms

In this study, the laboratory algorithm recommended by the CLSI 2014 guideline [13], in which NSCR and MTC were used to interpret the results, was employed as the reference algorithm. The diagnostic data from routine LA testing of 1801 patients were re-evaluated by applying the alternative algorithms advocated by the ISTH SSC 2009 [11], BCSH 2012 [12], CLSI 2014 [13], and ISTH SSC 2020 [20] guidelines with different interpretation parameters. The diagnostic performance of each LA detection algorithm was assessed and compared with the others.

2.8 | Evaluation of optimal methods of interpretation for LA detection

To determine which set of interpretation parameters was the most suitable method of interpretation for LA detection, the data from 395 plasma samples with positive LA screening, including 93 samples with positive aPTT screening and 392 samples with positive dRVVT screening, were analyzed. The methods of interpretation were generated by combining 1 of the interpretation parameters for the sequential mixing test, including ICA or MTC, and 1 of the LA confirmatory interpretation parameters, including %Correct, SCR, or NSCR. The performance characteristics of each interpretation method were calculated and compared.

2.9 | Statistical analysis

Statistical analysis was performed using IBM SPSS Statistics for Windows, version 20.0 (IBM Corp), and GraphPad Prism, version 9 (GraphPad Software). The Anderson–Darling test was performed to confirm the Gaussian distribution of the data. To evaluate the diagnostic performance of each guideline, the sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), and percentage of accuracy (%accuracy) were calculated and compared between guidelines. However, in tests with absence of a gold standard assay, the terms positive percent agreement (PPA), negative percent agreement (NPA), and overall rate of agreement (ORA) were recommended in place of sensitivity, specificity, and %accuracy, respectively. The PPA, NPA, and ORA were calculated as follows: PPA = (number of cases with test-positive / number of cases with the reference-positive) × 100, NPA = (number of cases with test-negative / number of cases with the reference-negative) × 100, and ORA = ([PPA + NPA] / total cases) × 100 [22]. McNemar's test was used to compare the diagnostic values between the 2 LA detection algorithms. Cohen's kappa coefficient was assessed to determine the diagnostic algorithm's agreement with the reference algorithm. In addition, the area under the receiver operating characteristic curve (AUC) for each analytical assay was analyzed. A P value of less than .05 was considered statistically significant.

3 | RESULTS

3.1 | Patient characteristics

Among the 1938 patient samples, 137 samples, including 123 samples obtained from patients receiving anticoagulant treatment, 3 samples from patients with FVIII inhibitors, and 11 samples from patients with other coagulopathies, were excluded. The remaining 1801 plasma samples obtained from nonanticoagulant patients clinically suspected of being investigated for APS were analyzed (Figure 1). The demographic and clinical characteristics of the 1801 patients are shown in Supplementary Table S2. Of these, 71.3% (1285 of 1801) and 28.7% (516 of 1801) were females and males, respectively. The median age of the study population was 41 years (IQR, 31–58 years). Altogether, 15.2% (275 of 1801) of the patients were diagnosed with APS according to the 2006 revised classification criteria for APS [23]. The patients were classified into 2 groups based on their LA test results interpreted by using NSCR and MTC under the CLSI 2014 guidelines [13] (the reference algorithm): LA-positive (n = 188) and LA-negative (n = 1613). All 188 LA-positive cases were repeated and confirmed positive after at least 12 weeks to evidence the antibody persistence. Although the different positive pattern of aPTT and dRVVT tests was observed in some samples, all cases demonstrated consistent positive results whether the 97.5th or 99.0th percentile cutoffs were used. Based on this reference algorithm, there were 8 patients who had negative mixing test results but positive LA confirmatory test results. The clinical and laboratory characteristics of these patients are shown in Supplementary Table S3. Only 3 patients who had positive LA mixing test results but negative confirmatory test results were observed; however, they demonstrated clearly negative results when the tests were repeated after 12 weeks.

Among the 188 LA-positive cases, 67.0% (126 of 188), 7.5% (14 of 188), and 25.5% (48 of 188) were considered LA-positive by dRVVT alone, aPTT alone, and both dRVVT and aPTT, respectively. Of these, 72.3% (136 of 188) were diagnosed with APS. Approximately 56.4% of LA-positive patients (106 of 188) experienced arterial or venous thrombosis, whereas 16.0% (30 of 188) of the patients presented with obstetrical and gynecological comorbidities. As expected, among the LA-positive patients, 52.7% (99 of 188) and 31.4% (59 of 188) had positive aβ2-GPI and aCL results, respectively. It was reported that the majority of patients diagnosed with APS were not triple–positive antiphospholipid antibodies [24]. In the present study, we found that only 20.7% (57 of 275) of these patients were triple-positive APS, whereas the remaining 79.3% (218 of 275) were considered non–triple-positive APS.

3.2 | Normal RIs and cutoffs

The RIs and the cutoff values for all assays and interpretation parameters are shown in Supplementary Table S4. The data distributions for the 120 normal samples were confirmed as Gaussian using the Anderson–Darling test (P > .05), with the exception of %Correct.

