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ORIGINAL ARTICLE

Direct Oral Anticoagulant removal by a DOAC filter: Impact on lupus anticoagulant testing – Evaluation on spiked and patient samples

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Abstract

Background: DOAC Filter (DF) is a new device to overcome interference in lupus anticoagulant (LAC) testing by direct oral anticoagulants (DOACs).

Objectives: We evaluated DOAC removal from plasma and elimination of DOAC interference in LAC testing by DF, and impact of DF on LAC assays in a representative patient cohort, including a comparison with DOAC-Stop (DS).

Methods: Normal pooled plasma (NPP) was spiked with increasing concentrations of apixaban, rivaroxaban, edoxaban, and dabigatran. DOAC and LAC was measured on untreated, DF-treated, and DS-treated spiked samples. Coagulation parameters and thrombin generation were measured on patient samples (n = 20) before and after DF. Patients treated with DOAC, vitamin K antagonist, or heparin and nonanticoagulated patient samples (n = 139) were tested for LAC before and after DF.

Results: In spiked NPP, levels were below the lower limit of quantification (LLoQ) after DF/DS treatment for all DOAC concentrations. Following DF, levels were below LLoQ for 53 of 56 DOAC-containing patient samples. Twenty-eight of 33 LAC-positive DOAC-containing samples became negative after filtration, whereas 5 remained LAC-positive (1/5 from a patient with antiphospholipid syndrome [APS]). Four LAC-positive DOAC-containing samples (from patients without APS), became negative after filtration, whereas they remained LAC positive after DS. In the non-DOAC patient groups following DF, LAC changed from positive to negative in 8 (due to a procoagulant effect) and vice versa in 2 cases.

Conclusion: DF reduces DOAC interference in LAC testing. As incomplete DOAC removal may occur, DOAC measurements should be performed after filtration. A procoagulant effect after filtration may lead to erroneous LAC results in non–DOAC-containing samples. Therefore, using DF should be restricted to DOAC-containing samples.

KEYWORDS
clotting times, direct oral anticoagulant, DOAC adsorption, DOAC Filter, lupus anticoagulant

Essentials

- DOAC Filter (DF) is an alternative for direct oral anticoagulant (DOAC) adsorbents.
- DF efficiently removes DOACs and eliminates their impact on lupus anticoagulant assays.
- A procoagulant effect occurs by filtration with DF.
- The use of DF should be restricted to samples containing DOAC.

1 | INTRODUCTION

Direct oral anticoagulant (DOAC) interference on clotting assays is a well-known issue in the coagulation clinical laboratory.[1-7] The degree of interference depends on the characteristics and concentration of the anticoagulant, the assay principle, and reagent and analyzer used.[1-4,8,9] DOACs are widely prescribed in clinical practice for treatment and prevention of thrombosis, since they have demonstrated benefits in pharmacokinetics and safety profile.[10-13] Although discouraged to test during anticoagulant therapy,[14] there may be a need to test for lupus anticoagulant (LAC) during anticoagulation in some conditions[15] and, consequently, laboratories will receive increasing numbers of DOAC-containing samples for a thrombophilia workup, including LAC detection.[15] Inherently to their mechanism of action, being a direct factor X inhibitor (apixaban, rivaroxaban, and edoxaban) or a direct thrombin inhibitor (dabigatran),[12,13] DOACs may interfere with the diluted Russell's viper venom time (dRVVT) and activated partial thromboplastin time (aPTT) clotting assays during LAC detection.[4,6,14-18] The Clinical and Laboratory Standards Institute guidelines and the recently updated guidelines on LAC detection from the Scientific and Standardization Committee for LAC/antiphospholipid antibodies (LAC/aPL) of the ISTH recommend not to perform LAC testing in patients receiving DOAC treatment.[14,15,19] If feasible, SSC LAC/aPL of the ISTH recommends to interrupt DOAC treatment for at least 48 hours before sample collection for LAC testing.[14,15] This, however, may not be clinically possible due to an increased risk of adverse effects.[6] In addition, a high interindividual variability in trough levels has been reported.[20] Several other strategies have already been proposed to overcome the effect of DOAC interferences in LAC testing, but all with limitations. Adding DOAC neutralizing agents, such as idarucizumab, to plasma before LAC testing is quite expensive[21,22] and the use of DOAC adsorbing agents, such as DOAC-Stop (DS; Haematex Research, Hornsby, Australia), DOAC-Remove (5 Diagnostics, Basel, Switzerland) or activated charcoal,[22-30] may lead to false-positive or false-negative results due to incomplete DOAC removal, a prolongation of the clotting time (CT) or a procoagulant effect.[30-33] An alternative method to remove DOAC is filtration of the plasma before LAC analysis. Recently, a new device, the DOAC Filter (DF; Diagnostica Stago, Parsippany, NJ, USA), is available. The name of the device could be somehow misleading, since the DF is a ready-to-use device using a solid-phase extraction principle to trap DOACs,[34] and not a real filtration procedure as historically was used to deplete plasma from platelets for LAC testing.[35,36]

