

Effects of Common Interferents in Hexagonal Phase Phospholipid Neutralization Testing

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Aim

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), has been described as having desirable diagnostic sensitivity, but inferior specificity.¹ We examined the effects of common coagulation interferents on two different HPNTs to understand how they may affect the interpretation of results in LA testing.

Method

Patient plasmas that were negative and weakly positive for LA were spiked with high concentrations of common coagulation interferents. The plasmas were then tested using Stago's Staclot[®] LA and Precision BioLogic's cryocheck[™] Hex LA reagents on a Stago STA-R Evolution[®] automated analyser, as per the manufacturer's instructions. In the case of C-reactive protein, the dose-dependent effect of the interferent was quantified via titration.

Results

- The most common coagulant interferents (i.e., hemolyzed, icteric and lipemic samples) had negligible effects on either HPNT assay.
- Oral anticoagulants, rivaroxaban and dabigatran, showed interference with LA positive samples as an increase in delta result on both assays, but this did not affect the diagnostic outcome.
- Heparin caused substantial interference with Staclot LA but did not interfere with Hex LA.
 - The impact of the interference with Staclot LA was dependent on the type of heparin — unfractionated heparin may cause false negative results by decreasing the apparent delta result.
- C-reactive protein (CRP) interfered with both assays, regardless of the LA status of the sample.
 - Particularly noteworthy was that CRP had a statistically significant effect on the Staclot LA result at concentrations as low as 3 mg/L.
 - With Staclot LA, CRP interference impacting diagnostic outcome was observed at CRP concentrations of ≥ 20 mg/L, despite a higher assay cut-off. This interference causing false positive results occurred at considerably lower concentrations of CRP than observed in previous studies.^{2,3} In contrast, interference impacting diagnostic outcome was not observed with Hex LA until concentrations of ≥ 40 mg/L CRP were added.

Conclusions

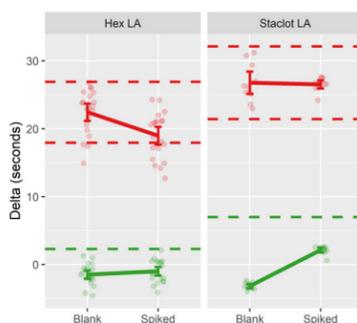
Labs must be cautious when interpreting HPNT results in the detection of LA, ensuring that the potential for various interfering substances has been accounted for. This includes, but is not limited to, elevated C-reactive protein concentrations due to inflammation, and patients being treated with heparin.

References

- Banki K, Aleksiejuk P, Patel J, Perl A. Hexagonal Phase Phospholipid Neutralization Assay Is the Most Sensitive but Least Specific Among Nine Tests for Detecting APS in SLE or Non-SLE Patients [abstract]. *Arthritis Rheumatol.* 2016; 68 (suppl 10).
- Schouwers SME, Devreese KMJ, Delanghe JR. Lupus anticoagulant testing in patients with inflammatory status: Does C-reactive protein interfere with LAC test results? *Thrombosis Research.* 2010; 125: 102–104.
- ECAT Foundation International External Quality Assessment Programme in Haemostasis and Thrombosis. Exercise 2010–4. Print date 28 Feb 2011.

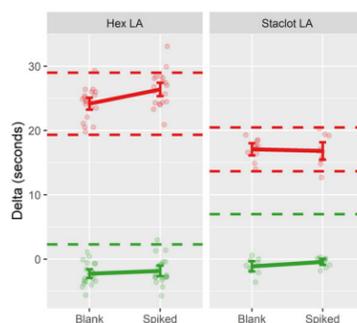
Hemolysis

(as hemoglobin, 500 mg/dL)



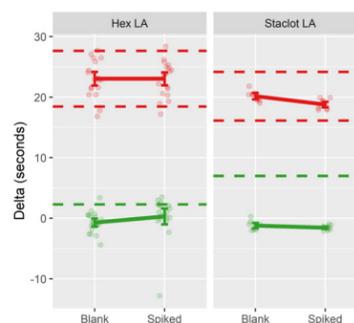
Icterus

(as unconjugated bilirubin, 20 mg/dL)