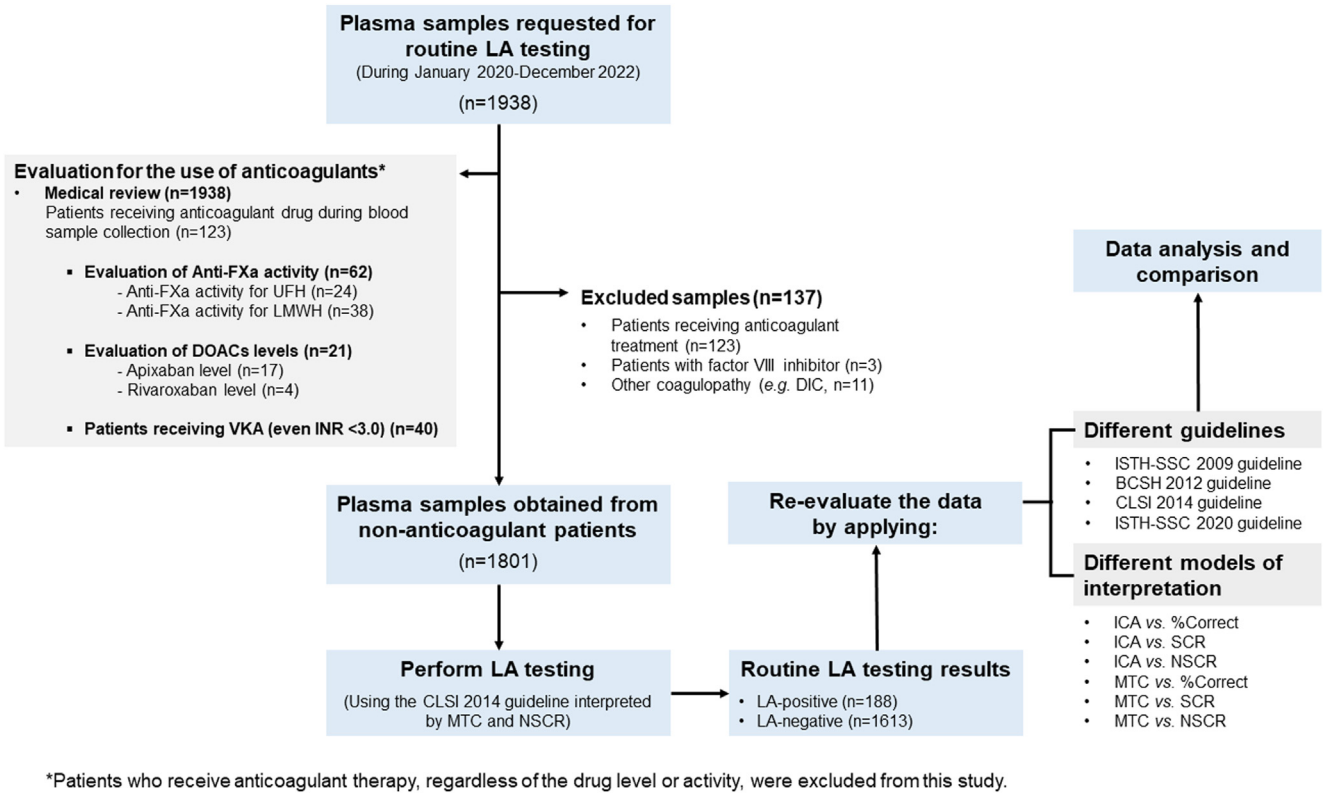

FIGURE 1 Schematic workflow of the study. BCSH, British Committee for Standards in Haematology; CLSI, Clinical and Laboratory Standards Institute; %Correct, percentage of correction; DIC, disseminated intravascular coagulation; DOAC, direct oral anticoagulant; FXa, factor Xa; ICA, index of circulating anticoagulant; INR, international normalized ratio; ISTH, International Society on Thrombosis and Haemostasis; LA, lupus anticoagulant; LMWH, low-molecular-weight heparin; MTC, mixing test–specific cutoff; NSCR, normalized screen-to-confirm ratio; SCR, screen-to-confirm ratio; SSC, Scientific and Standardization Committee; UFH, unfractionated heparin; VKA, vitamin K antagonist.

Therefore, %Correct was logarithmically transformed before its 97.5th and 99.0th percentiles were calculated.

3.3 | The positive results of LA vary between different approaches of laboratory algorithms and guidelines

Routine LA test data of 1801 patients were re-evaluated by applying the algorithms recommended by the ISTH SSC 2009 [11], BCSH 2012 [12], CLSI 2014 [13], and ISTH SSC 2020 [20] guidelines and interpreted using various methods of interpretation. It was observed that the positive results of LA varied based on algorithms and methods of interpretation used, as shown in Figures 2 to 5. Considering all algorithms recommended by each guideline, we found that only 172 samples were mutually identified as LA-positive.

3.4 | Diagnostic performance of the LA detection algorithms recommended by different guidelines

The diagnostic performance indices, including sensitivity/PPA, specificity/NPA, PPV, NPV, and %accuracy/ORA, for each LA detection algorithm are listed in Table 1. It was observed that the diagnostic performance for detecting LA varied with the algorithm and method of interpretation used. Even so, all laboratory algorithms displayed exceptional diagnostic performance, with >90% of PPA, NPA, PPV, NPV, and ORA for LA detection. Additionally, a nearly perfect agreement was observed between each algorithm and the CLSI 2014 [13] guidelines interpreted by NSCR and MTC as the reference assays, with Cohen's kappa coefficients greater than 0.90 (95% CI, 0.94-1.00) for each.

To evaluate the diagnostic value of using the 99.0th and 97.5th percentile cutoffs, the algorithms recommended by the ISTH SSC 2009 [11] and BCSH 2012 [12] guidelines, which share a similar testing priority (screen-mix-confirm), were compared. It was revealed that using different cutoffs led to inconsistent LA-positive results with all algorithms (McNemar's test *P* value for each algorithm was less than .05). Considering each particular algorithm, using the 97.5th percentile cutoff yielded a higher PPA (median PPA, 97.3% vs 93.6%) but lower PPV than the 99.0th percentile cutoff (median PPV, 95.6% vs 98.9%). Although it did not reach statistical significance, the number of cases requiring complete 3-step testing was less when using the 99.0th percentile cutoff than when using the 97.5th percentile cutoff (*P* = .448; average number of cases requiring complete 3-step testing, 185 vs 199 cases) (Table 1).

