ORIGINAL ARTICLE

Lupus anticoagulant mixing tests for multiple reagents are more sensitive if interpreted with a mixing test-specific cut-off than index of circulating anticoagulant

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Abstract

Background: Lupus anticoagulant (LA) is classified in the antibody family that is recognized as antiphospholipid antibodies. Guidelines for LA detection recommend mixing test interpretation with either a mixing test specific cut-off (MTC) or index of circulating anticoagulant (ICA). We previously evidenced that MTC was superior to ICA in detecting the in vitro inhibition of LA with a single dilute APTT (activated partial thromboplastin time) and dRVVT (diluted Russell's viper venom time) pairing.

Objectives: The objective in the present study was to compare the LA diagnostic effectiveness of MTC and ICA by multiple APTT and dRVVT reagents.

Methods: One hundred-five samples from non-anticoagulated patients positive for LA in the dilute APTT (dAPTT) and dRVVT reagent pairing employed for diagnostic examination were performed by undiluted and in a 1:1 mix with normal pooled plasma with four additional APTT reagents and another dRVVT reagent (dRVVT B).

Results: Frequencies of MTC and ICA positivity were determined from samples LA positive in undiluted plasma. MTC positivity in mixing test were 63%, 77%, 80%, 84%, 46%, 81%, and 72% in 4 APTT, dAPTT and 2 dRVVT, respectively. ICA positivity were 47%, 67%, 58%, 54%, 42%, 47%, and 29%, respectively. There were no samples of ICA-positive/MTC-negative with any reagent.

Conclusions: The data indicate that MTC is superior to ICA for LA detection in mixing tests in multiple reagents and reagent types. Although mixing tests may make weak LA samples appear negative, the efficacy of LA detection can be improved by the method to interpret the results.

KEYWORDS
activated partial thromboplastin time, antiphospholipid antibodies, antiphospholipid syndrome, diluted Russell's viper venom time, lupus anticoagulant

Essentials
- Various approaches are available for interpreting lupus anticoagulant mixing tests.
- Mixing test specific cut-off (MTC) was compared with index of circulating anticoagulant (ICA).
- MTC was superior to ICA in detecting inhibition by lupus anticoagulant (LA) in multiple reagents.
- Detection rates of LA can be improved by the method to interpret the results.

1 | INTRODUCTION

Antiphospholipid antibodies (aPL) have clinical significance due to their association with thrombosis, pregnancy morbidity, obstetrical complications, neurological issues, and cutaneous manifestations.[1] Diagnosis of antiphospholipid syndrome (APS) is performed when clinical laboratory examinations reveal the presence of persistent aPL in patients with appropriate clinical presentation, predominantly thrombosis or pregnancy morbidity.[2] When APS is diagnosed, anticoagulant therapy for a long-term period is considered due to the high risk of recurrent thrombosis.[3] Thus, accurate laboratory detection of aPL is critical. Solid phase assays are employed to detect two of the criteria antibodies, anti-cardiolipin antibodies (aCL) and anti-β2-glycoprotein I antibodies (aβ2GPI), whilst the other criterion antibody, lupus anticoagulant (LA), is detected in clotting time assays. Issues such as antibody heterogeneity, between-reagent and between-platform variability, and differences in raw data manipulation and interpretation, conspire to make standardization an elusive goal. Consequently, gold standard assays and reference plasmas are not yet established.[4,5] While aCL and aβ2GPI assays can be calibrated to generate quantitative results in semi-arbitrary units to aid interpretation, a medley of phospholipid-dependent coagulation assays are employed for LA detection and the presence of LA is inferred or excluded from the result patterns obtained. This additional complexity to LA detection further complicates diagnosis and some LA guidelines with broad but not complete agreement are available to lead best practices.[6-8]

There is no single test to detect all LA[1,6-8] and guidelines recommend performance of two different tests that represent different assay principles, the diluted Russell's viper venom time (dRVVT) and a LA-sensitive activated partial thromboplastin time (APTT).[6-8] The assay medley for test types involves: (i) a screening test with low phospholipid concentration to find the effect of LA, (ii) mixing test in the screening test by a 1:1 mixture of index and normal pooled plasma (NPP) to show inhibition, and (iii) recapitulation of the screening test but with high phospholipid concentration to evidence phospholipid dependence as the confirm procedure.[6,7] Mixing tests are important and useful in LA detection for the diagnosis because they can achieve differentiation between factor deficiency and the presence of an inhibitor, although the Clinical and Laboratory Standards Institute (CLSI) guideline supports initial performance of LA screening and confirmatory assays to show the phospholipid dependence of the antibody[8] and performance of mixing tests only when initial testing is not clear-cut.

