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

Abstract: PB1147

**Mushfiqur Rahman, Ali Sadeghi-Khomami, Karen M. Black:** Precision BioLogic Inc., Dartmouth, Nova Scotia, Canada

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PrecisionBioLogic

## 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 |

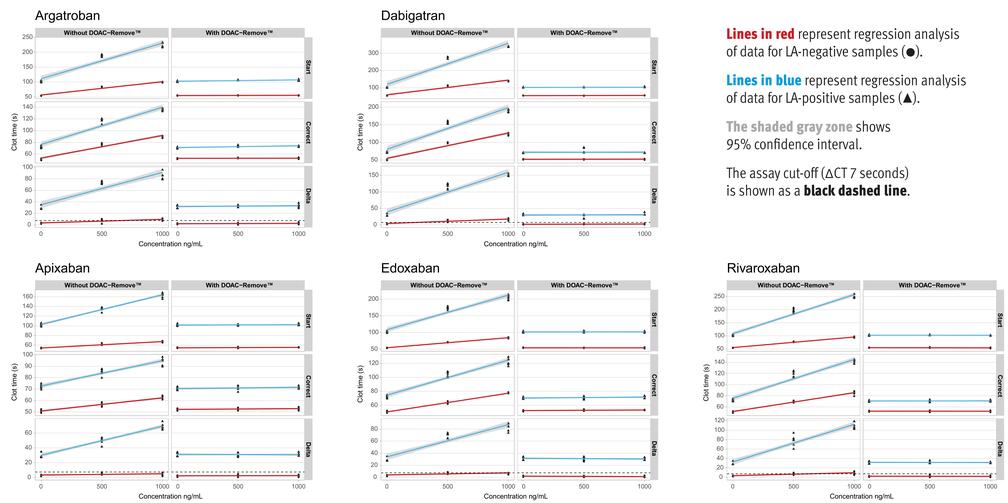
**Figure 1.**

**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**.