Comparison of Six Dilute Russell Viper Venom Time Lupus Anticoagulant Screen/Confirm Assay Kits

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Abstract

Background The normalized dilute Russell viper venom time (DRVVT) ratio provides a robust assay methodology for lupus anticoagulant (LA) detection.

Objectives We evaluated six normalized DRVVT LA screen and confirm systems for inter-method consistency. Reagents were purchased from Diagnostica Stago, Inc. (Parsippany, NJ); Precision BioLogic Inc. (Halifax, Nova Scotia, Canada); Siemens Healthcare Inc. (Deerfield, IL); TCoag (Parsippany, NJ); Instrumentation Laboratories (Bedford, MA); and Sekisui Diagnostics (Pfungstadt, Germany).

Methods For all assays, we employed the STA-R Evolution automated coagulometer, adhering to manufacturers' instructions. LA-positive and LA-negative plasma controls were purchased from Diagnostica Stago and pooled normal plasma (PNP) was purchased from Precision BioLogic. We computed the mean of the reference interval (MRI) and action limits for all kits using LA-negative aliquots from locally sourced normal subjects ($n = 42$). We then assayed locally sourced LA-positive plasmas ($n = 43$) and using analysis of variance compared uncorrected screen/confirm ratios and screen/confirm ratios that were normalized using MRI and mean PNP results.

Results The grand mean action limit, MRI + 3 SD, derived from the local normal plasmas, was 1.2, confirming the manufacturers' recommended limits; however, limits must be locally computed. The all-sample p value was < 0.001, indicating heterogeneity among ratios. When Sekisui ratios were excluded, the p value was 0.14, thus indicating that this method introduced the major difference among methods. Mean screen/confirm ratios computed from LA-positive specimens were 1.91 to 2.04 for reagent systems other than Sekisui, which instead yielded a mean ratio of 1.198, indicating that this method was relatively insensitive to LA. A negative bias was recorded by two lots from the Sekisui system for LA-positive specimens. Screen/confirm ratios from combined LA-positive and LA-negative samples generated a combined range of 1.59 to 1.67 for all reagents except Sekisui, which instead yielded 1.09. The within-run percent coefficient of variation (CV%) was $< 5.0\%$ using all samples. Between-run CV% using Diagnostica Stago LA-positive and LA-negative controls was $< 5.5\%$.

Conclusions DRVVT screen/confirm ratios discriminate between LA-positive and LA-negative samples and generally provide acceptable reproducibility. Ratio results may vary among reagent–instrument combinations. In this study, normalization added little to the clinical result interpretation.

Keywords

- dilute Russell viper venom time
- lupus anticoagulant
- antiphospholipid antibody
- LA-sensitive partial thromboplastin time
- mean of the reference interval
- normalization

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Table 1 Recommendations for the optimal laboratory detection of LA

Choose two assays based on different laboratory principles: • Low-PL DRVVT is the first test considered • LA-sensitive low-PL aPTT with silica activator is the second test considered • Suspect that LA is present if either of the two tests is prolonged beyond the upper limit of the reference interval
When the screen is prolonged, confirmatory tests are performed using bilayer or hex-phase high-PL reagents: • Locally compute the mean percent corrections • Establish the 99th percentile reference limit by testing LA-negative plasmas using low- and high-PL (screen/confirm) reagents • Locally compute the individual percent corrections by using [(screen–confirm)/screen × 100]
If patient plasma % correction is ≥ 99th percentile correction, LA is confirmed

Abbreviations: aPTT, activated partial thromboplastin assay; DRVVT, dilute Russell viper venom time; LA, lupus anticoagulant; PL, phospholipid.