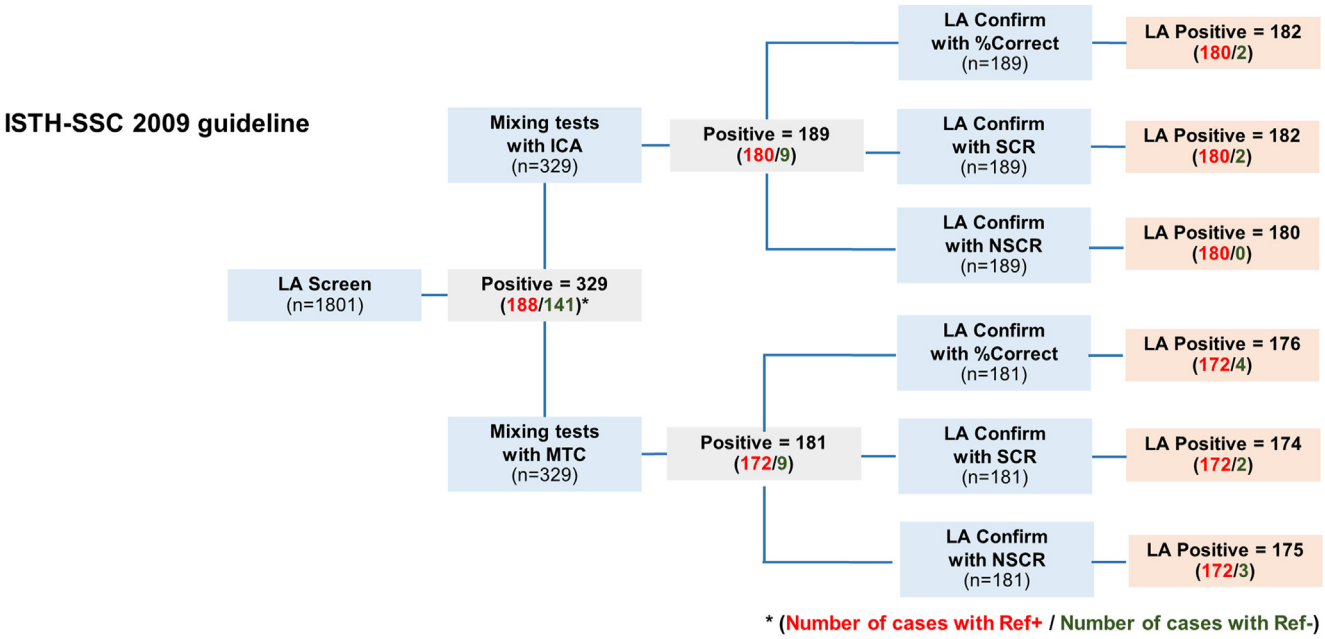

FIGURE 2 The results of lupus anticoagulant (LA) testing in 1801 samples using laboratory algorithms recommended by the International Society on Thrombosis and Haemostasis (ISTH) Scientific and Standardization Committee (SSC) 2009 guidelines for LA detection [11]. The number of cases with LA-positive and LA-negative results defined by the reference algorithm (the Clinical and Laboratory Standards Institute 2014 guideline [13] interpreted using normalized screen-to-confirm ratio [NSCR] and mixing test–specific cutoff [MTC]) is represented by red and green letters, respectively. %Correct, percentage of correction; ICA, index of circulating anticoagulant; Ref, reference; SCR, screen-to-confirm ratio.

Additionally, the diagnostic performance between the algorithms recommended by the ISTH SSC 2009 guideline [11] and their latest updated guideline in 2020 [20] was compared. It was revealed that the PPA was clearly improved by applying algorithms following the updated ISTH SSC 2020 guideline compared with the previous guideline in 2009 (median PPA, 93.6% vs 100.0%) (Table 1). Interestingly, when the ISTH SSC 2020 guideline [20] was employed, a maximum of an additional 8 patients who were classified LA-negative by the reference algorithm were considered LA-positive with a "negative mixing but positive confirmatory tests" pattern. Among these, 4 patients were persistently positive with the same pattern when the tests were re-examined after 12 weeks, and they were classified as weakly LA-positive.

3.5 | The optimal method of interpretation for LA detection

The sample distributions with PPA, NPA, and ORA for each interpretation method are shown in Figure 6. For aPTT-based assays, using ICA and NSCR for mixing and LA confirmatory test interpretations showed a superior ability to identify both LA-positive and LA-negative cases, with an ORA of 57.0% (PPA = 59.3% and NPA = 52.9%) (Figure 6C). In contrast, the ideal interpretation method for dRVVT-based assays was combining the mixing test interpreted by MTC with LA confirmation interpreted by NSCR, which resulted in an ORA of 91.3% (PPA = 86.3% and NPA = 95.7%) (Figure 6L).

3.6 | AUC analysis for each LA detection algorithm

The AUCs were analyzed using the sequential test data for both the aPTT and dRVVT assays in each LA detection algorithm. The results are shown in Table 2. All algorithms employing dRVVT-based assays demonstrated excellent performance in LA detection, with AUCs greater than 0.90 ($P < .001$). In contrast, almost all of the algorithms using the aPTT-based assays showed moderate to good diagnostic performance, with an average AUC of 0.83. Notably, excellent performance was observed in only ICA-containing algorithms applied to the aPTT-based assays.

4 | DISCUSSION

Although the current guidelines have helped standardize the LA testing process and interpretation, there is no gold standard assay available for LA detection. Technical and practical issues, including test algorithms, interpretation parameters, cutoff values, and methods of interpretation, are problematic in many laboratories [6,8,9,21]. Furthermore, there is a lack of empirical data to support any strong evidence-based recommendations. This study aimed to evaluate the diagnostic performance of different laboratory algorithms suggested by the existing guidelines for detecting LA.

In this study, we reanalyzed the LA test data of 1801 patients using various laboratory algorithms recommended by different guidelines to detect LA. Additionally, 120 normal plasma samples

