Letter to the Editor

Improving Lupus Anticoagulant Detection in Heparinized Patients: An Automated Heparin-Resistant Recalcifying Solution

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received July 10, 2025 | **accepted after revision** September 12, 2025 | **article published online** 2025
Bibliography Semin Thromb Hemost **DOI** 10.1055/a-2701-0113 **Art ID** STH-25-0150-LET

Issue Theme Laboratory Diagnostics for Thrombosis and Hemostasis Testing—Part V **Guest Editors** Kristi J. Smock, MD, and Karen A. Moffat, BEd, MSc, ART, FCSMLS(D)

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Lupus anticoagulant (LA) detection poses significant challenges for diagnosis laboratories.

The International Society on Thrombosis and Haemostasis (ISTH) recommends using two chronometric assays for LA detection: activated partial thromboplastin time (aPTT) and dilute Russell's viper venom time (dRVVT).[1] Laboratories typically use paired assays, combining a screening test, which contains a low concentration of phospholipids, and a confirmation test, containing a higher phospholipids concentration. This pairing is designed to demonstrate the phospholipid dependence of any observed prolongation in clotting time.

Yet, only a limited number of paired aPTTs are commercially available, among which Silica Clotting Time (Werfen), actin FSL/Actin FS (Siemens), Staclot LA (Diagnostica Stago), Cephen LS/Cephen (Hyphen Biomed), or Cryocheck HexLA (Precision BioLogic). This could partly be the reason why only about a third of diagnosis laboratories conducting LA testing actually use a confirmation aPTT test,[2] as revealed by a recent study on external quality assessment.

Another major challenge in LA detection is anticoagulation, as most of these treatments interfere with clotting assays. Heparin, for instance, is found in up to 11% of samples referred for LA detection.[3] The latest ISTH guidelines on LA testing in anticoagulated patients[4] recommend quenching unfractionated heparin (UFH) in vitro using reagents that contain heparin neutralizers. However, while dRVVTs reagents generally include such neutralizers, most aPTTs do not. As a result, aPTTs are often sensitive to UFH, which may cause significant interference in LA detection at therapeutic levels. As we previously demonstrated with Cephen LS/Cephen,[5] this could potentially cause false-negative LA results. Such interference may also affect other aPTTs used for LA detection—e.g., actin FSL and actin FS[6]—or even reagents including heparin neutralizers—e.g., Staclot LA[7]—when UFH levels are supratherapeutic. Additionally, false-positive LA results have also been reported under such conditions.[8]

Previous studies have shown that, while activated charcoal is ineffective at removing UFH interference,[7] heparin neutralizers—such as polybrene,[6,9–11] heparinase (Dade Hepzyme for instance),[12,13] or protamine[10,11]—can mitigate this interference in aPTTs.

Hence, to address this issue, laboratories may manually add a heparin neutralizer to the sample. However, this procedure is time-consuming and lacks reproducibility. A more reliable alternative is to use an automated method. In this context, polybrene can be added either before activation of the contact phase (Staclot LA or HexLA) or after (Silica Clotting Time). However, a study has shown that polybrene may interfere with the activation of the contact phase, resulting in prolonged clotting times.[14] Therefore, adding polybrene after activation seems preferable. For instance, standard calcium, which triggers coagulation in aPTTs, can be replaced by a heparin-resistant recalcifying solution (HRRS), such as antiHepCa (Haematex). This solution contains polybrene and can neutralize, according to the manufacturer, up to 1.0 IU/mL of UFH. This innovative solution is compatible with most aPTT reagents.

To date, to our best knowledge, there are no published data evaluating its performance—or that, in general, of any commercially available, broadly adaptable and automated method that introduces polybrene after contact phase activation—for LA detection in heparinized patients.

Therefore, this study aims to assess whether performing a paired aPTT, namely Cephen LS/Cephen, using HRRS can effectively mitigate UFH interference without interfering in LA detection.