Antiphospholipid antibodies (APLs) are a family of immunoglobulins (primarily G isotypes) that bind phospholipid-bound proteins.[1,2] Lupus anticoagulants (LAs) are members of the APL family that prolong clot-based assays such as the activated partial thromboplastin time (aPTT) and occasionally the prothrombin time (PT).[3] Laboratory testing for LAs is common due to a relative high occurrence rate and their chronic presence associated with several arterial and venous thrombotic events (including transient ischemic attacks, ischemic cerebrovascular accidents, acute myocardial infarction, peripheral artery disease, venous thromboembolic disease, and recurrent spontaneous abortion).[1–3] In an effort to detect all relevant LAs, and since no single assay is 100% sensitive or specific, laboratory methodologies typically involve applying two clot-based low-phospholipid (PL) screening assays designed to be sensitive to LA.[4,5] Prolonged initial screens are then followed by high-PL confirmatory assays, designed to specifically neutralize LA. The most commonly employed two-test system is based on a LA-sensitive aPTT-based screen/confirm assay and a LA-sensitive dilute Russell viper venom time (DRVVT) screen/confirm assay.[1–4] Historically, other assays have been proposed, which used kaolin clotting time and tissue thromboplastin inhibition (dilute PT).[6] Although these are still used by many laboratories, the aPTT and DRVVT combination is the most currently used, to fulfill the International Society on Thrombosis and Haemostasis (ISTH) Scientific Standardization Committee guidelines, as synthesized in ►**Table 1**.[4]

The DRVVT may be a more robust LA test platform than the aPTT because it triggers coagulation at the level of factor X (►**Fig. 1**), thus bypassing potential interferences by deficiencies or inhibitors of factors VIII, IX, or XI. Due to LA heterogeneity, both assays are required, however.[1,7,8] The DRVVT is based on the time-honored Russell viper venom time, using a 1:500 dilution of the original venom reagent, and it is performed like a PT.[9,10]

Both the low-PL aPTT and the DRVVT screen/confirm systems are available in kit form.[11] We compared the capability of six commercially available DRVVT screen/confirm kits to detect LA in plasma aliquots from previously diagnosed LA patients. We also compared the validity of results using uncorrected screen/confirm clotting interval ratios and screen/confirm clotting interval ratios normalized to the mean of the reference interval (MRI) and pooled normal plasma (PNP) control results. The normalization approach is thought to correct differences in instrument–reagent combinations and is effective for improving discrimination between normal and positive LA samples.[11–13]

Materials and Methods

We evaluated six DRVVT LA screen/confirm reagents for assay consistency using the STA-R Evolution automated coagulometer (Diagnostica Stago, Inc. [DSI], Parsippany, NJ). We purchased kits from the distributors listed in ►**Table 2**. All respective kit testing was otherwise performed as per manufacturer recommendations.

We purchased positive and negative LA control plasmas from DSI. From Precision BioLogic Inc. (PBI, Halifax, Nova Scotia, Canada), we purchased strong and weak positive control plasmas and used PNP for daily precision testing.

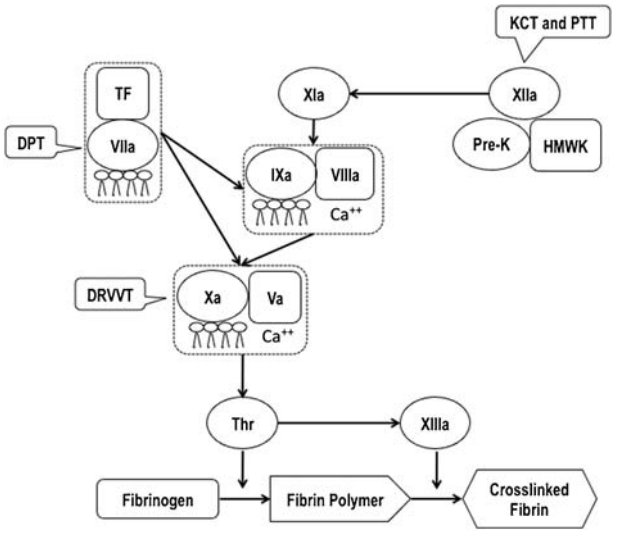

Fig. 1 Kaolin clotting time (KCT), partial thromboplastin time (PTT), dilute prothrombin time (DPT), and dilute Russell viper venom time (DRVVT). The plasma coagulation cascade illustrates that the KCT and activated PTT initiate coagulation at the level of factor XII, the DRVVT at factor X, and the DPT (tissue thrombin inhibition) at factor VII. HMWK, high-molecular-weight kininogen (Fitzgerald factor); Pre-K, prekallikrein (Fletcher factor); TF, tissue factor; Thr, thrombin (IIa).