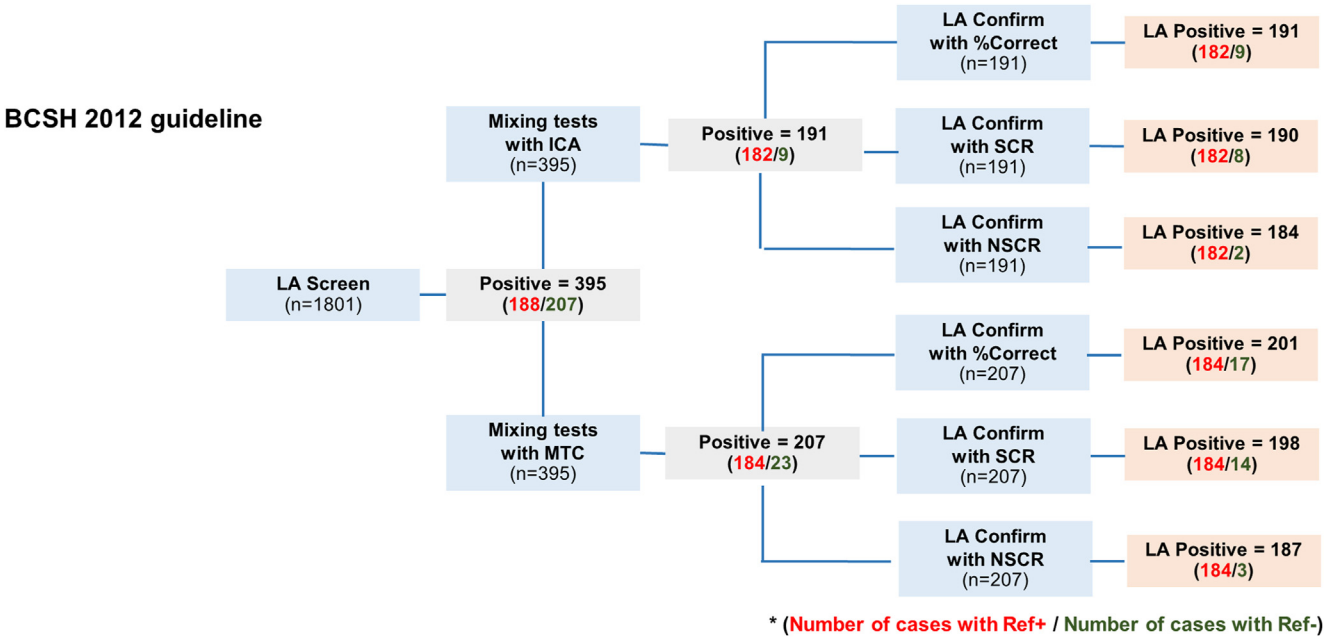

FIGURE 3 The results of lupus anticoagulant (LA) testing in 1801 samples using laboratory algorithms recommended by the British Committee for Standards in Haematology (BCSH) 2012 guidelines for LA detection [12]. The number of cases with LA-positive and LA-negative results defined by the reference algorithm (the Clinical and Laboratory Standards Institute 2014 guideline [13] interpreted using normalized screen-to-confirm ratio [NSCR] and mixing test–specific cutoff [MTC]) is represented by red and green letters, respectively. %Correct, percentage of correction; ICA, index of circulating anticoagulant; Ref, reference; SCR, screen-to-confirm ratio.

were used to establish and validate the specific local RIs and cutoff values. The findings showed inconsistencies in the LA results for each approach. Although the LA-positive case number varied by the algorithm and method of interpretation used, overall, the laboratory algorithms showed satisfactory diagnostic performance, as shown by their high PPA, NPA, PPV, NPV, and ORA. Furthermore,

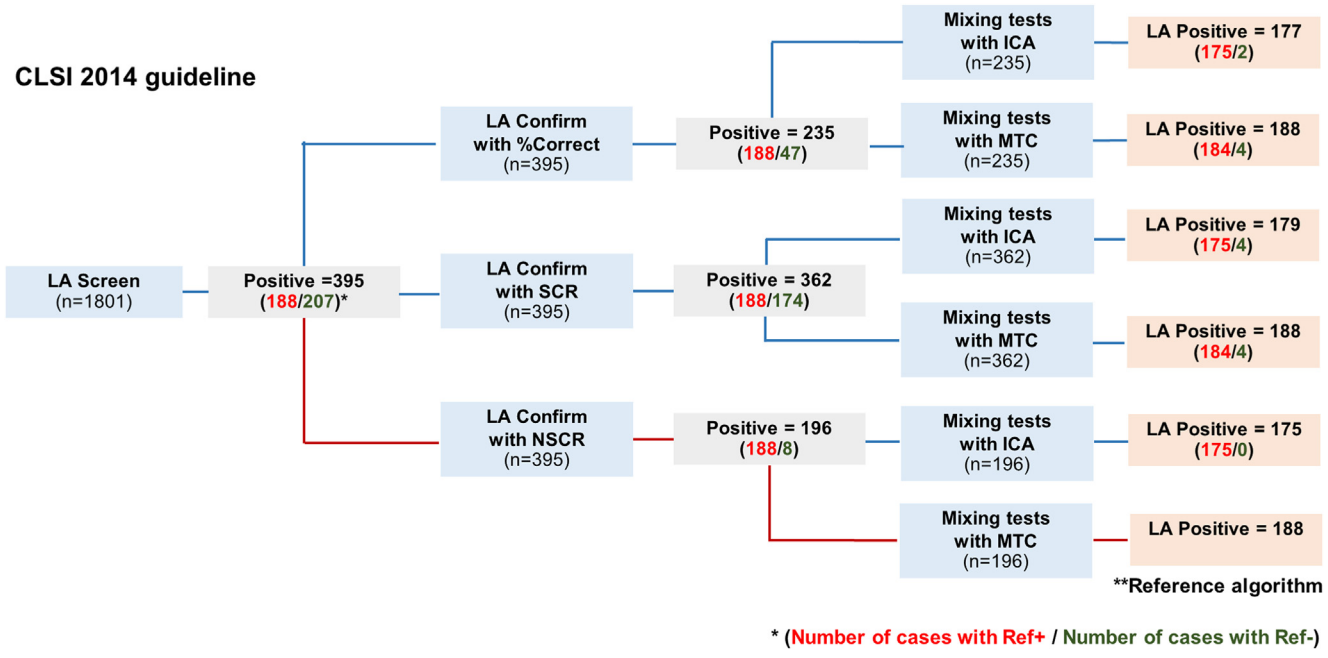

FIGURE 4 The results of lupus anticoagulant (LA) testing in 1801 samples using laboratory algorithms recommended by the Clinical and Laboratory Standards Institute (CLSI) 2014 guidelines for LA detection [13]. The number of cases with LA-positive and LA-negative results defined by the reference algorithm (the CLSI 2014 guideline [13] interpreted using normalized screen-to-confirm ratio [NSCR] and mixing test–specific cutoff [MTC]) is represented by red and green letters, respectively. %Correct, percentage of correction; ICA, index of circulating anticoagulant; Ref, reference; SCR, screen-to-confirm ratio.

