

# Effects of Anticoagulants and Common Interfering Substances on Four Commercially Available dRVVT Assays

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## Background

Antiphospholipid syndrome (APS) is defined as the persistent (>12 weeks) presence of circulating AP antibodies and a history of thrombosis or pregnancy morbidity.<sup>1</sup> The clinical manifestations correlate best with PL-dependent clotting assays, i.e. lupus anticoagulant (LA) testing.<sup>2</sup> The dilute Russell's Viper Venom Time (dRVVT) test is a popular LA assay for detection of thrombotic risk factors. Anticoagulant-free plasma samples are ideal for LA testing but not always available because anticoagulant therapy is a common thrombosis treatment.<sup>3</sup> Anticoagulant interference may impact dRVVT results, affecting diagnosis.<sup>4-8</sup>

## Aim

Evaluate the effect of numerous anticoagulants and common interferents on four commercial dRVVT assays to compare impact on LA diagnosis.

## Material and Methods

Precision BioLogic Inc. (PBI) dRVVT screening and confirmatory assays were performed on three analyzers – Stago STA-R Evolution (mechanical), Instrumentation Laboratory (IL) ACL TOP (optical), and Siemens BCS XP (optical) – and compared with the analyzer manufacturer's dRVVT assay (Table 1).

*crvocheck*<sup>TM</sup> Normal Donor Sets (NDS), N=50, were tested with the four reagents to verify dRVVT cutoff. Citrated plasma samples and corresponding matrix controls were prepared *in vitro* by spiking various potential interferents, at fixed concentrations, into *crvocheck* controls: Pooled Normal Plasma (CCN), Weak LA Positive (CCWLP), and LA Positive (CCLP).

Samples were aliquoted, frozen in liquid nitrogen, and stored in -80 °C freezers.

Before testing, samples were thawed in a 37 °C water bath and then assayed in quintuplicate using dRVVT.

## Results

- Normal Donor Sets were tested with each dRVVT assay to verify 1.20 as the general cutoff (Table 2). Due to a relatively short confirm clot time, a high percent correction for normal samples was detected with IL's dRVVT.
- No diagnostic interferences were observed in hemoglobin and bilirubin spiked samples. Clotting times for lipemic samples (~500 mg/dL) could not be detected by optical analyzers due to turbidity. The effect of LA was partially impaired in lipemic samples on the Stago analyzer (Figure 1).
- Heparin neutralizers, used in commercial dRVVT reagents, were effective against indirect FIIa/FXa inhibitors at the concentrations tested (Figure 2).
- Direct FXa (DOACs) and direct FIIa (Argatroban) inhibitors significantly prolonged both the screen and confirm clotting times. However, the extent of prolongation was not the same for each anticoagulant and it also varied between commercial reagents. PBI's dRVVT screen assay was less sensitive to prolongation of direct FXa inhibitors than the other screen reagents (Figure 3).
- The effect of a Vitamin K antagonist (Warfarin, INR 1-3) was measured on 10 clinical plasma samples. A high rate of false positivity (60-80%) was observed for Stago, Siemens and IL dRVVT assays (Figure 4).

## Conclusions

Diagnostic interference of direct FXa inhibitors depends on the type of dRVVT assay.

Rivaroxaban can cause false positive result with Stago, Siemens, and IL dRVVT assays but not with PBI's dRVVT reagent.

Unlike other commercial dRVVT reagents, PBI's dRVVT did not show the risk of false positivity in VKA clinical samples.

### References

- J Thromb Haemost 2006; 4: 295-306
- Blood 2003; 101: 1827-32
- https://doi.org/10.1111/jth.14846
- Am J Clin Pathol 2017; 147: 632-640
- Autoimmun Rev 2017; 16: 173-178
- Thromb Res 2018; 165: 101-106
- Pathology 2019; 3: 292-300
- https://doi.org/10.1111/jth.14829

**Note:** Spiked normal pooled and weak LA-positive plasma with the other direct FIIa inhibitors (Dabigatran 0.4 µg/mL and Lepirudin 1.5 µg/mL) were tested by PBI's dRVVT on STA-R Evolution, without any false diagnostic result (data not shown).