Table 2 DRVVT screen/confirm kits purchased from six manufacturers/distributors

Distributor	Screen/confirm kit trademark	Identifier
Diagnostica Stago, Inc. (Parsippany, NJ)	STA Staclot DRVV Screen, STA Staclot DRVV Confirm	DSI
Precision BioLogic Inc. (Halifax, Nova Scotia, Canada)	CRYOcheck LA Check, CRYOcheck LA Sure	PBI
Siemens Healthcare Inc. (Deerfield, IL)	LA1 Screening reagent/LA2 Confirmation reagent	SI
Instrumentation Laboratory (Bedford, MA)	HemosIL LAC Screen, HemosIL LAC Confirm	IL
TCoag (Parsippany, NJ)	TriniCLOT Lupus Screen, TriniCLOT Lupus Confirm	TC
Sekisui Diagnostics LLC (Pfungstadt, Germany)	DVVtest, DVVconfirm	SK

Abbreviation: DRVVT, dilute Russell viper venom time.

We used aliquots of 43 locally sourced plasmas with documented LA positivity by low-PL aPTT and DRVVT screen/confirm assays from several manufacturers. Aliquots of 42 locally provided LA-negative plasmas were used to establish the MRI and standard deviation (SD). We used MRI + 3 SD to provide action limits, which approximates the 99th percentile as specified by the ISTH while using a reasonable number of normal subjects' aliquots. We assayed all controls, daily PNPs, and positive and negative LA plasma aliquots using identical sample volumes on the Stago Evolution.

Screen and confirm intervals in seconds were recorded for all six DRVVT screen/confirm kits and screen/confirm ratios were computed for each of the positive and negative plasmas. We also used the following formula to normalize screen/confirm ratios to the MRI and the PNP screen/confirm results.[9]

$$\text{MRI-normalized ratio} = \frac{\text{Subject screen/MRI screen}}{\text{Subject confirm/MRI confirm}}$$

Or

$$\text{PNP-normalized ratio} = \frac{\text{Subject screen /PNP screen}}{\text{Subject confirm/PNP confirm}}$$

where MRI-normalized ratio is the screen/confirm ratio normalized to the mean of the reference interval and PNP-normalized ratio is the screen/confirm ratio normalized to the value of the pooled normal plasma performed daily.

The normalized ratio is calculated by dividing the raw DRVVT screen/confirm values in seconds by the laboratory's MRI or PNP value for each reagent.

Results were compared using analysis of variance (ANOVA).

Results

The within-run percent coefficient of variation (CV%) for the combined LA-positive and LA-negative samples was $< 5.0\%$ and the between-run CV% for PBI-combined LA-positive and LA-negative controls was $< 5.5\%$. The MRIs for 42 normal subjects for all six kits and for uncorrected, MRI-normalized, and PNP-normalized ratios were statistically indistinguishable using the ANOVA test for within or between samples (►**Table 3**). The MRI + 3 SD action limits for the uncorrected screen/confirm ratio confirmed distributors' recommended cutoff of 1.2 (►**Table 3**). The MRI + 3 SD action limits for five of six kits, including uncorrected, MRI-normalized, and PNP-normalized ratios, were statistically indistinguishable using the ANOVA test for within or between samples (►**Table 3**), when the SK ratios were excluded. Two lots of SK reagents (Sekisui Diagnostics, Pfungstadt, Germany) generated a negative bias and lower action limits for normal samples when using uncorrected, MRI-normalized, or PNP-normalized ratios (►**Table 3**).