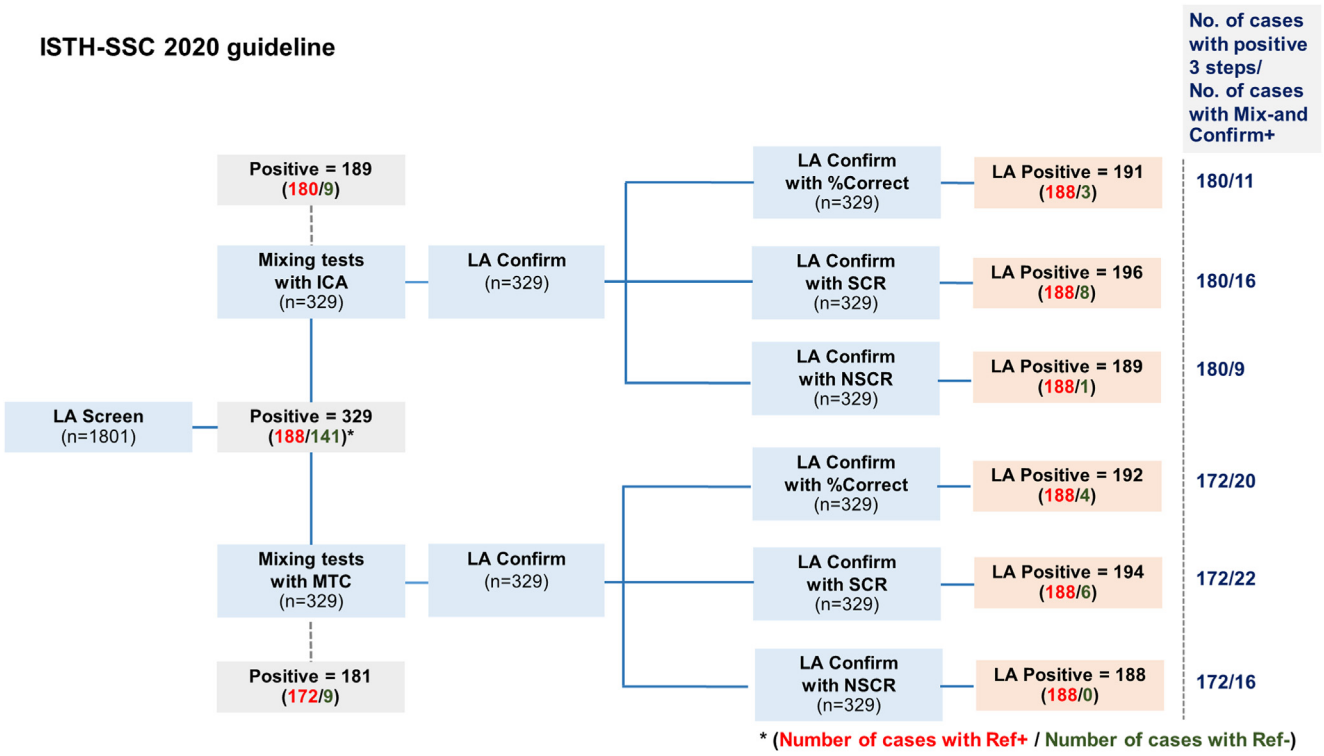

FIGURE 5 The results of lupus anticoagulant (LA) testing in 1801 samples using laboratory algorithms recommended by the International Society on Thrombosis and Haemostasis (ISTH) Scientific and Standardization Committee (SSC) 2020 guidelines for LA detection [20]. The number of cases with LA-positive and LA-negative results defined by the reference algorithm (the Clinical and Laboratory Standards Institute 2014 guideline [13] interpreted using normalized screen-to-confirm ratio [NSCR] and mixing test–specific cutoff [MTC]) is represented by red and green letters, respectively. %Correct, percentage of correction; ICA, index of circulating anticoagulant; Ref, reference; SCR, screen-to-confirm ratio.

all approaches displayed excellent agreement when compared with the reference algorithm. Given these results, it is challenging to select which of these algorithms and guidelines is ideal for LA detection.

We also analyzed the diagnostic performance of the algorithms recommended by the ISTH SSC 2009 [11] in comparison with its updated version in 2020 [20]. Focusing on the testing priority, the previous version proposed the screen-mix-confirm platform, whereas the mixing and confirmatory tests were advised to be performed simultaneously in the updated ISTH SSC 2020 guideline if the screening tests revealed the existence of LA. Our results indicated that employing the algorithms based on the ISTH SSC 2020 recommendations resulted in significantly improved PPA when compared to their previous version. Interestingly, using the algorithms following the ISTH SSC 2020 guideline, we identified 4 additional patients as weak LA-positive, who were classified as LA-negative according to our reference algorithm. In addition, the findings were validated by repeating the tests 12 weeks later, and the results demonstrated a persistently positive with "negative mixing but positive confirmatory tests" pattern. These findings suggest that applying this updated guideline not only improves sensitivity but also increases the chance of detecting patients with weak LA presence by reducing the chance of a false negative result involving the mixing step. However, larger studies to support this issue, as well as their effect on clinical outcomes, are needed.

To determine the effectiveness of using the 99.0th vs the 97.5th percentile as the cutoff, we compared the ISTH SSC 2009 [11] and BCSH 2012 [12] guidelines, in which the screen-mix-confirm algorithms are employed. Under the ISTH SSC 2009 recommendations [11], using the 99.0th percentile as the cutoff can increase specificity while decreasing sensitivity [6,8,20]. On the other hand, the BCSH 2012 guideline [12] suggests using the 97.5th percentile as the cutoff. Our study observed that the latter guideline yielded greater sensitivity. Although there were no significant variations in specificity between the 2 guidelines, the PPVs decreased when the 97.5th percentile cutoff was applied. Considering the cost and amount of work required for testing, the algorithms recommended by the ISTH SSC 2009 [11] guideline appeared to be the most suitable approach for routine practice. This is because they require less testing of cases in 3 steps compared with other guidelines. On the contrary, while the algorithms recommended by the ISTH SSC 2020 guideline [20] demonstrated significantly improved sensitivities or PPAs, they required more subsequent cases with 3-step testing than others. Nevertheless, we did not evaluate the costs and labor associated with each algorithm and guideline in our study, so further research comparing the cost-effectiveness of these guidelines is needed to draw a firm conclusion.

To determine the most suitable method of interpretation for aPTT- and dRVVT-based assays, 395 plasma samples with positive

TABLE 1 The overall diagnostic performance of the lupus anticoagulant detection assays using algorithms recommended by different guidelines.