In this study, approved by the institution's Ethics Board (CE-2024-145), adult patients' samples who were referred to the Hematology Laboratory of Strasbourg's University Hospital (France) for LA investigation were included between March and May 2025. Exclusion criteria were as follows: underaged patients, insufficient plasma volume, patients anticoagulated with direct oral anticoagulants, or low molecular weight heparin.

Blood samples were drawn in 3.2% sodium citrate Vacuette PET tubes (Greiner Bio One, Kremsmünster, Austria). Platelet-poor plasma was collected after double spinning at 2,500 g for

10 minutes each, accordingly to the guidelines of the French Society on Thrombosis and Haemostasis.[15] Aliquots were quickly frozen and stored in polypropylene tubes at −20°C and analyzed within a week.

Anti-Xa activity (AXA) was measured using STA Liquid anti-Xa on a STA-R Max (both Diagnostica Stago, Asnières-sur-Seine, France). Cephen LS and Cephen (Hyphen Biomed, Neuville-sur-Oise, France) aPTTs were performed on a STA-R Max analyzer using either standard calcium 0.025 M $CaCl_2$ (Diagnostica Stago) or the HRRS AntiHepCa (Haematex, Horsnby, Australia), containing 0.025 M of $CaCl_2$ and polybrene. Briefly, 50 µL of plasma was incubated with 50 µL of Cephen LS or Cephen reagents for 240 seconds at 37°C, after which coagulation was triggered by the addition of 50 µL of either $CaCl_2$ or HRRS.

Positivity thresholds were determined using samples from healthy blood donors from Strasbourg's Etablissement Français du Sang. Cephen LS $CaCl_2$ and Cephen LS HRRS thresholds were defined as the 99th percentile of the ratio to the mean time of those healthy controls, while thresholds of normalized ratio (NR) $CaCl_2$ and NR HRRS were calculated as the 99th percentile of the ratios of Cephen LS/Cephen $CaCl_2$ or of Cephen LS/Cephen HRRS NR.

To assess whether HRRS (namely, AntiHepCa) effectively neutralizes UFH without compromising LA detection, AXA, Cephen LS $CaCl_2$/Cephen $CaCl_2$, and Cephen LS HRRS/Cephen HRRS were performed simultaneously on normal pooled plasma spiked with UFH or on patients' samples.

First, the effect of HRRS on Cephen LS and Cephen was evaluated on patients who were not anticoagulated with UFH and who were either LA positive or LA negative with Cephen LS/Cephen $CaCl_2$. This step aimed to assess whether HRRS could interfere in LA detection.

Second, spiking experiments were conducted using Cryocheck normal pooled plasma (Cryopep, Montpellier, France), which was spiked with increasing concentrations of UFH Héparine Choay (Cheplapharm, Levallois-Perret, France).