So far, the information published on DF has mainly aimed to show that DOACs are efficiently removed and that sample integrity after DF treatment remains.[34] The impact of DF on LAC testing in nonanticoagulated and anticoagulated large patient groups has not been studied yet. As is stated by Sevenet et al,[34] additional studies are needed to further evaluate the impact of the device on the plasma coagulation profile. In this study, we aim to assess the ability of this new device to remove DOACs from plasma on spiked and patient samples. We will evaluate the impact of DF treatment on LAC testing in a large representative patient cohort including nonanticoagulated patients and patients receiving vitamin K antagonists (VKAs), heparin, or DOAC therapy. In addition, we will compare the effect of a pretreatment by DF versus DS on spiked samples and patient samples treated with DOAC.

2 | MATERIALS AND METHODS

2.1 | Spiking experiments

Citrated whole blood (BD vacutainer citrate 3.2%, 2.7 mL; Becton Dickinson, Franklin Lakes, NJ, USA) was collected from healthy volunteers (n = 70) after informed consent. Platelet-poor plasma (PPP) was obtained following double centrifugation at 2230 g for 15 min at room temperature. PPP was pooled to create normal pooled plasma (NPP) and stored at −80°C. Before analysis, NPP was thawed at 37°C for 5 minutes. Stock solutions of apixaban (15 mg/mL), rivaroxaban (15 mg/mL) and edoxaban (7.5 mg/mL) in dimethyl sulfoxide (DMSO) were provided by Agro-Bio (La Ferté saint Aubin, France). Working solutions, prepared in DMSO and further diluted in physiological saline solution, were added to NPP at eight different concentration levels. Final dilution was the same for each concentration level and never exceeded 10% of the total sample volume. A dabigatran spiking experiment was performed by reconstituting commercial calibration plasma (Hyphen BioMed, Neuville-sur-Oise, France) in NPP and adding neat NPP up to seven concentration levels. DOAC-spiked NPP was pretreated with DF and DS at all levels or left untreated before DOAC quantification analysis and LAC testing.

2.2 | Patient samples

A total of 134 patient samples with LAC request were included. Within this cohort, there were patients treated with DOAC, VKA,

low-molecular-weight heparin (LMWH), and unfractionated heparin (UFH) and patients not taking anticoagulants. All citrated plasma samples were collected and pretreated according to the ISTH guidelines and stored at −20°C for up to 1 week until LAC analysis.[14] For all samples, LAC testing was performed before and after DF pretreatment. LAC testing of DOAC-containing samples was also measured after incubation of plasma with DS. DOAC concentrations were measured for DOAC-containing samples before and after DF/DS treatment. Anti-Xa activity was determined in patient samples with LMWH or UFH, and prothrombin time (PT) in patient samples containing VKA. In addition, 21 DOAC-containing patient samples without LAC request were included for evaluation of DOAC removal efficacy. This study was approved by the ethical committee of Ghent University Hospital. An overview of the analysis performed with DF and DS pretreatment is presented in Table S1.

