ORIGINAL ARTICLE

Application of DOAC-Stop in a diagnostic laboratory

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ABSTRACT

Objectives: A study was set-up to validate DOAC-Stop at Waikato Hospital laboratory for lupus and thrombophilia testing at the request of a clinician. The study was limited to the DOACs, rivaroxaban and dabigatran.

Methods: Samples had coagulation tests pre- and post-treatment. Tests included: INR, APTT, fibrinogen, TCT, dabigatran assay, rivaroxaban assay, non-sensitive APTT, antithrombin III assay, dRVVC, and dRVVT.

Results: DOAC-Stop significantly removed dabigatran and rivaroxaban from the residual plasma. It had little effect on non-DOAC plasma.

Conclusions: This study indicated that DOAC-Stop can remove the effects of dabigatran and rivaroxaban from plasma to allow the testing for Lupus and Thrombophilia. DOAC-Stop has been implemented at Waikato Hospital and is used routinely, with the provision that either a TCT for dabigatran or rivaroxaban assay for rivaroxaban is added post-treatment to prove a successful reversal. Regardless, it is important to interpret treated plasma with caution as DOAC-Stop has demonstrated some variability in the coagulation cascade.

Key words: Direct oral anticoagulants; rapid reversal; dabigatran; rivaroxaban; activated charcoal; DOAC-Stop.

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INTRODUCTION

Direct oral anticoagulants (DOACs) have taken the coagulation world by storm with their effectiveness and decreased need for monitoring. The effect of DOACs on clotting tests reliant on Factors IIa and Xa is a recognised problem and can cause false positive and negative results (1). DOAC-Stop is a first-generation agent available to remove DOACs for laboratory testing (2). DOAC-Stop by Haematex is activated charcoal of specifically high grade chosen for its ability to remove DOACs, based on their molecular weight (3). There are similar products on the market that remove DOACs. These include: DOAC-Remove®, DP-Filter®, and DOAC Filter®.

Multiple evaluations have been published on the uses of DOAC removal agents which conclude that DOACs can be successfully removed, notably a study by McGlasson and Fritsma compared DOAC-Stop®, DOAC-Remove®, DP-Filter®, and DOAC Filter® (4). These agents are expected to eliminate DOACs from plasma by adsorption, filtration, and precipitation. The authors concluded that these agents could successfully remove dabigatran and rivaroxaban. The aim of our study was to validate DOAC-Stop at Waikato laboratory for lupus and thrombophilia testing at the request of a clinician. This study was limited to the DOACs, rivaroxaban and dabigatran.

MATERIALS AND METHODS

A total of 51 samples were used in the initial study. There was no age or gender discrimination in the selection of samples. Samples were run on either the STA-R® Evolution or STAR Max 2® (Stago). Ethical approval was not required as residual plasma was used. The citrate samples were already spun. Samples were not included if the plasma was haemolysed. All lupus samples were double spun and frozen. The lupus samples were from within Waikato Hospital laboratory, satellite hospitals, and community laboratories. Samples were thawed for 5 minutes in a water bath at 37 degrees. The DOAC-Stop removal method (2) was as follows:

- Add 1 ml of plasma to a plastic tube (There was an allowance of plasma from 0.5 ml to 1.5 ml; however, for this validation 1 ml was used).
- Add 1 tablet of DOAC-Stop to the plasma and mix.
- After 5 minutes mix again.
- Centrifuge for 10 minutes at 4000 rpm. Transfer supernatant into a separate tube for testing, avoiding charcoal particles.

Six normal non-DOAC patient samples were randomly selected. These samples were treated with DOAC-Stop as negative controls to ensure that the DOAC-Stop did not have any interfering effects. Pre- and post-treatment samples were run in parallel for a coagulation screen.