**Table 1**  
Comparison of dRVVT protocols on different analyzers.

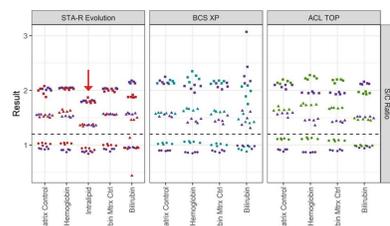
Analyzer	Wavelength	Sample Incubation	Data Acquisition time*	Sample Volume	Reagent Volume	Clot detection
STA-R Evolution	N/A	240 s	240 s	100 µL	100 µL	Mechanical detection
BCS XP	405 nm	230-250 s	180 s	100 µL	100 µL	Fixed absorbance (threshold = 200 mA)
ACL TOP	671 nm	150-360 s	240 s	75 µL	75 µL	Second derivative (greatest maxima peak)

\* for testing anticoagulant interference, the original acquisition times were extended to 350 s

**Table 2**  
Verification of 1.20 dRVVT cutoff using NDS plasma samples (N=50).

Analyzer	dRVVT Manufacturer	Screen (sec)			Confirm (sec)			S/C Ratio		
		Mean	SD	%CV	Mean	SD	%CV	Mean	SD	%CV
STA-R Evolution	PBI	37.9	3.4	8.9	38.7	1.9	4.8	0.98	0.07	6.9
	Stago	39.0	3.0	7.6	35.8	1.5	4.2	1.09	0.06	5.6
BCS XP	PBI	33.3	2.7	8.1	36.2	1.8	5.0	0.92	0.06	6.4
	Siemens	37.1	2.8	7.5	34.8	1.4	4.0	1.07	0.06	5.8
ACL TOP	PBI	33.8	2.9	8.6	36.3	1.9	5.2	0.93	0.06	6.8
	HemosIL	36.6	3.2	8.8	30.7*	1.5	5.0	1.19*	0.09	7.5

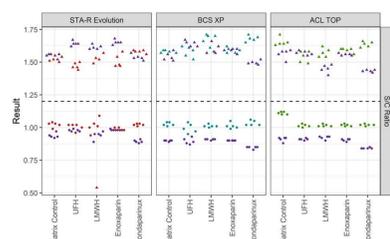
**Figure 1**



◀ Hemoglobin (250 mg/dL), Bilirubin (25 mg/dL), and Intralipid (~500 mg/dL) impact at various LA levels on different dRVVT assays.

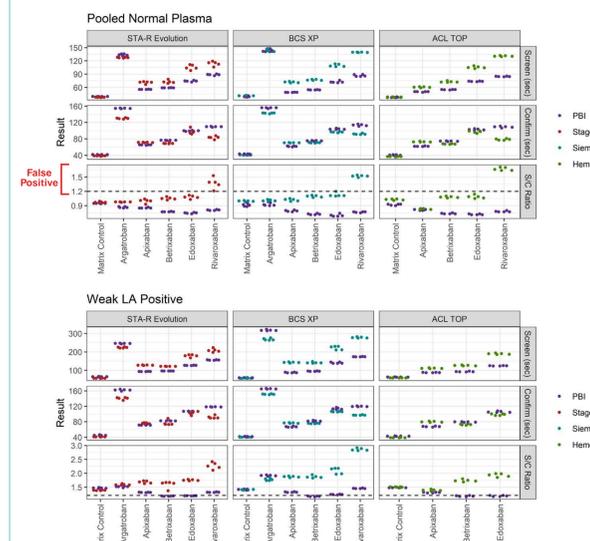
No clot was detected for lipemic samples on BCS XP and ACL TOP and the LA effect was partially impaired on the Stago analyzer (red arrow).

**Figure 2**



◀ Interference of indirect FIIa/FXa inhibitors in the presence and absence of LA on different dRVVT assays: UFH, LMWH, Enoxaparin (1 IU/mL); and Fondaparinux (1.25 µg/mL).

**Figure 3**



Interference of direct FIIa (Argatroban) and direct FXa inhibitors (DOACs) in the absence and the presence of LA on different dRVVT assays: Argatroban (1.5 µg/mL), Apixaban (0.3 µg/mL), Betrixaban (50 ng/mL), Edoxaban (0.4 µg/mL), and Rivaroxaban (0.4 µg/mL).

No clot was detected by ACL TOP for Argatroban and weak LA-positive containing Rivaroxaban.

**Figure 4**

VKA interference on different dRVVT assays: plasma samples (N=10) from patients on Warfarin (INR 1-3) showed a risk of false positivity with dRVVT assays except for PBI's dRVVT.