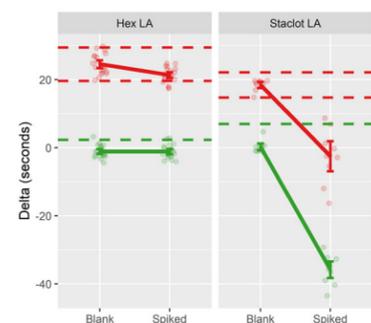
Lipemia

(as Intralipid, 500 mg/dL)



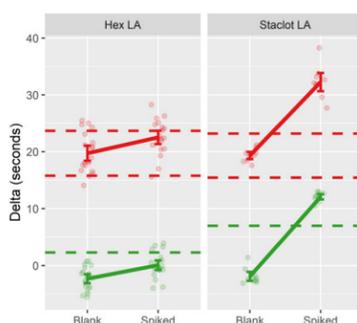
Unfractionated Heparin

(at 2 IU/mL)



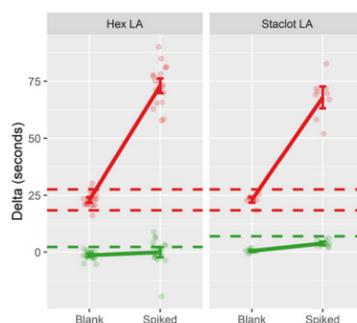
C-Reactive Protein

(added at 40 mg/L)



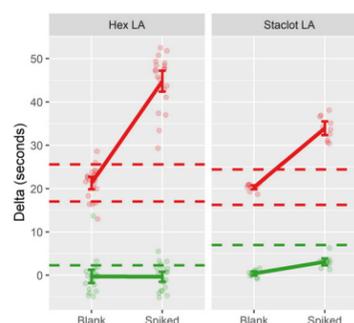
Dabigatran

(at 200 ng/mL)



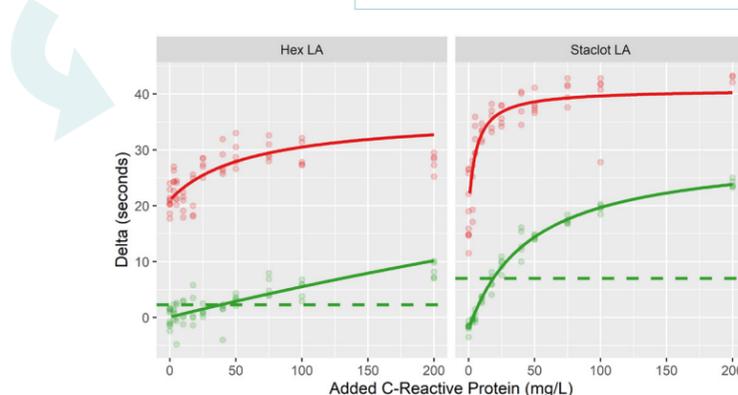
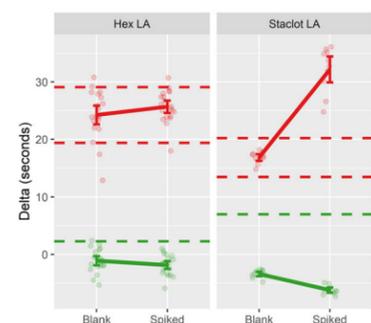
Rivaroxaban

(at 400 ng/mL)



LMW Heparin

(at 2 IU/mL)



The figures here show a comparison of samples that have been:

- spiked** with a high concentration of interferent
- blanked** with just the interferent matrix (e.g., buffer or solvent)
- The **green dashed lines** represent the lot-specific cut-off for each assay, above which a result should be interpreted as positive for lupus anticoagulant
- The **red dashed lines** represent a change of +/- 20% of the test result for the weak LA positive plasma sample