Mean results for MRI- and PNP-normalized ratios from five kits generated from the previously confirmed LA-positive samples were indistinguishable when the SK ratios were excluded (►**Table 4**). Mean results for the uncorrected screen/confirm ratios of LA-positive specimens (excluding SK ratios) showed within-run bias, attributable to the slightly lower PBI mean ratios (►**Table 4**).

The DSI kit correctly classified all LA-positive samples when using the uncorrected screen/confirm and the MRI- and PNP-normalized screen/confirm ratio limits. The IL kit (Instrumentation Laboratory, Bedford, MA) correctly classified all samples except when applying MRI-normalized ratio limits, whereupon one sample was classified as LA negative. The PBI, TC (TCoag, Parsippany, NJ), and SI (Siemens Healthcare Inc., Deerfield, IL) kits all misclassified two positive samples (the same two samples for each kit) using all three ratios. The SK kit misclassified 10 LA-positive samples as LA negative by all three ratios (►**Table 5**). The SK results generated a negative bias using two consecutive kits (►**Fig. 2**).

Discussion

We assayed a series of normal samples using six DRVVT screen/confirm kits and expressed the results as ratios, compared the ratios, computed the MRI for each, and established the ratio action limits for each as MRI + 3 SD. We used three means of ratio expression: uncorrected, MRI normalized, and PNP normalized. We also assayed 43 LA-positive specimens to compare results among the six kits.

The relevance of these findings to standard laboratory practice is highlighted by the following example of problems

Table 3 Normal MRI and +3 SD action limits for six DRVVT kits, uncorrected and normalized

A. 42 normal subjects' MRIs			
	MRI-normalized screen/confirm	PNP-normalized screen/confirm	Uncorrected screen/confirm
DSI	1.000	0.941	1.014
PBI	1.002	1.007	1.028
SI	1.000	0.983	1.048
IL	1.000	1.006	1.064
TC	1.000	1.035	1.060
SK	1.001	0.932	1.020
ANOVA revealed no significant differences between or within samples			
B. 42 normal subjects' normal MRI + 3 SD action limit			
	MRI-normalized screen/confirm	PNP-normalized screen/confirm	Uncorrected screen/confirm
DSI	1.157	1.088	1.173
PBI	1.189	1.195	1.220
SI	1.163	1.143	1.220
IL	1.163	1.170	1.237
TC	1.152	1.194	1.222
SK	1.147	1.068	1.118
Mean of uncorrected screen/confirm (confirms distributors' recommended value of 1.2)			1.198

Abbreviations: DRVVT, dilute Russell viper venom time; MRI, mean of the reference interval; PNP, pooled normal plasma; SD, standard deviation.
Notes: By ANOVA, the all-sample p is < 0.001; but when Sekisui ratios are excluded, $p = 0.14$.

associated with proper LA identification. The 2011 College of American Pathologists' (CAP) extended coagulation proficiency testing survey CGE-1 provided a PNP specimen diluted to 10% with cryoprecipitate. This resulted in 10 to 20% coagulation factors except for factor VIII, 33%; factor XIII, 27%; fibrinogen, 140 mg/dL; and VWF, 76%. The CAP PNP was negative for coagulation inhibitors and LA, confirmed by

Table 5 Percent of LA-positive specimens that are correctly classified using MRI + 3 SD as the action limit

	N	Correctly classified	Correctly classified (%)
SDI	43	43	100
PBI	42	40	95
SI	43	41	95
IL	41	41[a]	100
TC	42	40	95
SK	35	25	71

Abbreviations: LA, lupus anticoagulant; MRI, mean of the reference interval; SD, standard deviation.
[a]One specimen was incorrectly classified when using the MRI-normalized action limit.

independent DRVVT uncorrected screen/confirm ratios. Participants using the kaolin clotting time, dilute PT, PT, and aPTT 1:1 mix studies reported false abnormal (LA-positive) results at a rate of 57, 40, 28.4, and 25.3%, respectively. For the nonrecommended tests especially, factor deficiencies can cause false-positive LA results. Overall, 97 laboratories used the uncorrected DRVVT screen/confirm ratio; 86.6% of these correctly identified the specimen as normal. Of the 62 laboratories that used the normalized screen/confirm ratio (the normalization mean was unspecified), 89.9% correctly identified the specimen as normal.