Guideline/laboratory algorithm	No. of cases that required 3-step testing[a]	No. of positive cases[a]	No. of positive cases with Ref+/Ref−[a]	Diagnostic performance (%)					Cohen's kappa coefficient (95% CI)
				Sens/ PPA	Spec/ NPA	PPV	NPV	Accuracy/ ORA	
ISTH SSC 2009 guideline (using 99.0th cutoff values)									
Screen→mix (ICA)→confirm (%Correct)	189	182	180/2	95.7	99.9	98.9	99.5	99.4	0.97 (0.95-0.99)
Screen→mix (ICA)→confirm (SCR)	189	182	180/2	95.7	99.9	98.9	99.5	99.4	0.97 (0.95-0.99)
Screen→mix (ICA)→confirm (NSCR)	189	180	180/0	95.7	100.0	100.0	99.5	99.6	0.97 (0.96-0.99)
Screen→mix (MTC)→confirm (%Correct)	181	176	172/4	91.5	99.8	97.7	99.0	98.9	0.94 (0.91-0.97)
Screen→mix (MTC)→confirm (SCR)	181	174	172/2	91.5	99.8	98.9	99.0	99.0	0.95 (0.92-0.97)
Screen→mix (MTC)→confirm (NSCR)	181	175	172/3	91.5	99.9	98.3	99.0	98.9	0.94 (0.92-0.97)
Median	185	178		93.6	99.9	98.9	99.3	99.2	0.96 (0.93-0.98)
BCSH 2012 guideline (using 97.5th cutoff values)									
Screen→mix (ICA)→confirm (%Correct)	191	191	182/9	96.8	99.4	95.3	99.6	99.2	0.96 (0.93-0.98)
Screen→mix (ICA)→confirm (SCR)	191	190	182/8	96.8	99.5	95.8	99.6	99.2	0.96 (0.94-0.98)
Screen→mix (ICA)→confirm (NSCR)	191	184	182/2	96.8	99.9	98.9	99.6	99.6	0.98 (0.96-0.99)
Screen→mix (MTC)→confirm (%Correct)	207	201	184/17	97.8	98.9	91.5	99.8	98.8	0.94 (0.91-0.97)
Screen→mix (MTC)→confirm (SCR)	207	198	184/14	97.8	99.1	93.0	99.8	99.0	0.95 (0.92-0.97)
Screen→mix (MTC)→confirm (NSCR)	207	187	184/3	97.8	99.8	98.4	99.8	99.6	0.98 (0.96-1.00)
Median	199	191		97.3	99.5	95.6	99.7	99.2	0.96 (0.93-0.98)
CLSI 2014 guideline (using 97.5th cutoff values)									
Screen→confirm (%Correct)→mix (ICA)	235	177	175/2	93.0	99.9	98.9	99.2	99.2	0.95 (0.93-0.98)
Screen→confirm (%Correct)→mix (MTC)	235	188	184/4	97.8	99.8	97.9	99.8	99.6	0.98 (0.96-0.99)
Screen→confirm (SCR)→mix (ICA)	362	179	175/4	93.0	99.8	97.8	99.2	99.1	0.95 (0.92-0.97)
Screen→confirm (SCR)→mix (MTC)	362	188	184/4	97.8	99.8	97.9	99.8	99.6	0.95 (0.93-0.98)
Screen→confirm (NSCR)→mix (ICA)	196	175	175/0	93.0	100.0	100.0	99.2	99.3	0.96 (0.94-0.98)
Screen→confirm (NSCR)→mix (MTC)[b]	196	188	N/A	100.0	100.0	100.0	100.0	100.0	Ref[b]
Median	235	184		95.4	99.8	98.4	99.5	99.5	0.95 (0.92-0.97)
ISTH SSC 2020 guideline (using 99.0th cutoff values)									
Screen→mix (ICA) and confirm (%Correct)	329	191	188/3	100.0	99.8	98.4	100.0	99.8	0.99 (0.98-1.00)
Screen→mix (ICA) and confirm (SCR)	329	196	188/8	100.0	99.5	95.9	100.0	99.6	0.98 (0.96-1.00)
Screen→mix (ICA) and confirm (NSCR)	329	189	188/1	100.0	99.9	99.5	100.0	99.9	0.99 (0.99-1.00)
Screen→mix (MTC) and confirm (%Correct)	329	192	188/4	100.0	99.8	97.9	100.0	99.8	0.99 (0.98-1.00)
Screen→mix (MTC) and confirm (SCR)	329	194	188/6	100.0	99.6	96.9	100.0	99.7	0.98 (0.97-1.00)
Screen→mix (MTC) and confirm (NSCR)	329	188	188/0	100.0	100.0	100.0	100.0	100.0	1.00 (1.00-1.00)
Median	329	192		100.0	99.8	98.2	100.0	99.6	0.99 (0.98-1.00)

BCSH, British Committee for Standards in Haematology; CLSI, Clinical and Laboratory Standards Institute; %Correct, percentage of correction; ICA, the index of circulating anticoagulant; ISTH, International Society on Thrombosis and Haemostasis; MTC, mixing test–specific cutoff; N/A, not applicable; NPA, negative percent agreement; NPV, negative predictive value; NSCR, normalized screen-to-confirm ratio; ORA; overall rate agreement, PPA; positive percent agreement; PPV, positive predictive value; Ref, reference; SCR, screen-to-confirm ratio; Sens, sensitivity; Spec, specificity; SSC, Scientific and Standardization Committee.

[a]This study was conducted among the Thai population.

[b]The reference algorithm used in this study is the Clinical and Laboratory Standards Institute 2014 guidelines interpreted using normalized screen-to-confirm ratio and mixing test–specific cutoff.