Guidelines recommend mixing tests are interpreted with either a mixing test specific cut-off (MTC) or the index of circulating anticoagulant (ICA). In our previous study, we suggested that MTC had higher sensitivity than ICA for detection in the in vitro inhibition of LA. However, the study was performed with only one dAPTT and one dRVVT reagent.[9] Sensitivity and specificity of different APTT and dRVVT reagents to LA varies, predominantly due to differences in phospholipid composition and concentration.[10-12] Additionally, mixing plasma with NPP shows a dilution factor that can make weaker LA samples appear negative in the mixing test despite clear positivity in the screening and confirmation results on undiluted plasma,[7,8,13,14]

and a less sensitive reagent is therefore more likely to generate a false-negative mixing test. The aim of the present study is to compare the LA diagnostic effectiveness of MTC and ICA by multiple APTT and dRVVT reagents.

2 | MATERIALS AND METHODS

2.1 | Plasma samples

The methods for blood collection and sample preparation were previously described.[9] We examined plasma samples from 105 non-anticoagulated patients who were LA-positive with routine diagnostic testing. Forty-four had definite APS, 12 were patients with systemic lupus erythematosus (SLE) and persistent LA, 9 had SLE and were LA-positive at the time of testing, and 40 were receiving diagnostic testing for aPL/APS in response to relevant clinical findings. Routine diagnostic testing for LA employed Life Diagnostics LA Screen and LA Confirm reagents, and Stago PTT-LA (Diagnostica Stago UK, Theale, UK) were employed for dRVVT test and the screen in dilute APTT (dAPTT), respectively and addition of Bio/Data Corporation LA Confirmation Reagent (Alpha Labs, Eastleigh, UK) for the confirmatory test were used for dAPTT. Screen and confirm clotting times were converted to normalized ratios by dividing test clotting times by reference interval mean clotting times.[8,9,15] Samples were recognized as LA-positive when one or both screening test ratios were elevated according to previously established local reference intervals, and corrected by ≥10% with their confirmatory test ratio.[8,14,15] All patients were positive in dRVVT and/or dAPTT. The LA assays were performed on CS-2000i analyzer (Sysmex UK, Milton Keynes, UK).

2.2 | Coagulation screening tests

Factor deficiencies and undisclosed anticoagulation were excluded by performing coagulation screening tests prior to the LA assays. APTT, prothrombin time (PT), thrombin time, and fibrinogen for Clauss method tests were measured by a Sysmex CS-2100i (Sysmex UK) using Actin FS, Dade Innovin, Thromboclotin, and Thrombin-Reagent (Siemens Healthineers, Marburg, Germany), respectively. As a LA-insensitive routine APTT reagent, Actin FS was employed and thus the reagent is suitable for exclusion of other cause of clotting time prolongation.[8,16]

2.3 | Additional LA assays

Four APTT reagents having high LA sensitivity, results from the routine dAPTT and dRVVT reagents, and an additional dRVVT screening reagent were included in this study. Thrombocheck APTT-SLA (SLA) (Sysmex Corporation, Kobe, Japan), Actin FSL (FSL) (Siemens Healthineers), APTT-SP (SP) (Instrumentation Laboratory Company, Bedford, MA, USA), and Cephen 2.5 LS (Cephen) (Hyphen BioMed, Neuville sur Oise, France) were the APTT reagents and LA1 Screening reagent (dRVVT B) (Siemens Healthineers) was the additional dRVVT.