From our data and the information provided in the 2011 CAP coagulation extended proficiency testing survey, we conclude that screen/confirm ratios and interpretations vary little among five of the six kits tested. It is essential, however, that for all methods in use, local action limits be established using normal samples. Action limits may be established using MRI + 3 SD, which approximates the 99th percentile while using a reasonable 40 LA-negative subject specimens. In two previous studies comparing DRVVT reagents, the SK kit was the least sensitive to weak LA-positive samples.[7,9] In our study, it was the least sensitive to all the positive LA specimens over the entire range of testing.

Table 4 MRIs for LA-positive plasma specimens for six DRVVT kits, normalized and uncorrected

	N	MRI-normalized screen/confirm	PNP-normalized screen/confirm	Uncorrected screen/confirm
DSI	43	2.010	1.891	2.038
PBI	42	1.829	1.838	1.877
SI	43	1.949	1.916	2.042
IL	42	1.845	1.856	1.963
TC	42	1.797	1.862	1.905
SK	35	1.314	1.224	1.281

Abbreviations: DRVVT, dilute Russell viper venom time; LA, lupus anticoagulant; MRI, mean of the reference interval; PNP, pooled normal plasma.
Notes: By ANOVA, both the normalized and uncorrected screen/confirm ratios produced a p value < 0.001. When Sekisui ratios are excluded, $p = 0.31$ for normalized ratios and 0.005 for uncorrected screen/confirm ratios.

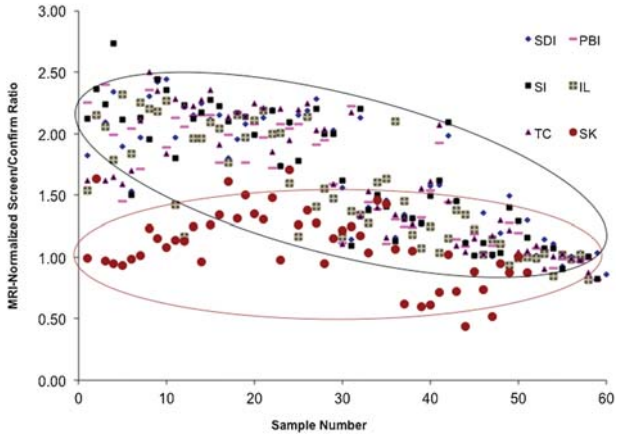

Fig. 2 MRI-normalized screen/confirm ratios for combined LA-positive and LA-negative samples. Ovals indicate divergence of Sekisui population from results of the other five kits, which instead provide similar patterns. LA, lupus anticoagulant; MRI, mean of the reference interval.

Conclusions

We recommend that all laboratories apply the DRVVT screen/confirm as a primary means for LA detection and confirmation as specified in ISTH guidelines. In distinction from the ISTH, we suggest that laboratories may in general avoid the added demands of screen/confirm normalization. Normalization adds mathematical complexity while offering little to reduce statistical variability within a method and adding little to clinical diagnosis providing that laboratories establish clear and appropriate cutoffs locally using the same methodology. Indeed, considering its low CV%, the clinical efficacy of DRVVT screen/confirm diagnosis exceeds that of the commonly used international normalized ratio (INR) and is enhanced by practice standards that include additional confirmatory steps. However, nonnormalized ratios may be disparate among methods. Therefore, normalized ratios are important for reducing inter-method differences. This would be important for cross-laboratory studies, potentially including proficiency testing exercises, when inter-method differences would produce higher inter-assay CVs using nonnormalized ratios. Accordingly, and similar to the INR, normalization will expectantly increase the portability of LA resulting across methods and across laboratories, but is not strictly required for within method LA identification.

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