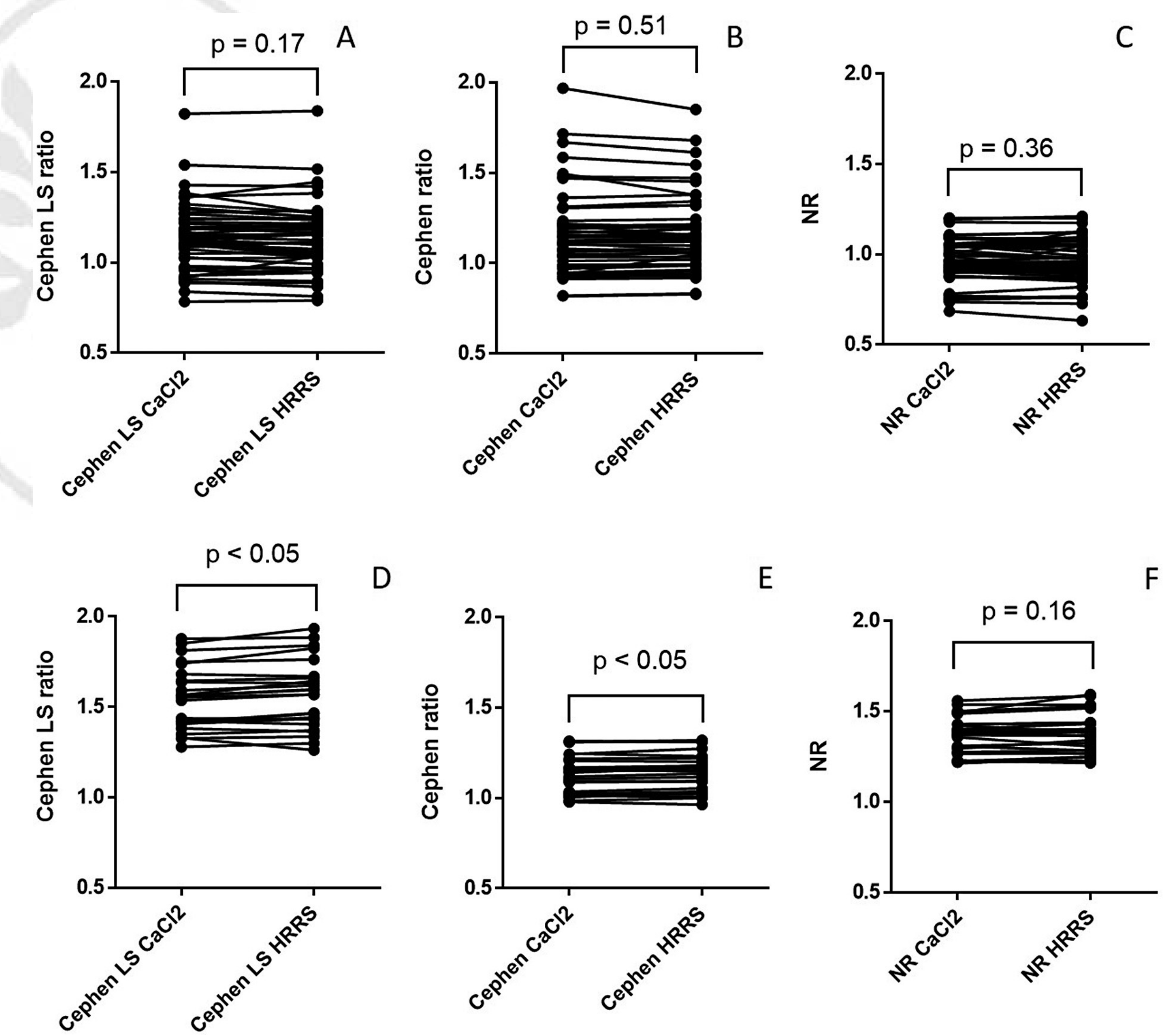

Fig. 1 Cephen LS, Cephen, and NR on samples from patients LA negative (**A**, **B**, and **C**, respectively) or LA positive (**D**, **E**, and **F**, respectively) using $CaCl_2$ or HRRS. HRRS, heparin-resistant recalcifying solution; LA, lupus anticoagulant.

Lastly, the effect of HRRS was assessed on patients anticoagulated with UFH to determine whether HRRS could efficiently mitigate the interference of UFH on Cephen LS/Cephen.

Statistical tests were performed on Prism v6.05 (GraphPad Software). Ratios of Cephen LS $CaCl_2$ versus Cephen LS HRRS and NR $CaCl_2$ and NR HRRS were compared using Wilcoxon or Student's *t*-tests on paired samples. Correlation between AXA and Cephen LS $CaCl_2$, Cephen $CaCl_2$, NR $CaCl_2$, Cephen LS HRRS, Cephen HRRS, and NR HRRS was tested using Pearson's test.

Samples from 40 healthy blood donors were used to determine positivity thresholds. Cephen LS $CaCl_2$ and Cephen $CaCl_2$ mean times were 34.5 and 32.0 seconds, respectively. Cephen LS $CaCl_2$ and NR $CaCl_2$ positivity cutoffs were 1.20 and 1.19. Cephen LS HRRS and Cephen HRRS mean times were 32.0 and 30.5 seconds, respectively. Cephen LS HRRS and NR HRRS positivity cutoffs were 1.20 and 1.21, respectively, which aligns with previous findings.[5,16]

First, we assessed the effect of HRRS versus $CaCl_2$ on 53 patients that was not anticoagulated with UFH and who were LA negative. Samples were evaluated with Cephen LS/Cephen using both $CaCl_2$ and Ca HRRS (**Fig. 1A–C**).