TABLE 1 Characteristics of APTT, dAPTT and dRVVT reagents

Principles	Reagent	Abbreviation	Activator	Phospholipids
APTT	Thrombocheck APTT-SLA	SLA	Ellagic acid	Synthetic
	Actin FSL	FSL	Ellagic acid	Soy and rabbit phosphatides
	APTT-SP	SP	Silica	Synthetic
	Cephen 2.5 LS	Cephen	Silica	Undisclosed proprietary information
dAPTT	PTT-LA	PTT	Silica	Rabbit
dRVVT	Life Diagnostics LA Screen	dRVVT A	Russell's Viper Venom-X	Undisclosed proprietary information
	LA 1 Screening reagent	dRVVT B	Russell's Viper Venom-X	Undisclosed proprietary information

APTT, Activated Partial Thromboplastin Time; dAPTT, dilute APTT; dRVVT, diluted Russell's viper venom time.

The characteristics of these reagents are shown in Table 1. Actin FS (Siemens Healthineers) was used as a paired confirmatory reagent for the LA-sensitive APTTs. All samples with elevated APTT screen ratios achieved significant correction by the Actin FS ratio to confirm LA activity in undiluted plasma.[17–20] Similarly, LA2 confirmation reagent (Siemens Healthineers) confirmed LA activity in undiluted plasma for all samples with elevated LA1 screening results. Screen and confirm clotting times were converted to normalized ratios by dividing test clotting times by reference interval mean clotting times. These tests were measured by a CS-2400 (Sysmex Corporation).

2.4 | Mixing test

Normal and patient plasmas were mixed with NPP in a ratio of 1:1, and mixing tests were performed without incubation. CRYOcheck frozen Pooled Normal Plasma (Precision BioLogic Inc., Dartmouth, Canada) was used as the NPP. The mixing test ratios were calculated by dividing the clotting time of the mixture by that of the NPP to reflect the effect of any LA on the plasma in which it was mixed.[9] The ICA was calculated as follows: ([Screen 1:1 Mix [sec] – Normal Pooled Plasma [sec])/Screen Patient (sec).[8] All mixing tests were performed on the CS-2400 (Sysmex Corporation) employing the automatic dilution function. All elevated screen ratios indicating that the known LA was reacting in a given alternative reagent were followed with a mixing test whose data were converted to normalised ratio and ICA.

2.5 | Reference intervals and cut-off values

Cut-off values for screen, confirm, and mixing test in each additional reagent were determined from upper limits of the distribution of 50 normal samples in each reagent, and for ICA with the routinely employed LA reagents, ICA cut-off values were previously established and used in each additional reagent.[9,21–23] The normal donor plasmas were from sets of commercial frozen plasmas, Normal Donor Set (Precision BioLogic, Inc.).

2.6 | Statistical analysis

Data for the various parameters were compared using a Wilcoxon signed rank test, P-values below .01 were considered to be statistically significant. Reference intervals were calculated as the mean ± 2 standard deviations.[7,8,22,24–27] Shapiro-Wilk test was performed to confirm the Gaussian distribution of the reference intervals.

3 | RESULTS

3.1 | Cut-off values for each index and reagent

The cut-off values for screen ratio, mix ratio, ICA and confirm ratio for all reagents are shown in Table 2. The confirm ratio of the four APTTs were calculated from the same Actin FS ratio. The population distributions for the normal donor samples were confirmed as Gaussian.

3.2 | Screen ratios, mix ratios, and ICA of LA positive samples in each reagent

Screen ratios of the 105 LA positive samples were calculated in each reagent. Mixing tests were performed on the samples and assays where LAs were detected in undiluted plasmas. Mix ratio and ICA were calculated in each reagent and samples were grouped according to whether they were mixing test-positive by both MTC and ICA, MTC only or negative in both MTC and ICA (Table 3). The range, mean, and median values of screen ratio in undiluted plasma are shown in each group. Mean and median screen ratios were higher in samples positive by MTC and ICA than those positive by MTC alone in all reagents. There were no positive samples for inhibition by ICA alone in all reagents.

3.3 | Comparison of distribution in MTC and ICA

Mix ratio values for the groups of MTC- and ICA-positive, MTC-positive only, and MTC- and ICA-negative were compared (Figure 1).