The coagulation screen included activated partial thromboplastin time (APTT: TriniCLOT aPTT HS), INR (STA-NeoPTimal), TCT (THROMBIN 10), and fibrinogen (STA®-Liquid Fib). A further eight non-DOAC control patient samples were selected for lupus testing using dilute Russell viper venom time (dRVVT: STA® - Staclot® dRVV Screen 5), non-sensitive APTT (Dade® Actin® FS Activated PTT Reagent), and dilute Russell viper venom confirm. (dRVVC: STA® - Staclot® dRVV Confirm). Upon reviewing the non-DOAC non-sensitive APTT results, it was decided that more data was required, and ten extra control patient samples were added to the study. Pre- and post-treatment samples were run in parallel. The reference values for dRVVT and dRVVC remained continuous between batches. They were the mean of the respective reference ranges calculated and provided by Stago. (6)

STA® - Staclot® dRVV screen: The final result was expressed as a screen ratio: screen ratio= screen clotting time (seconds) of the patient tested / screen clotting time (seconds) of reference pool (6).

STA® - Staclot® dRVV Confirm: The final result was expressed as a normalised ratio: Confirm ratio= Confirm clotting time (seconds) of the patient tested/Confirm clotting time of the reference pool (6).

Normalized ratio = Screen ratio/confirm ratio (6).

The reference pool time was determined in-house as per the manufacturer's instructions (6). A total of 27 normal donors were tested and the reference time for STA® - Staclot® dRVV Screen was 38.4 seconds and the reference time for STA® - Staclot® dRVV Confirm was 35.5 seconds. The package insert has a reference time of 39.8 seconds for STA® - Staclot® dRVV Screen (5).This differs from our in-house reference time.

However, it goes on further to state that a standard deviation of 3 seconds is associated with a mean time of 39.8 seconds, range. This accounted for the variation in reference times (5).

Ten samples from patients in Waikato hospital known to be on dabigatran were randomly selected. Full coagulation screens on pre- and post-treatment samples were run in parallel. Additionally, 12 more known dabigatran patient samples were randomly selected and tested for DRVVT (pre- and post-treatment samples were run in parallel). A further six dabigatran samples were used to demonstrate the effect of DOAC-Stop on the dabigatran assay (in-house *BIOPHEN*^{TM} dabigatran assay). Two of the samples were from patients known to be on dabigatran, the other four samples were normal patients pooled together and spiked with dabigatran. The spiked concentrations were derived from a Pradaxa capsule 150 mg that was crushed and added to 1ml of distilled water. This "liquid" Pradaxa was then spiked to 1 ml of pooled normal plasma. The 4 pooled samples were spiked at different concentrations. The concentrations for each spiked pool were as followed; 100 microliters; 1.5 x 10^12 ng/ml, 80 microliters; 1.2 x 10^12 ng/ml, 60 microliters; 0.9 x 10^12ng/ml and 40 microliters; 0.6 x 10^12 ng/ml.

Nine samples from patients known to be on dabigatran were randomly selected and run for antithrombin III assay (STA®-*Stachrom*® AT *III)*. Pre- and post-treatment samples were run in parallel.

An additional 21 samples from patients known to be on rivaroxaban were selected for a rivaroxaban study. Five patients had INR and rivaroxaban assays (STA®-Liquid Anti-Xa), with pre-and post-treatment samples run in parallel. 16 samples were selected and run for DRVVT and non-sensitive APTT assays (four samples from Pathlab and 12 from Waikato hospital). All 16 samples had a rivaroxaban assay run to confirm the DOAC-Stop had removed the rivaroxaban successfully.

RESULTS AND DISCUSSION

There was an insignificant difference between the treated and non-treated results with any variation between the results being within the uncertainty of measurement for the initial six normal non-DOAC control samples. (Figures 1-4). Measurement of uncertainty limits were as follows: INR: 5%; APTT: 5%; TCT: 5%; fibrinogen: 5%.