Mean [min–max] ratios of Cephen LS $CaCl_2$ (1.14 [0.78–1.82]), Cephen $CaCl_2$ (1.21 [0.82–2.48]), and Cephen $CaCl_2$ NR (0.97 [0.69–1.20]) were not statistically different from mean ratios of Cephen LS HRRS (1.14 [0.79–1.84]; $p = 0.58$), Cephen HRRS (1.20 [0.83–2.53]; $p = 0.83$), and NR HRRS (0.96 [0.63–1.21]; $p = 0.87$).

Second, the effect of HRRS versus $CaCl_2$ was assessed on 25 patients who were positive for LA with Cephen LS/Cephen $CaCl_2$, and who were not anticoagulated with UFH (**Fig. 1D–F**).

Mean ratios of Cephen LS $CaCl_2$ (1.56 [1.28–1.88]) and Cephen $CaCl_2$ (1.14; 0.98–1.32]) were statistically different ($p < 0.05$) from mean ratios of Cephen LS HRRS (1.59 [1.26–1.93]) and Cephen HRRS (1.15 [0.97–1.32]). However, these differences were within laboratory's reproducibility limits and did not alter LA interpretation. Meanwhile, mean NR $CaCl_2$ (1.37 [1.21–1.56]) was not statistically different from mean NR HRRS (1.38 [1.22–1.59]; $p = 0.16$).

Third, the effect of UFH on Cephen LS and Cephen was assessed by comparing Cephen LS $CaCl_2$, Cephen $CaCl_2$ and NR $CaCl_2$ to Cephen LS HRR, Cephen HRRS and NR HRRS on normal pooled plasma spiked with increasing concentrations of UFH. Cephen $CaCl_2$ clotting time was beyond measuring range whenever AXA was ≥ 1.0 IU/mL.

Cephen LS $CaCl_2$, Cephen $CaCl_2$, and NR $CaCl_2$ were significantly different ($p < 0.05$) from Cephen LS HRRS, Cephen HRRS, and NR HRRS, respectively.

Also, Cephen LS $CaCl_2$ ($r = 0.93$; slope = 4.77), Cephen $CaCl_2$ ($r = 0.92$; slope = 8.03), NR $CaCl_2$ ($r = 0.97$; slope = 0.67), Cephen LS HRRS ($r = 0.93$; slope = 0.07), Cephen HRRS ($r = 0.96$; slope = 0.18), and NR HRRS ($r = 0.78$; slope = 0.08) were significantly correlated to UFH levels ($p < 0.05$).

Neither Cephen LS HRRS nor Cephen HRRS were above positivity cutoffs up to an AXA of 1.2 IU/mL, indicating that UFH did not significantly prolong clotting times when using HRRS (**Fig. 2A**).

Finally, 32 patients who were anticoagulated with UFH were tested with Cephen LS/Cephen using $CaCl_2$ and HRRS.

Mean AXA was 0.35 IU/mL [0.10–0.79]. Cephen LS $CaCl_2$ ($r = 0.62$), Cephen $CaCl_2$ ($r = 0.74$), and NR $CaCl_2$ ($r = -0.76$) were correlated with AXA ($p < 0.05$), whereas Cephen LS HRRS ($r = -0.13$), Cephen HRRS ($r = -0.16$), and NR HRRS ($r = 0.14$) were not ($p = 0.50$, 0.39, and 0.44, respectively; **Fig. 2B**).

Mean ratios of Cephen LS $CaCl_2$ (1.86 [1.00–3.32]), Cephen $CaCl_2$ (3.2 [1.12–7.27]), and NR $CaCl_2$ (0.66 [0.41–1.03]) were statistically different ($p < 0.05$, **Fig. 3A–C**) from mean ratios of Cephen LS Ca HRRS (1.08 [0.89–1.29]), Cephen Ca HRRS (1.22 [1.01–1.54]), and NR Ca HRRS (0.89 [0.82–1.00]). Hence, using HRRS, an AXA up to 0.8 IU/mL did not seem to impact Cephen LS, Cephen or NR.