	APTT				dAPTT	dRVVT	
	SLA	FSL	SP	Cephen	PTT	dRVVT A	dRVVT B
Screen ratio	1.13	1.12	1.15	1.09	1.20	1.17	1.12
Mix ratio	1.07	1.07	1.08	1.04	1.15	1.07	1.06
ICA	12.4	10.4	13.6	12.0	13.2	11.9	12.0
Confirm ratio	1.16				1.18	1.10	1.08

TABLE 2 Cut-off values for each index and reagent

The medians of those groups respectively in APTT and dAPTT were 1.45, 1.13, 1.07 for SLA, 1.16, 1.09, 1.06 for FSL, 1.61, 1.12, 1.06 for SP, 1.37, 1.07, 1.03 for Cephen, and 1.35, 1.18, 1.05 for PTT-LA. The median mix ratios for those groups respectively in dRVVT were 1.36, 1.11, 1.06, and 1.53, 1.13, 1.04 in dRVTT A and dRVVT B, respectively. The mix ratio values of samples positive in mixing test by both MTC and ICA were significantly higher than those of MTC only and negative in both MTC and ICA in all reagents. In addition, the values of MTC only positive were significantly higher than those of negative in both MTC and ICA in all reagents. The same analysis was performed for ICA data, and ICA values for the three groups were also compared (Figure 2). The medians of those groups respectively in APTT and dAPTT were 29.7, 10.3, 5.9 for SLA, 14.5, 9.7, 8.1 for FSL, 32.3, 11.6, 7.1 for SP, 25.2, 7.5, 4.4 for Cephen, and 26.0, 12.1, 3.9 for PTT-LA. The medians for each group respectively in dRVVT were 20.7, 8.2, 3.0 and 27.0, 7.6, 0.6 in dRVVT A and dRVVT B, respectively. The ICA values of samples positive in mixing test by both MTC and ICA were significantly higher than those of MTC only and negative in both MTC and ICA in all reagents. Although the values in two groups such as MTC only positive and negative in both MTC and ICA were under the cut-off value, the values in MTC only positive group were significantly higher than those of negative both MTC and ICA group in all reagents.

3.4 | Comparison of positivity in MTC and ICA

Frequencies of MTC and ICA positivity in samples that were LA-positive in undiluted plasma are shown for each reagent (Table S1). Overall MTC positivity combines the samples positive by both MTC and ICA and by MTC only. On the other hand, ICA positivity only includes those that were MTC- and ICA-positive because none were only ICA-positive. Frequency of MTC-positivity was higher for ICA in all reagents.

4 | DISCUSSION

The present study compared detection rates of LA in 1:1 mixing studies via MTC and ICA in multiple reagents. Without exception, MTC showed higher sensitivity than ICA in detection of known LA with every reagent. Mixing tests are recommended in all current guidelines[6–8] although different approaches are advocated. The guidelines of Lupus Anticoagulant/Phospholipid-Dependent Antibodies Subcommittee of the Scientific and Standardisation Committee (SSC) of International Society on Thrombosis and Haemostasis (ISTH)

advocates performing the mixing test immediately after recognizing an elevated screening test and the results of mixing test are suggestive of LA when their clotting times or ratios are above the local cut-off value, or when ICA is greater than the local cut-off value.[6] The British Society for Haematology (BSH) guideline also suggests performance of the mixing test in response to finding an elevated screening test and goes on to indicate the limitation introduced by the dilution effect and how LA can, in certain circumstances, be detected despite a negative mixing test.[7] On the other hand, the CLSI guideline supports initial performance of screening and confirmatory assays to evidence phospholipid dependence. If phospholipid dependence cannot be demonstrated at the LA confirmatory assay, or the confirmatory test and/or coagulation screen suggest the possibility of an alternative or co-existing abnormality, the mixing test is performed to assess for inhibition. The guideline also recommends Mix ratio or ICA for calculating and interpreting mixing test results.[8] Since two guidelines give substantiated statements that LA can be confidently detected without mixing tests in certain sample types yet all three indicate situations where they are diagnostically valuable and improve specificity, it is clinically crucial to investigate performance characteristics of mixing test indexes to maximize diagnostic efficacy. We evaluated 105 LA-positive samples to compare the detection rate of Mix ratio and ICA in multiple reagents and reagent types.