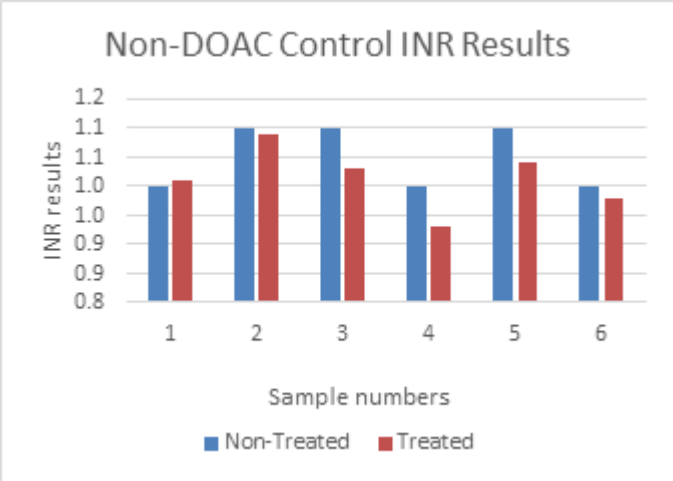

Figure 1.

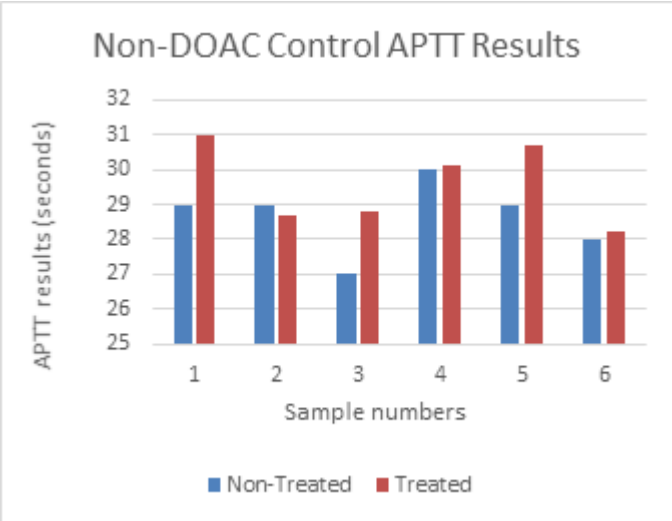

Figure 2.

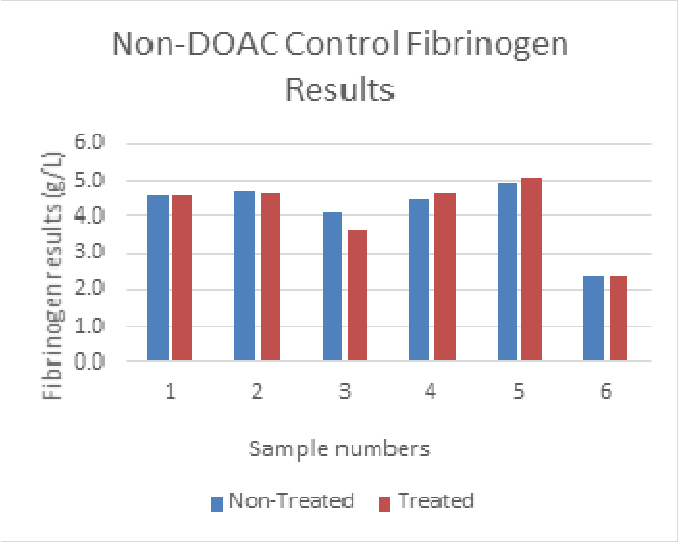

Figure 3.