When comparing patients anticoagulated with UFH to LA-negative patients, no significant differences were observed in Cephen LS HRRS (1.08 vs. 1.14; $p = 0.08$) or Cephen HRRS (1.20 vs. 1.22; $p = 0.07$). However, the NR was significantly different between the two groups (0.96 vs. 0.89; $p < 0.05$), which might be due to a lack of population homogeneity.

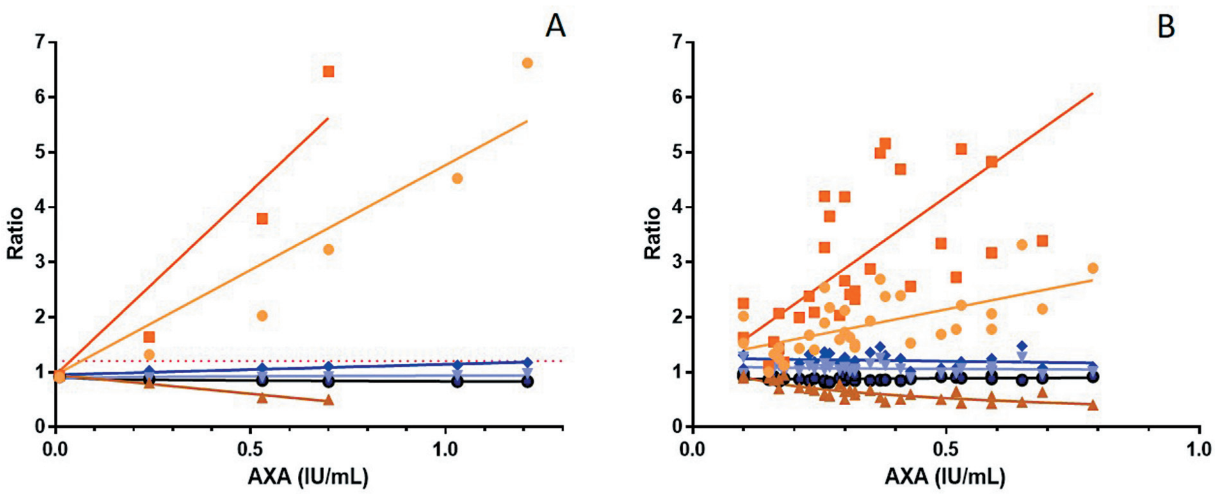

Fig. 2 ratios of Cephen LS $CaCl_2$ (●) or HRRS (▼), Cephen $CaCl_2$ (■) or HRRS (♦), and NR $CaCl_2$ (●) or HRRS (▲) according to AXA in normal pooled plasma spiked with increasing concentrations of UFH (**A**) or in patients anticoagulated with UFH samples (**B**). AXA, anti-Xa activity; HRRS, heparin-resistant recalcifying solution; UFH, unfractionated heparin.