From screen and confirm data in undiluted plasma, between 33% and 63% of samples were positive in APTT-based assays and 62%-70% in dRVVTs (Table 3). This is a reflection of the well-described phenomena of antibody heterogeneity and reagent variability,[2,6–8,10–12,26–29] which at its most extreme, can result in a given dRVVT and APTT pairing detecting a particular antibody while another pairing would not. Screen and mix ratios and ICA values were similar between the two dRVVT reagents, possibly due, at least in part, to normalizing the data reducing between reagent differences.[28,29]

More samples were positive in mixing tests when applying MTC than ICA in all reagents studied. In addition, there were no positive samples for inhibition by ICA alone in any reagent. Therefore, it was considered that MTC had higher sensitivity than ICA for detection in the in vitro inhibition of LA, as previously described with single APTT and dRVVT reagent pairings.[9,30] The LA-sensitivity of a given reagent is a crucial contributor to the efficacy of mixing tests performed with it, and interpretation via MTC appears to enhance mixing test sensitivity. The mixing of weak LA samples may introduce loss of detectable LA activity and the false-negativity give rise to inaccurate interpretation if the mixing test result is employed as a decision point for subsequent confirmatory test performance.[14,31–33] For samples from

TABLE 3 Mixing test results of MTC and ICA for samples positive for lupus anticoagulant in undiluted plasma: (A) APTT and (B) dRVVT

(A)	APTT				dAPTT
	SLA	FSL	SP	Cephen	PTT
The number of LA positive in undiluted plasma (total 105 samples)	35 (33%)	60 (57%)	40 (38%)	61 (58%)	66 (63%)
Positive in mixing test by MTC & ICA	17 (49%)	40 (67%)	23 (58%)	33 (54%)	28 (42%)
Screen ratio in undiluted plasma (range)	1.16-2.64	1.14-2.14	1.17-5.42	1.19-3.50	1.23-3.82
Mean	1.83	1.41	2.21	1.74	1.86
Median	1.58	1.30	1.86	1.54	1.58
Positive in mixing test by MTC only	5 (14%)	6 (10%)	9 (22%)	18 (30%)	2 (3%)
Screen ratio in undiluted plasma (range)	1.19-1.31	1.17-1.44	1.22-1.44	1.10-1.38	1.35-1.59
Mean	1.23	1.25	1.30	1.19	1.47
Median	1.21	1.18	1.28	1.18	1.47
Negative in mixing test by MTC & ICA	13 (37%)	14 (23%)	8 (20%)	10 (16%)	36 (55%)
Screen ratio in undiluted plasma (range)	1.15-1.42	1.13-1.23	1.16-1.33	1.12-1.24	1.21-1.64
Mean	1.19	1.17	1.20	1.15	1.33
Median	1.16	1.16	1.18	1.15	1.28

| (B)	dRVVT	
	dRVVT A	dRVVT B
The number of LA positive in undiluted plasma (total 105 samples)	73 (70%)	65 (62%)
Positive in mixing test by MTC & ICA	34 (47%)	19 (29%)
Screen ratio in undiluted plasma (range)	1.24-3.26	1.19-3.79
Mean	1.80	2.08
Median	1.56	2.22
Positive in mixing test by MTC only	25 (34%)	28 (43%)
Screen ratio in undiluted plasma (range)	1.18-1.83	1.16-1.58
Mean	1.29	1.34
Median	1.26	1.32
Negative in mixing test by MTC & ICA	14 (19%)	18 (28%)
Screen ratio in undiluted plasma (range)	1.18-1.42	1.13-1.28
Mean	1.26	1.21
Median	1.25	1.22