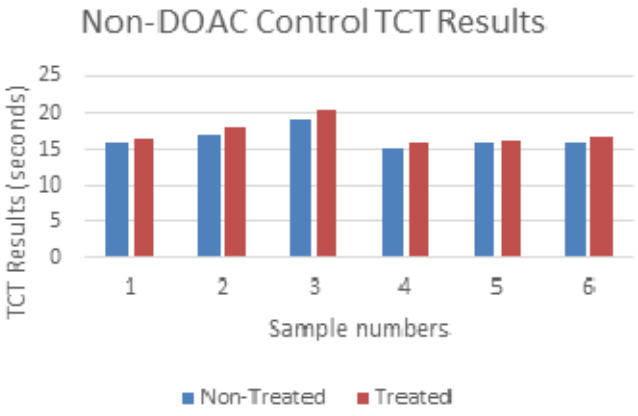

Figure 4.

Table 1 (a). Non- DOAC control lupus results.

	Non-treated non-sensitive APTT Reference interval: 25-36 seconds.	**Non-treated DRVV screen normalised ratio** Reference Interval: 0.8-1.2.	**Non-treated DRVV Confirm normalised ratio** Reference interval: 0.8-1.2.	**Non-treated Normalised ratio** Reference interval: 0.8-1.2.	**Treated non-sensitive APTT** Reference interval: 25-36 seconds.	**Treated DRVV screen Normalised ratio** Reference interval: 0.8-1.2.	**Treated DRVV Confirm normalised ratio** Reference interval: 0.8-1.2.	**Treated normalised ratio** Reference interval: 0.8-1.2.
1	32.0	0.9			32.9	1.01		
2	36.0	1.1			35.7	1.21		
3	26.0	1.4	0.94	1.49	26.5	1.03		
4	25.1	1.31	0.94	1.39	33.1	1.29	0.93	1.39
5	20.7	1.32	1.11	1.19	24.6	1.47	1.03	1.4
6	24.5	1.27	1.04	1.22	29.8	1.1	0.95	1.2
7	23.4	1.42	1.16	1.22	27.6	1.4	1.18	1.2
8	24.5	1.26	1.03	1.22	29.4	1.31	1.01	1.3

Table 1(b). Additional non-DOAC non-sensitive APTT results.

	Non-treated non-sensitive APTT Reference interval: 25-36 seconds.	**Treated non-sensitive APTT** Reference interval: 25-36 seconds.
1	29.1	28.2
2	27.4	26.7
3	28.8	27.5
4	28.6	27.7
5	47.4	48.3
6	27.4	27.5
7	27.1	27.6
8	32.9	35.6
9	43.0	41.4
10	27.9	27.5

Table 2. Coagulation screen dabigatran results.

	Pre-treatment dabigatran TCT Reference interval: <20 seconds.	**Pre-treatment dabigatran** INR Reference interval: 0.8-1.2.	**Pre-treatment dabigatran** APTT Reference interval: 25-38 seconds.	**Pre-treatment dabigatran** Fibrinogen Reference interval: 1.5-5.0 g/L.	**Post-treatment dabigatran** TCT Reference interval: <20 seconds.	**Post-treatment dabigatran** INR Reference interval: 0.8-1.2.	**Post-treatment dabigatran** APTT Reference interval: 25-38 seconds.	**Post- treat-ment dabigatran** Fibrinogen Reference interval: 1.5-5.0 g/L.
1	>150	2.0	73.0	4.9	19.6	1.2	24.9	4.4
2	124.0	1.3	45.0	3.2	18.1	1.2	33.9	3.1
3	115.0	1.3	47.0	3.2	17.7	1.2	32.2	3.1
4	95.0	1.1	37.0	4.4	16.4	1.0	29.3	4.3
5	96.0	1.3	53.0	4.6	16.3	1.2	46.0	4.8
6	>150	1.2	89.0	4.7	17.8	1.1	52.6	4.7
7	164.0	1.4	64.0	4.7	17.9	1.1	53.3	4.7
8	>150	1.8	58.0	3.0	18.6	1.1	32.1	3.2
9	>150	1.1	50.0	4.7	17.7	1.0	24.5	4.9
10	>150	1.4	89.0	5.4	16.7	0.9	31.5	5.6

Table 3. DRVV screen normalised ratio dabigatran results.

