Comparison of Hexagonal Phase Phospholipid Neutralization Assays for Lupus Anticoagulant Detection

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Background

Numerous assays exist to detect lupus anticoagulants (LA), varying greatly in their principle, sensitivity, and specificity. One such assay, the hexagonal phase phospholipid neutralization test (HPNT), detects LA by measuring shortened activated partial thromboplastin times (aPTT) in the presence of excess hexagonal phase phospholipid. We compared two different HPNTs using a range of both LA positive and LA negative plasmas as well as plasmas referred for LA testing of unknown status. We also tested plasmas spiked with common coagulation interferents.

Staclot LA® is an HPNT sold by Diagnostica Stago (Asnières-sur-Seine, France). Staclot LA is sold as a five-component lyophilized kit and can be stored long term at 4 °C. Reconstitution of the kit takes 30–60 minutes.

CRYO*check*™ Hex LA is an HPNT marketed by Precision BioLogic (Dartmouth, Canada). Hex LA is a three-component kit that is presented in a frozen, ready-to-use format. It requires 5 minutes of thawing time and is ready to use after 15 minutes total. It is intended for use on various automated coagulation analyzers.[1]

Methods

We measured 446 venipuncture-collected plasma samples in 3.2% citrate, representing a mix of LA negative and LA positive plasmas, using two different HPNTs, both on the Stago STA-R Evolution® automated coagulation analyzer.

The measured corrections of each plasma were interpreted relative to the assay's cut-off.

We also quantified the effects of common interferents in coagulation testing: C-reactive protein (CRP); heparin (low molecular weight and unfractionated); and the direct oral anticoagulants dabigatran and rivaroxaban.

Results

Staclot LA and CRYO*check* Hex LA showed excellent agreement across both LA negative and LA positive plasma samples. Of the 308 samples negative by Staclot LA, 294 were negative by CRYO*check* Hex LA as well. Similarly, 129 of 135 LA positive samples by Staclot LA were also LA positive by Hex LA.

Heparins (2 IU/mL), dabigatran (200 ng/mL) and rivaroxaban (400 ng/mL) interfered with both assays similarly. The correction of LA negative samples spiked with anticoagulant drugs remained below both assays' cut-offs, but LA positive samples showed an exaggerated correction relative to the matrix-matched control plasmas. Elevated concentrations of C-reactive protein (CRP) showed potential to interfere with the interpretation of the assays.

Using CRYO*check* Hex LA, up to 40 mg/L of added CRP has no significant effect on the interpretation of an LA negative plasma sample. Staclot LA was significantly more sensitive to CRP interference, with LA negative plasma reading falsely positive by Staclot LA at 25 mg/L of added CRP. In both assays, elevated CRP concentrations made LA positive samples look more pronounced.

Conclusions

We observed excellent agreement between the two HPNTs when they are used to measure clinical samples. However, labs must be cautious when interpreting HPNT results, ensuring that the potential for various interfering substances, especially elevated CRP, has been considered. CRYO*check* Hex LA provides HPNT results with less potential for interfering agents and offers improved specificity without compromising on accuracy.

References

1. C.D. Douglas, R. Clarke, N. Kesavan, D. Lamont, A. Sadeghi-Khomami, A. Wood, K.M. Black (July 2020). *Laboratory Validation of a Novel Hexagonal Phase Phospholipid Neutralization Assay for Lupus Anticoagulant Detection.* Poster PB0675, presented at the ISTH Virtual Congress.

Acknowledgements

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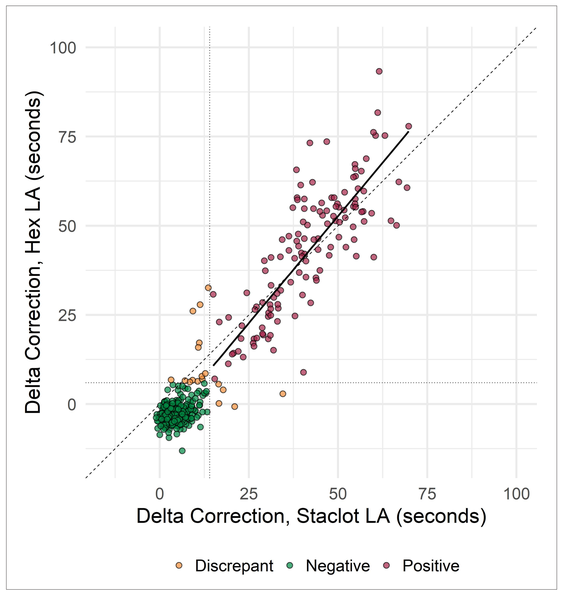
Method Comparison

Comparison of 446 plasmas tested by two different HPNTs. Plasmas that tested negative by both assays are shown in **green**, while plasmas positive by both assays are shown in **red**. Discrepant results are shown in **orange**. The **solid black line** shows the line of best fit for LA positive plasmas (slope = 1.16, $R^2 = 0.868$), and the **dashed black line** shows the line of perfect correlation (i.e., y = x). Plasmas with absolute corrections greater than 100 seconds (n = 3) are removed for clarity.

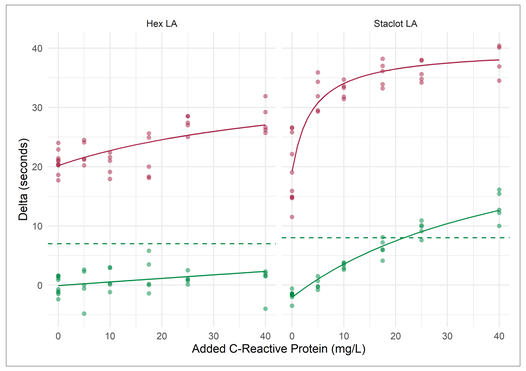
	Agree / Total	% Agreement	95% Conf. Int.
Negative	295 / 310	95.2%	92.1 – 97.3%
Positive	130 / 136	95.6%	90.6 – 98.4%
Total	425 / 446	95.2%	92.8 – 97.1%



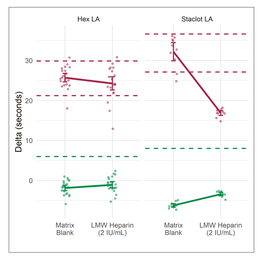
Interferents

Effects of five common interferents on two HPNTs. Effects on LA negative plasma are shown in **green**, while the effects on LA positive plasma are shown in **red**. The **red dashed line** shows a drift of ± 20% of the correction of the LA positive plasma, and the **green dashed line** represents the assay- and lot-specific cut-off, the point above which a result should be treated as a positive.

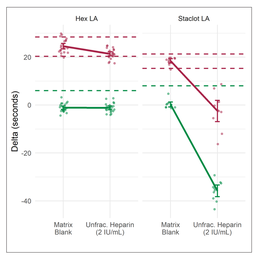
C-Reactive Protein



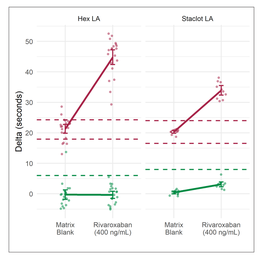
Low Molecular Weight Heparin



Unfractionated Heparin



Rivaroxaban



Dabigitran