patients with no other causes of clotting time prolongation and LA assay confirmatory test results within reference intervals, the increasingly popular paradigm of integrated testing is sufficient to detect the presence of LA without performing the mixing test at all.[6–9,26–28,31,33,34] However, in situations where alternative or co-existing coagulation abnormalities are present, mixing tests can improve specificity of LA testing.[34–37] While some authors contend that LA can be detected with integrated testing alone, even in situations such as anticoagulant therapy,[31,36] other studies have evidenced improved diagnostic accuracy where initial analysis is not clear cut.[34,38] The onus is on diagnostic practitioners to recognize when mixing tests can be omitted, and perform them where they will enhance interpretive and diagnostic outcomes.[7–9,14,26,27,34] Thus, it is valuable to maximise diagnostic efficacy of mixing tests, so for the present study, we specifically assessed mixing tests in the "ideal" situation of otherwise uncompromised samples. Cut-offs were generated using readily available statistical models
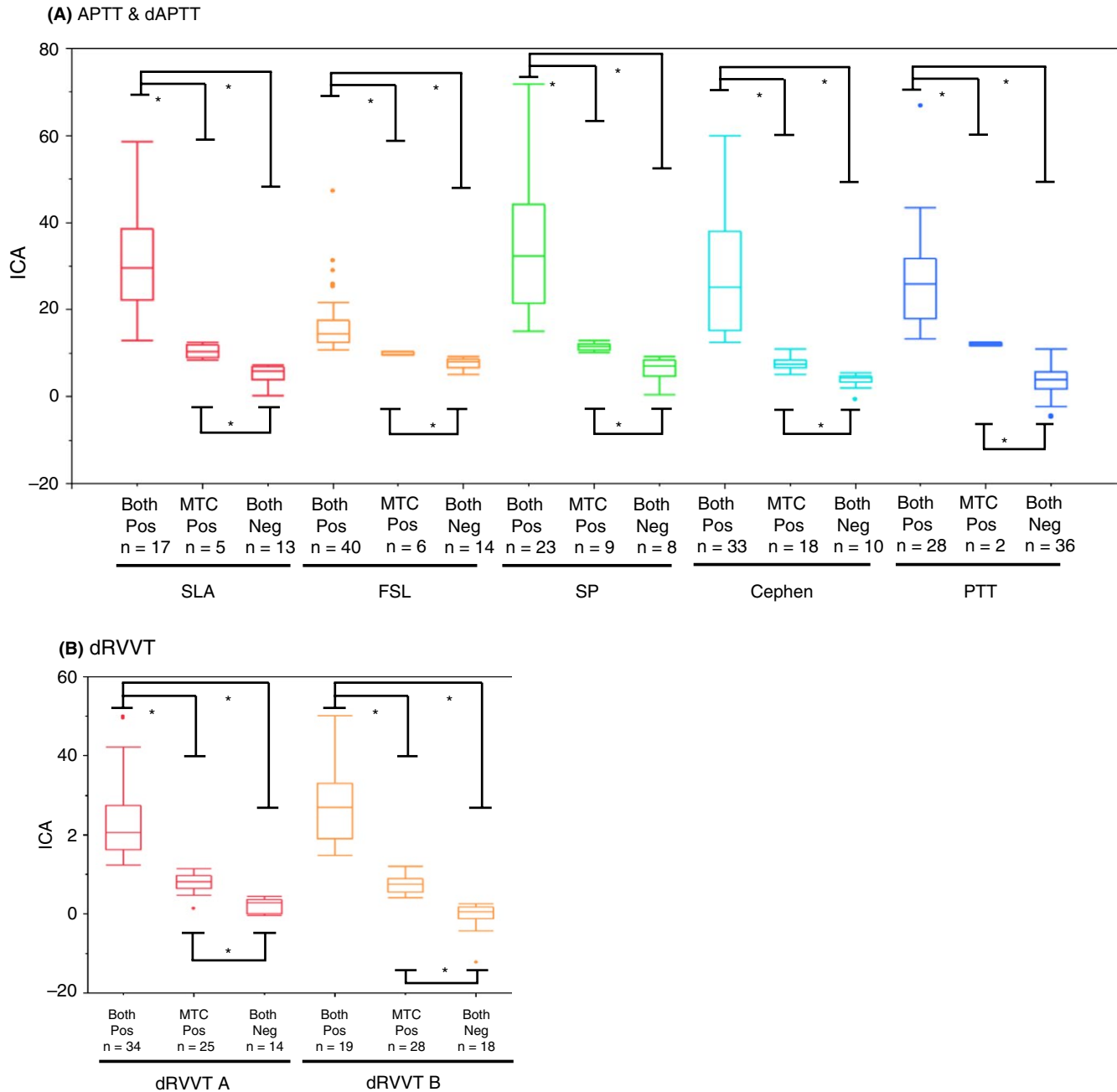
(A) APTT & dAPTT
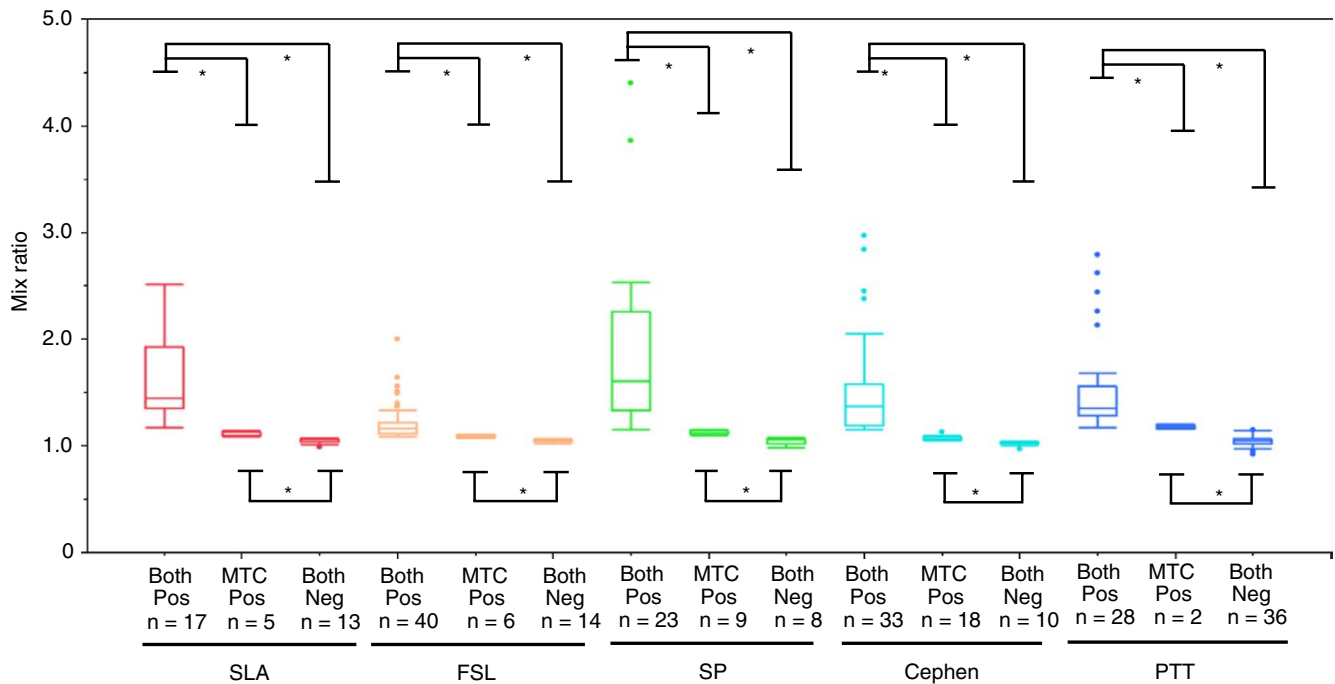
(B) dRVVT
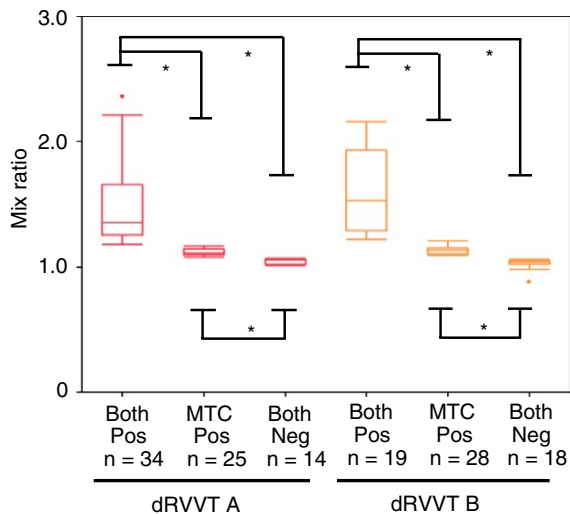
FIGURE 1 Comparison of Mix ratio values for each group in all reagents. Both Pos, positive in both Mix ratio and ICA; MTC Pos, positive in only Mix ratio; Both Neg, negative in both Mix ratio and ICA. The outliers were beyond the ± 2SD distribution in each group. Middle, lower and upper bars indicate median, minimum and maximum value for each group in the distribution. Asterisks indicate statistical significance (*P* < .01). (A) The boxes of red, orange, green, light blue and deep blue indicate SLA, FSL, SP, Cephen, and PTT, respectively. (B) The boxes of red and orange indicate dRVVT A and dRVVT B, respectively. ICA, index of circulating anticoagulant; MTC, mixing test specific cut-off; SLA, APTT-SLA; FSL, Actin FSL; SP, APTT-SP; Cephen, Cephen 2.5 LS; PTT, PTT-LA

