Effect of High-Dose Direct Anticoagulant Drugs and DOAC-Remove™ on Lupus Anticoagulant Detection by a Hexagonal Phase Phospholipid Assay

Abstract: PB1147

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Background

Direct oral anticoagulants (DOACs) and Argatroban are known to interfere with Lupus Anticoagulant (LA) testing. CRYOcheck™ Hex LA™ is a hexagonal phase phospholipid neutralization test (HPNT) with a heparin neutralizer.[1] LA is detected in citrated plasma samples by a Delta Correction (ΔCT) of a prolonged APTT using hexagonal phase phospholipid.

Previously, we reported that Dabigatran (200 ng/mL) and Rivaroxaban (400 ng/mL) did not diagnostically impact the assay, although DOACs could prolong the clotting times (CTs) and ΔCT of LA-positive samples.[2] The capacity and diagnostic compatibility of DOAC-Remove, an activated charcoal anticoagulant neutralizer,[3] in treating samples prior to testing by the Hex LA assay is unknown.

Objective

To evaluate the effect of high-doses of direct anticoagulant drugs (500 ng/mL and 1000 ng/mL) on the detection of LA by CRYOcheck Hex LA, with and without DOAC-Remove treatment.

Methods

LA-positive plasma was pooled from three unique donors who tested positive for lupus anticoagulant by multiple assays. LA-negative plasma was pooled from 21 unique normal donors.

LA-negative and LA-positive pooled plasma samples were spiked at two final concentrations of 500 ng/mL and 1000 ng/mL for each of Argatroban, Dabigatran, Apixaban, Edoxaban, and Rivaroxaban **(Table 1)**.

Each sample was tested seven times using the Hex LA assay (Precision Biologic, Dartmouth, Canada) on a Stago STA-R Evolution analyzer, with and without DOAC-Remove (5-Diagnostics, Switzerland) treatment per manufacturers' instructions.

DP-Filter, a DOAC filtration device, was also used in our pre-evaluation study. However, its DOAC neutralization performance was inferior to DOAC-Remove and further evaluation was not performed.

Results

In the absence of anticoagulant drugs, the effect of DOAC-Remove treatment vs. non-treatment on ΔCT was not statistically significant ($p < 0.05$) for either neat LA-negative (2.0 vs 2.9 seconds) or LA-positive samples (31.5 vs 29.9 seconds). This indicates that DOAC-Remove does not adversely impact the LA analytical sensitivity of the Hex LA assay.

Direct anticoagulants increased the ΔCT results in a dose-dependent manner; the increase was noticeably higher in LA-positive samples compared to LA-negative, with Dabigatran having the largest ΔCT increase for both sample types **(Table 2)**.

Table 1.

Anticoagulants tested, with final concentrations in plasma vs. reported peak concentration from PK studies.

Anticoagulant tested	Tested anticoagulant concentrations (ng/mL)	95th percentile, anticoagulant peak concentration* (ng/mL)[4,5]
Argatroban	500, 1000	538.6
Dabigatran	500, 1000	443
Apixaban	500, 1000	321
Edoxaban	500, 1000	250
Rivaroxaban	500, 1000	419

*All were determined by HPLC-MS/MS, except Argatroban (C_{max} mean) which was measured by HPLC-fluorescence assay

Conclusions

Hex LA accurately detected LA even in the presence of high-dose direct anticoagulants (500–1000 ng/mL). However, the presence of such anticoagulant drugs can increase the ΔCT values, which may approach the assay cut-off.

In this *in vitro* research study, DOAC-Remove mitigated CT prolongation caused by direct anticoagulant drugs and reverted Hex LA results to those observed in the absence of such drugs in both LA-negative and LA-positive plasma samples. Thus, LA detection by the Hex LA assay is compatible with plasma samples treated with DOAC-Remove.

References

1. CRYOcheck Hex LA™ Instructions for Use. Precision Biologic Inc.; 2023.
2. Colin Douglas, Rachel Clarke, Navya Kesavan, Derek Lamont, Ali Sadeghi-Khomami, Amanda Wood, and Karen M. Black. *Comparison of Hexagonal Phase Phospholipid Neutralization Assays for Lupus Anticoagulant Detection.* Presented at THSNA Summit (October 2020).
3. DOAC-Remove™ Instructions for Use. 5-Diagnostics.; 2023.
4. B.T. Samuelson, A. Cuker, *Blood Reviews* 2017, *31*, 77.
5. Swan SK, Hursting MJ, Pharmacotherapy 2000, 20, 318.

Table 2.