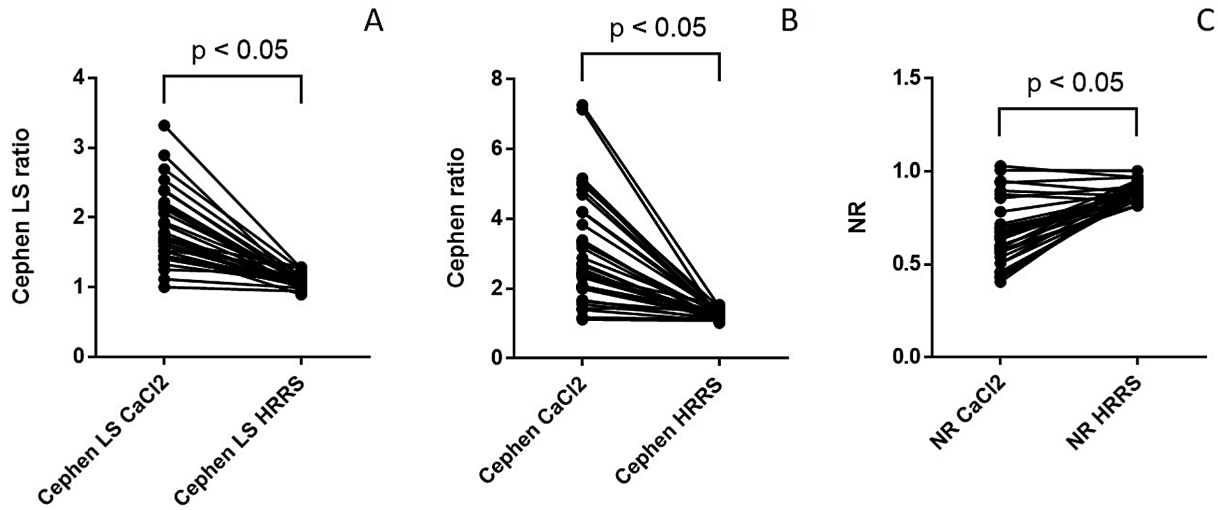

Fig. 3 Cephen LS (**A**), Cephen (**B**), and NR (**C**) on samples from patients anticoagulated with UFH using $CaCl_2$ or HRRS. HRRS, heparin-resistant recalcifying solution; UFH, unfractionated heparin.

Our findings support the use of HRRS to minimize UFH interference during LA testing with Cephen LS/Cephen. First, we demonstrated that using HRRS instead of a standard calcium did not interfere on Cephen LS, Cephen, or NR interpretation on LA negative samples, which is consistent with previous findings.[11]

Second, we showed on LA-positive samples (from nonanticoagulated patients) that no significant difference was observed between NR using HRRS or $CaCl_2$. Although Cephen LS and Cephen $CaCl_2$ were statistically different from Cephen LS and Cephen Ca HRRS, differences were within laboratory's reproducibility limits and did not modify the interpretation of LA detection for any of those samples, and hence no clinically relevant difference was observed, as previously described.[6,11]

Third, we demonstrated that, when using HRRS instead of $CaCl_2$, UFH did not impact Cephen LS and Cephen on both normal pooled plasma spiked with UFH (up to an AXA of 1.2 IU/mL) and on samples from patients anticoagulated with UFH (up to an AXA of 0.8 IU/mL). These findings align with those of previous studies, who had demonstrated that adding polybrene could normalize aPTT ratios from samples spiked with UFH.[6,11]

Last, we highlighted that Cephen LS HRRS and Cephen HRRS were comparable between patients anticoagulated with UFH and non-UFH anticoagulated LA-negative patients, although NR were not—which might be due to a lack of population homogeneity.

However, it was noted that HRRS tended to slightly shorten aPTTs clotting times. This phenomenon has been previously described in literature,[9–11] depending on the concentration of polybrene used (which, in antiHepCa HRRS, is not specified by the supplier). Therefore, laboratories should be cautious and systematically establish assay-specific positivity cutoffs when using HRRS.

This study presents several limitations: first, we were not able to determine positivity cutoffs using 120 healthy donors as recommended by the ISTH,[1] due to difficulty in obtaining such samples. Second, we did not perform a mixing step. Lastly, experiments were conducted using a single batch of HRRS, Cephen LS, and Cephen.

In conclusion, this study emphasizes the interest of performing aPTTs in LA detection using a calcium containing a heparin neutralizer, to mitigate the interference of UFH. Indeed, unlike manual neutralization methods (such as adding heparinase), which are costly, time consuming, and poorly reproducible, this approach is automated, easy to implement, and compatible with most LA testing workflows.

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Statements and Additional Information

Conflict of Interest The authors declare that they have no conflict of interest.

Contributors' Statement A.H. designed the study, analyzed data and wrote the manuscript. N.D. and A.R. collected data. J.W., L.S., and L.M. revised intellectual content.

Funding Information Strasbourg University Hospital supported this study. This sponsor had no role on the design, methods, subject recruitment, data collections, analysis, or preparation of this report.

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