	Pre-Treatment (Dabigatran)	Post-Treatment (Dabigatran)
	DRVV screen normalised ratio Reference interval: 0.8-1.2	**DRVV screen normalised ratio** Reference interval: 0.8-1.2
1	2.3	1.1
2	1.6	1.2
3	1.4	1.0
4	2.6	1.0
5	2.4	1.4
6	2.6	1.6
7	2.0	1.0
8	1.1	1.1
9	1.6	1.1
10	1.8	1.0
11	4.0	1.0
12	2.0	1.0

Table 4. Dabigatran assay results.

	Pre-treatment TCT Reference interval: <20 seconds	**Pre-treatment dabigatran assay level** Reference interval: <10 ng/ml	**Post-treatment TCT** Reference interval: <20 seconds	**Post-treatment dabigatran assay level** Reference interval: <10 ng/ml ml)
1	>150	101	18.1	<10
2	>150	298	19	<10
Spiked 100 µl	>150	Mmax* >600	54.8	<10
Spiked 80 µl	>150	Mmax* >600	47.9	<10
Spiked 60 µl	>150	557.25	46.4	<10
Spiked 40 µl	>150	421.4	37.4	<10

Table 5. Antithrombin III dabigatran results.

	Pre-treatment antithrombin III (Dabigatran) Reference interval: 80-120 %	**Post-treatment antithrombin III** (Dabigatran) Reference interval: 80-120 %	**Post-treatment TCT** (Dabigatran) Reference interval: <20 seconds
1	95	93	18.7
2	94	86	19.1
3	92	93	17.2
4	64	64	16.3
5	73	71	18.2
6	110	100	18.8
7	105	99	18.2
8	75	81	18.3
9	101	99	18.4

Table 6. INR and rivaroxaban assay results**.**

	Pre-treatment		**Post-treatment**	
	INR Reference interval: 0.8-1.2.	**Rivaroxaban** Reference interval: <25 ug/L.	**INR** Reference Interval: 0.8-1.2.	**Rivaroxaban** Reference interval: <25 ug/L.
1	1.03	72	0.86	<25
2	1.15	38	1.0	<25
3	1.05	74	0.88	<25
4	1.32	91	0.95	<25
5	1.44	211	1.01	<25

Table 7. Non-sensitive APTT and DRVV screen normalised ratio rivaroxaban results.

Pre-treatment			Post-treatment		
Non-sensitive APTT Reference interval: 25-36 seconds		**DRVV screen normalised ratio** Reference interval: 0.8-1.2	**Non-sensitive APTT** Reference interval: 25-36 seconds	**DRVV screen normalised ratio** Reference interval: 0.8-1.2	**Rivaroxaban** Reference interval: <25 ug/L
1	35	2.9	25	1.0	<25
2	32	1.2	21	1.0	<25
3	43	3.39	31	1.0	<25
4	45	3.2	31	1.3	<25
5	51	4.0	26	1.2	<25
6	39	1.1	26	1.1	<25
7	45	1.5	38	1.0	<25
8	32	1.3	26.6	1.0	<25
9	28.5	1.3	25.3	1.1	<25
10	26.2	1.2	26.2	1.1	<25
11	36.0	3.00	36.0	3.0	<25
12	48	3.5	28	1.2	>25
13	41	3.1	24	1.0	<25
14	60.4	3.6	33.1	1.2	<25
15	43.2	3.4	29.7	1.3	<25
16	41.0	2.4	34.5	1.3	<25

Table 1(a) shows that for non-DOAC non-sensitive APTT it was noted that patient samples 4-8 had differences of greater than 5%, which is outside the measurement of uncertainty. Samples 5 and 7 pretreatment had APTT results below the reference range. Visual checks showed no visible fibrin clots. We suspect sample activation.