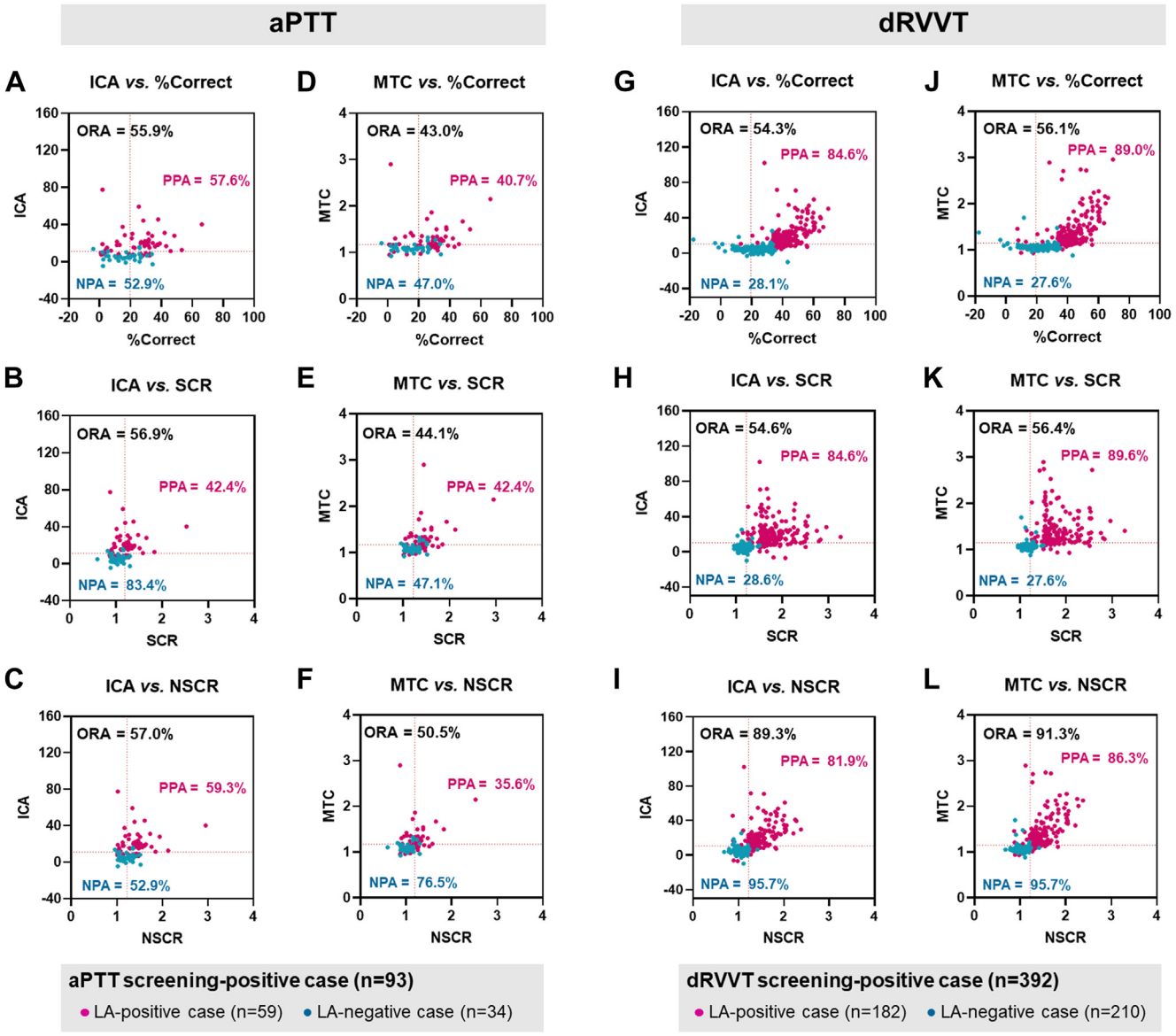

FIGURE 6 The interpretation methods generated by combining the interpretation parameters of the mixing and confirmatory tests of (A–F) activated partial thromboplastin time (aPTT)–based assays and (G–L) diluted Russell's viper venom time (dRVVT)–based assays using 99.0th-percentile cutoffs. The terms positive percent agreement (PPA), negative percent agreement (NPA), and overall rate of agreement (ORA) were used in place of sensitivity, specificity, and percentage of accuracy, respectively. %Correct, percentage of correction; ICA, index of circulating anticoagulant; LA, lupus anticoagulant; MTC, mixing test–specific cutoff; NSCR, normalized screen-to-confirm ratio; SCR, screen-to-confirm ratio.

LA screening results were analyzed. The results showed that a combination of ICA and NSCR was the best method of interpretation for aPTT-based assays, while MTC combined with NSCR was the best for dRVVT-based assays. The NSCR is recommended by the existing guidelines as a parameter for interpreting phospholipid-dependent assays [11–13,20] because it can reduce both inter-laboratory and interreagent variations [25]. Based on our findings, we encourage the use of NSCR as the most suitable parameter in both aPTT- and dRVVT-based assays for accurate detection of the presence of LA.

For the interpretation of the LA mixing test result, several studies have recommended the use of MTC over ICA as it offers greater sensitivity in detecting *in vitro* inhibition of LA [17–19]. The ICA was considered the most robust approach to express the LA mixing test results [26], and a previous study recommended the use of MTC for the dRVVT-based mixing test and ICA for the aPTT-based mixing test to achieve the highest sensitivity [27]. Similarly, our results demonstrated that MTC was optimal for interpreting the dRVVT-based test results, while using ICA for interpreting the aPTT-based mixing tests yielded the most precise results as it offered better positive agreement rates with the highest rate of overall agreements when compared with the reference assay.

Although MTC provides greater sensitivity for the detection of LA, especially when weak LA is present, it has a greater tendency to

TABLE 2 The areas under the receiver operating characteristic curves of activated partial thromboplastin time–and diluted Russell's viper venom time–based assays using different laboratory algorithms for lupus anticoagulant detection.