from population distributions that are relatively easy to perform and recommended in guidelines as applicable to the routine diagnostic environment.[6–8,25] Further work is planned to assess LA mixing tests in other situations, including anticoagulant therapy and factor deficiencies, in order to additionally apply Receiver Operator Characteristic (ROC) curve analysis to cut-off generation.[23]

Mix ratios and ICA values were significantly higher than those positive only by MTC or negative by both indexes, although there was some cross-over. Using screen ratio in undiluted plasma as indicator of antibody potency, the data suggest that stronger antibodies are more likely to generate elevations of both MTC and ICA. However, there was a degree of cross-over where some samples with screen ratios in undiluted plasma that were close to cut-offs also generating elevated mixing test values, while some others with moderately elevated screen ratios did not. Manifestation in mixing tests seems to be a function of more than just potency, other possible contributory

FIGURE 2 Comparison of ICA values for each group in all reagents. Both Pos, positive in both Mix ratio and ICA; MTC Pos, positive in only Mix ratio; Both Neg, negative in both Mix ratio and ICA. (A) The boxes of red, orange, green, light blue and deep blue indicate SLA, FSL, SP, Cephen, and PTT, respectively. (B) The boxes of red and orange indicate dRVVT A and dRVVT B, respectively. ICA, index of circulating anticoagulant; MTC, mixing test specific cut-off; SLA, APTT-SLA; FSL, Actin FSL; SP, APTT-SP; Cephen, Cephen 2.5 LS; PTT, PTT-LA.

factors include epitope specificity, antibody avidity, and reagent composition.[9] In regard to clinical significance, Hong et al. suggested that positive mixing tests indicated a higher LA titer and conferred a higher thrombotic risk than in cases where the mixing test was negative.[31] Both MTC- and ICA-positive samples might have higher thrombotic risk than MTC only positive and both negative samples. On the other hand, a number of studies have indicated populations of clinically significant LA despite the negative results in the mixing tests[14,32,36] and the potency alone does not necessarily correlate with clinical significance and thrombotic risk.[37,39] We performed the LA assays in a

clinically select and appropriate cohort, 53.3% of whom had established persistent LA.

Ellagic acid-based APTT reagents have been reported to have lower LA sensitivity than those employing silica as activator,[6,40] although this has been questioned and is considered to be coincidental to the phospholipid composition of each reagent.[12] Tripodi et al. and Kershaw et al. investigated LA sensitivity of commercial APTT reagents such as Pathromtin SL, Synthasil IL, APTT LT, and KPTT in addition to the PTT, FSL, and SP used in this study.[40,41] Their results showed that the LA sensitivity of ellagic acid-activated FSL was lower than that of PTT and

SP, silica-based reagents. In the present study, performed on a much larger cohort of LA-containing plasmas, the percentage of LA detected in the FSL screening test was 57%, much higher than the 38% and 33%, respectively, of SP and SLA, and similar to that of Cephen and PTT silica-based reagents. In addition, positivity by both MTC and ICA was higher with FSL than all other reagents in the study. These data provide further evidence, in a large clinically appropriate population, that ellagic acid-based APTT reagents are not necessarily less effective than others in detecting LA.

In conclusion, our data indicate that MTC had higher sensitivity than ICA for detecting LA in multiple reagents. Whilst integrated testing can be diagnostically accurate and logistically attractive, there are situations where additionally undertaking mixing tests achieves accurate and more confident diagnoses. Although mixing tests introduce a dilution factor and may make weak LA samples appear negative, maximizing diagnostic capability of mixing tests for when they are needed, by applying the most sensitive interpretive index, improves the efficacy of LA detection.

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RELATIONSHIP DISCLOSURES

O. Kumano is an employee of Sysmex Corporation. G.W. Moore is a Coagulation Advisory Board member for Roche Diagnostics International Ltd.

AUTHOR CONTRIBUTION

O. Kumano performed the measurement, analysis and interpretation of the data, and wrote the manuscript. G. W. Moore designed the research, interpreted data, and critically revised the manuscript.

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SUPPORTING INFORMATION

Additional Supporting Information may be found online in the supporting information tab for this article.