As a result of this discrepancy, another 10 non-DOAC patient samples were run to see if the same discrepancy could be replicated. [Table 1(b)]. These 10 samples were all were within the measurement of uncertainty. Therefore, it is speculated that on this occasion the discrepancy in Table 1(a) could have been sample related. Nonetheless, this initial discrepancy promoted further research on the effects DOAC-Stop had on the coagulation cascade.

Kopatz *et al*. found that by using Calibrated Automated Thrombography (CAT) that DOAC-Stop successfully absorbed DOACs from plasma (5). The treated plasma was found to be marginally more procoagulant in comparison to treated non-DOAC plasma. It was also found that there was a slight reduction in tissue factor pathway inhibitor. Another study by Exner *et al.* observed that DOAC-Stop bound to some cationic inhibitors of the APTT (8). Interestingly, a study by Baker *et al.* investigated TCT, anti - Xa activity, aPTT - SP, SCT, and dRVVT with non-DOAC samples (10). DOAC-Stop exhibited nil significant differences on the assays in their group mean. However, it was noted that there was individual variability between pre- and post-treatment. It was decided due to the small size of the control group that the effect of DOAC-Stop could not be completely excluded. It was also documented that some of the samples that did not contain apixaban did show higher SCT or dRVVT ratios post treatment. This was seen in lupus positive and negative control samples. The level of discrepancy was greater in neat samples than after a 1:1 mix with normal pool sera. It was thought probable that the DOAC-Stop process may intensify a factor deficiency by direct binding or denaturation.

Exner *et al.* documented a minor elongation of the APTT, pre- and post-treatment of DOAC-Stop in non- DOAC samples which was believed to be due to the high speed of centrifugation (9). The variation between observations and studies is evident; however, it is essential to understand that, although DOAC-Stop does mostly remove DOAC's from plasma, it can also exhibit variability in the coagulation cascade.

Dabigatran was removed successfully from the 10 samples as the TCT was normalized (Table 2). Table 3 illustrates two examples where the dRVVT ratios did not completely normalise. It is impossible to know if this was due to the effects of dabigatran as a TCT was not performed due to limited sample volume. A study by Slavik *et al.* using a sensitive method of liquid chromatography confirmed that DOAC-Stop does not completely remove DOAC's from plasma (10). Notably, the residual DOAC was low enough not to effect the dRVVT.

There were two samples that had a Mmax error, indicating that these results were potentially >600 ng/ml (Table 4). This figure was extrapolated from the calibration curve. Post-treatment the dabigatran assay result was <10 ng/ml, however the TCT did not completely correct which likely indicated that a small amount of dabigatran remained. In fairness to the product the probability of getting a patient sample with such a high dose of dabigatran is unlikely. It is also important to consider the biology of dabigatran being absorbed in-vivo versus in-vitro in regard to spiking normal plasma with a specific potency.

Table 5 shows that there was a marginal difference between the pre- and post-treatment samples and the normalised TCT proved that the DOAC reversal was successful (Table 5). The package insert from the STA®-*Stachrom*® AT III states that the presence of thrombin inhibitors can lead to an over-estimation of antithrombin III (11). However, it was noted that the difference in the results was marginal and was well within the uncertainty of measurement of 10%.

There were four patients with dRVVT ratios that did not completely normalise post-treatment (Table 7). It is unlikely to be due to rivaroxaban as the rivaroxaban assay was <10 ug/L for all four results. Unfortunately, there was not enough remaining plasma to perform further confirmatory lupus studies on the four samples with prolonged dRVVT ratios.

In conclusion, our study indicates that DOAC-Stop can remove the effects of dabigatran and rivaroxaban from plasma to allow testing for Lupus and Thrombophilia. DOAC-Stop has been implemented at Waikato Hospital and is used routinely, with the provision that either a TCT for dabigatran or rivaroxaban assay for rivaroxaban is added post-treatment to prove a successful reversal. Regardless of the success of this study and others like it, it is important to interpret treated plasma with caution as DOAC-Stop has demonstrated some variability in the coagulation cascade (5).

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