	aPTT-based assays			dRVVT-based assays		
Guideline/laboratory algorithm	**AUC**	**95% CI**	***P* value**	**AUC**	**95% CI**	***P* value**
ISTH SSC 2009 guideline (using 99.0th cutoff values)						
Screen→mix (ICA)→confirm (%Correct)	0.78	0.57–0.98	.110	0.98	0.95–1.00	**<.001**
Screen→mix (ICA)→confirm (SCR)	0.80	0.61–0.99	**.027**	0.99	0.97–1.00	**<.001**
Screen→mix (ICA)→confirm (NSCR)	0.89	0.76–1.00	**.014**	0.98	0.96–1.00	**<.001**
Screen→mix (MTC)→confirm (%Correct)	0.71	0.53–0.89	**.033**	0.98	0.96–1.00	**<.001**
Screen→mix (MTC)→confirm (SCR)	0.69	0.51–0.87	.088	0.96	0.90–1.00	**<.001**
Screen→mix (MTC)→confirm (NSCR)	0.67	0.47–0.87	.152	0.99	0.97–1.00	**<.001**
BCSH 2012 guideline (using 97.5th cutoff values)						
Screen→mix (ICA)→confirm (%Correct)	0.81	0.61–1.00	**.011**	0.99	0.97–1.00	**<.001**
Screen→mix (ICA)→confirm (SCR)	0.80	0.60–1.00	**.013**	0.98	0.97–1.00	**<.001**
Screen→mix (ICA)→confirm (NSCR)	0.85	0.71–0.99	**.012**	0.99	0.97–1.00	**<.001**
Screen→mix (MTC)→confirm (%Correct)	0.72	0.53–0.91	.056	0.99	0.97–1.00	**<.001**
Screen→mix (MTC)→confirm (SCR)	0.71	0.55–0.88	**.040**	0.98	0.97–0.99	**<.001**
Screen→mix (MTC)→confirm (NSCR)	0.73	0.57–0.89	**.012**	0.99	0.97–1.00	**<.001**
CLSI 2014 guideline (using 97.5th cutoff values)						
Screen→confirm (%Correct)→mix (ICA)	0.95	0.90–0.99	**<.001**	0.98	0.97–1.00	**<.001**
Screen→confirm (%Correct)→mix (MTC)	0.82	0.70–0.93	**<.001**	0.99	0.98–1.00	**<.001**
Screen→confirm (SCR)→mix (ICA)	0.96	0.85–1.00	**<.001**	0.98	0.97–0.99	**<.001**
Screen→confirm (SCR)→mix (MTC)	0.89	0.79–0.99	**<.001**	0.96	0.89–1.00	**<.001**
Screen→confirm (NSCR)→mix (ICA)	0.98	0.97–1.00	**<.001**	0.94	0.87–1.00	**<.001**
Screen→confirm (NSCR)→mix (MTC)[a]	0.84	0.67–1.00	**<.001**	0.99	0.98–1.00	**<.001**
ISTH SSC 2020 guideline (using 99.0th cutoff values)						
Screen→mix (ICA) and confirm (%Correct)	0.87	0.80–0.94	**<.001**	0.94	0.91–0.96	**<.001**
Screen→mix (ICA) and confirm (SCR)	0.85	0.78–0.92	**<.001**	0.96	0.94–0.98	**<.001**
Screen→mix (ICA) and confirm (NSCR)	0.89	0.83–0.95	**<.001**	0.99	0.98–1.00	**<.001**
Screen→mix (MTC) and confirm (%Correct)	0.77	0.68–0.85	**<.001**	0.96	0.94–0.98	**<.001**
Screen→mix (MTC) and confirm (SCR)	0.88	0.81–0.95	**<.001**	0.96	0.94–0.98	**<.001**
Screen→mix (MTC) and confirm (NSCR)	0.83	0.75–0.91	**<.001**	0.99	0.98–1.00	**<.001**

P < .05 is shown in bold.

aPPT, activated partial thromboplastin time; AUC, area under the receiver operating characteristic curve; BCSH, British Committee for Standards in Haematology; CLSI, Clinical and Laboratory Standards Institute; %Correct, percentage of correction; dRVVT, diluted Russell's viper venom time; ICA, index of circulating anticoagulant; ISTH, International Society on Thrombosis and Haemostasis; MTC, mixing test–specific cutoff; NSCR, normalized screen-to-confirm ratio; SCR, screen-to-confirm ratio; SSC, Scientific and Standardization Committee.

[a]The reference algorithm used in this study is the Clinical and Laboratory Standards Institute 2014 guidelines interpreted using normalized screen-to-confirm ratio and mixing test–specific cutoff.

produce false-positive results than ICA [16,18,19]. In contrast, using mixing tests interpreted with ICA demonstrates superior specificity as there are few false positives of inhibition by non-LA causes in cases of positive LA screening test results [16]. A recent study revealed that using ICA for interpreting the mixing tests of LA screening and confirming reagents greatly improved the capacity to distinguish between samples containing LA and direct FXa inhibitors with high specificity [28]. Additionally, in our receiver operating characteristic analysis, only ICA-containing algorithms demonstrated excellent performance for the aPTT-based assays. These findings suggest that using the ICA could improve the aPTT-based mixing test accuracy regardless of the phospholipid-dependent interpretation indices used. Compared with dRVVT, aPTT is a more complex assay involving multiple enzymatic steps and may be influenced by physiologic and pathologic factors

unrelated to the inhibition of LA [14,29]. Collectively, rather than using MTC, which relies on the RI_m of the normal mix ratio, using ICA calculated by the CT of NPP in each run might be more beneficial for interpreting the aPTT-based mixing test.

Our study had some limitations. First, the analyzed data were only applied to the results of clinical samples with 1 aPTT and 1 dRVVT reagent. Differences in reagent composition and concentration of phospholipids between various types of reagents are considered to be the cause of interreagent variation in LA sensitivity [30,31]. In addition, the Dade Actin FSL and Dade Actin FS aPTT reagents used in this study are ellagic acid–based reagents. Although the sensitivity of ellagic acid-based reagents to LA is comparable with that of other silica-based reagents, the commercial reagents employed in this study are potentially less sensitive to LA [31]. On the other hand, the aPTT reagent pair used in this study (Actin FSL for screening and Actin FS for confirmation) is one of only a few paired aPTT reagents offered by commercial manufacturers, with most manufacturers of silica-based aPTT assays offering an LA-sensitive reagent, but not providing an LA insensitive silica-based paired reagent [32]. Hence, an analysis with various types of reagents is suggested. Second, as the samples of patients who received anticoagulants were excluded, the interfering effect of the drugs on the diagnostic performance of LA testing was not evaluated in our study.

In conclusion, this large, single-center investigation compared the diagnostic performance of laboratory algorithms recommended by different guidelines for LA detection. To enhance test performance, determining the most suitable method of interpretation for each assay before applying it to laboratory practice is recommended. Our findings provide important information that could be implemented by laboratories and could be used to develop evidence-based recommendations in the future.

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ETHICS STATEMENT

The study protocol was approved as an exempt review study by the Institutional Review Board of the Royal Thai Army Medical Department (project number S050b/66_Xmp). It was conducted in accordance with the principles of the Declaration of Helsinki. The requirement for informed consent was waived by the Institutional Review Board of the Royal Thai Army Medical Department because this was a retrospective study of deidentified data retrieved from medical records.

AUTHOR CONTRIBUTIONS

D.A. developed the concept, collected patient cases, analyzed the data, and wrote the article. R.L., P.A., and C.R. supervised the project. W.C. developed the concept, supervised the project, and wrote and revised the article. All authors contributed to and approved the final version.

RELATIONSHIP DISCLOSURE

There are no competing interests to disclose.

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SUPPLEMENTARY MATERIAL

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