Hex LA assay results of plasma samples containing high-dose of direct anticoagulants, without and with DOAC-Remove treatment.

The addition of direct anticoagulants to both LA-negative and LA-positive plasma samples increased clot times measured for the Start and Correct components of the Hex LA assays.

The increase in CT was found to be more pronounced in LA-positive samples across all anticoagulant drugs tested **(Figure 1)**.

Plasma type	Anticoagulants	Concentration (ng/mL)	Hex LA ΔCT (Mean ±SD, N=7)	
			Without DOAC-Remove	With DOAC-Remove
LA-negative	Argatroban	500	7.1 ± 2.7	2.1 ± 1.2
		1000	9.0 ± 2.0	2.6 ± 1.6
	Dabigatran	500	13.5 ± 2.8	2.1 ± 0.9
		1000	16.4 ± 2.5	2.8 ± 1.3
	Apixaban	500	5.2 ± 1.2	3.0 ± 1.5
		1000	4.6 ± 2.1	2.2 ± 1.4
	Edoxaban	500	7.8 ± 1.3	1.2 ± 1.0
		1000	6.2 ± 1.1	1.3 ± 1.1
	Rivaroxaban	500	7.6 ± 2.0	2.9 ± 2.0
		1000	9.2 ± 3.0	1.6 ± 1.7
LA-positive	Argatroban	500	72.9 ± 1.9	32.8 ± 1.7
		1000	85.9 ± 7.2	32.8 ± 3.1
	Dabigatran	500	117.8 ± 7.7	27.0 ± 5.6
		1000	150.1 ± 3.2	32.3 ± 2.9
	Apixaban	500	50.2 ± 4.3	30.3 ± 2.4
		1000	69.0 ± 3.9	31.1 ± 1.9
	Edoxaban	500	68.8 ± 4.2	30.8 ± 3.2
		1000	83.4 ± 5.9	30.5 ± 2.1
	Rivaroxaban	500	77.9 ± 10.8	31.8 ± 2.3
		1000	109.4 ± 6.7	31.1 ± 1.6

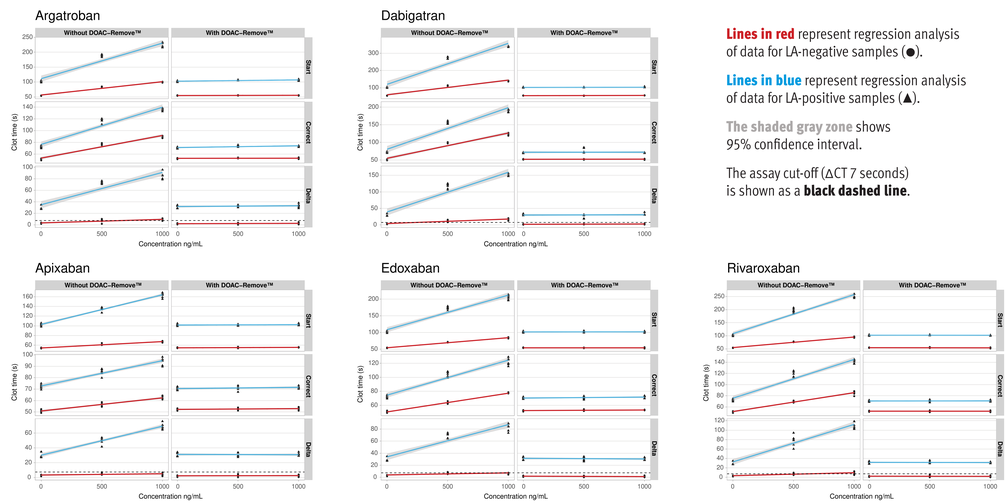
Figure 1.

Hex LA Start, Correct, and Delta CT Results (N=7) of various direct anticoagulants.

Plasma samples containing 500 ng/mL and 1000 ng/mL anticoagulants were tested using the CRYOcheck Hex LA kit.

In this study, which examined *in vitro* contrived plasma samples spiked with anticoagulant drugs, DOAC-Remove prevented the prolongation of CT and ΔCT caused by DOAC interference in both LA-negative and LA-positive samples in the Hex LA assay.

The effect of DOAC-Remove was observed at both 500 ng/mL and 1000 ng/mL concentrations for all anticoagulant drugs tested in this study.



Lines in red represent regression analysis of data for LA-negative samples (●).

Lines in blue represent regression analysis of data for LA-positive samples (▲).

The shaded gray zone shows 95% confidence interval.

The assay cut-off (ΔCT 7 seconds) is shown as a **black dashed line**.