2.3 | Coagulation assays

LAC testing was performed according to the ISTH guidelines by a three-step (screening-mixing-confirmatory) method using a dRVVT- and aPTT-based test system,[14] using STA-Staclot dRVV Screen, STA-Staclot dRVV Confirm, PTT-LA, and Staclot LA reagents (Diagnostica Stago), as previously described.[37] Results are expressed as normalized clotting ratio (NCR) or a difference of CT for Staclot LA aPTT.[14,37]

Apixaban, rivaroxaban, and edoxaban levels were measured using a chromogenic anti-Xa assay (STA-Liquid anti-Xa; Diagnostica Stago) calibrated for the corresponding DOAC. A diluted thrombin time (TT) assay (Hemoclot Thrombin Inhibitors, Hyphen BioMed) was used for measurement of dabigatran concentrations. Routine coagulation parameters, PT and aPTT, were determined using STA-NeoPtimal and STA-PTT Automate (Diagnostica Stago), respectively. Heparins were measured by a chromogenic anti-Xa assay (STA-Liquid anti-Xa, Diagnostica Stago). Intrinsic and extrinsic coagulation factors were measured by one-stage assays using STA-(immuno)deficient plasma and C.K. Prest or STA-NeoPtimal, respectively. All analyses were performed on a STA-R Evolution analyzer (Diagnostica Stago).

2.4 | Other assays

Von Willebrand factor antigen (VWF:Ag) and activity (VWF:GPIbR) were measured by chemiluminescense on AcuStar (Werfen Instrumentation Laboratory, Bedford, USA) using corresponding HemosIL reagents. Thrombin generation (TG) was performed by calibrated automated thrombinography using a fluorometer (Fluoroskan Ascent; Thermolab, Massachusetts, USA) with Thrombinoscope software (Diagnostica Stago) with PPP reagent (5 pM tissue factor on PPP. Free tissue factor pathway inhibitor (TFPI) antigen was measured by an ELISA using Asserchrom Free TFPI (Diagnostica Stago) and performed according to the manufacturer's recommendations.

2.5 | DF and DS procedure

DF and DS treatment was performed according to the manufacturer's instructions. Six hundred microliters of citrated PPP was loaded in the cartridge of the DF and was centrifuged at 300 *g* for 15 minutes at room temperature. Filtered PPP was collected in a STA-Microtainer. One DS minitablet was added to 1 mL PPP, which was subsequently incubated and mixed for 5 minutes at room temperature, followed by a centrifugation step of 15 minutes at 2230 *g*. The supernatant was collected for further analysis.

2.6 | Statistics

All statistical analyses were computed using MedCalc statistical software (MedCalc Software, Ostend, Belgium). Data are presented as median (range, minimum-maximum) and mean percentage difference/deviation (95% confidence interval [CI]) calculated as follows: $(\text{result}_{\text{DF/DS treated}} - \text{result}_{\text{untreated}})/(\text{result}_{\text{untreated}}) \times 100$. Statistical comparison was performed using Wilcoxon matched-pair signed-rank tests. A P value <.05 was considered statistically significant.

3 | RESULTS

3.1 | Volume recovery and efficacy of DF and DS for DOAC removal

3.1.1 | Residual volume of plasma after DF and DS treatment

Volume assessment was carried out after treatment with DF (600 μL per filter) on 30 plasma samples and after treatment with DS (1 mL per tablet) on 20 plasma samples. Mean volume recovered after DF treatment was 465 μL (95% CI, 456-472 μL) with a mean plasma recovery of 77.4%. After DS treatment, the mean recovered volume was 861 μL (95% CI, 843-880 μL), corresponding to a mean plasma recovery of 86.1%. The volume reproducibility of DF and DS treatment expressed in coefficient of variation was 4.1% and 4.6%, respectively.

3.1.2 | Efficacy of DOAC removal by DF and DS in spiked NPP and in patient samples

DOAC concentration in spiked untreated NPP, spiked NPP after DF treatment, and spiked NPP after after DS treatment are presented in Figure 1. Apixaban concentrations in spiked untreated NPP ranged from 32 to 894 ng/mL, for rivaroxaban from 34 to 962 ng/mL, for edoxaban from 32–908 ng/mL, and dabigatran ranged from 45 to 503 ng/mL. DOAC concentrations measured in spiked NPP samples after DF or DS treatment were all below the corresponding lower limit of quantification (LLoQ), except for apixaban at the highest

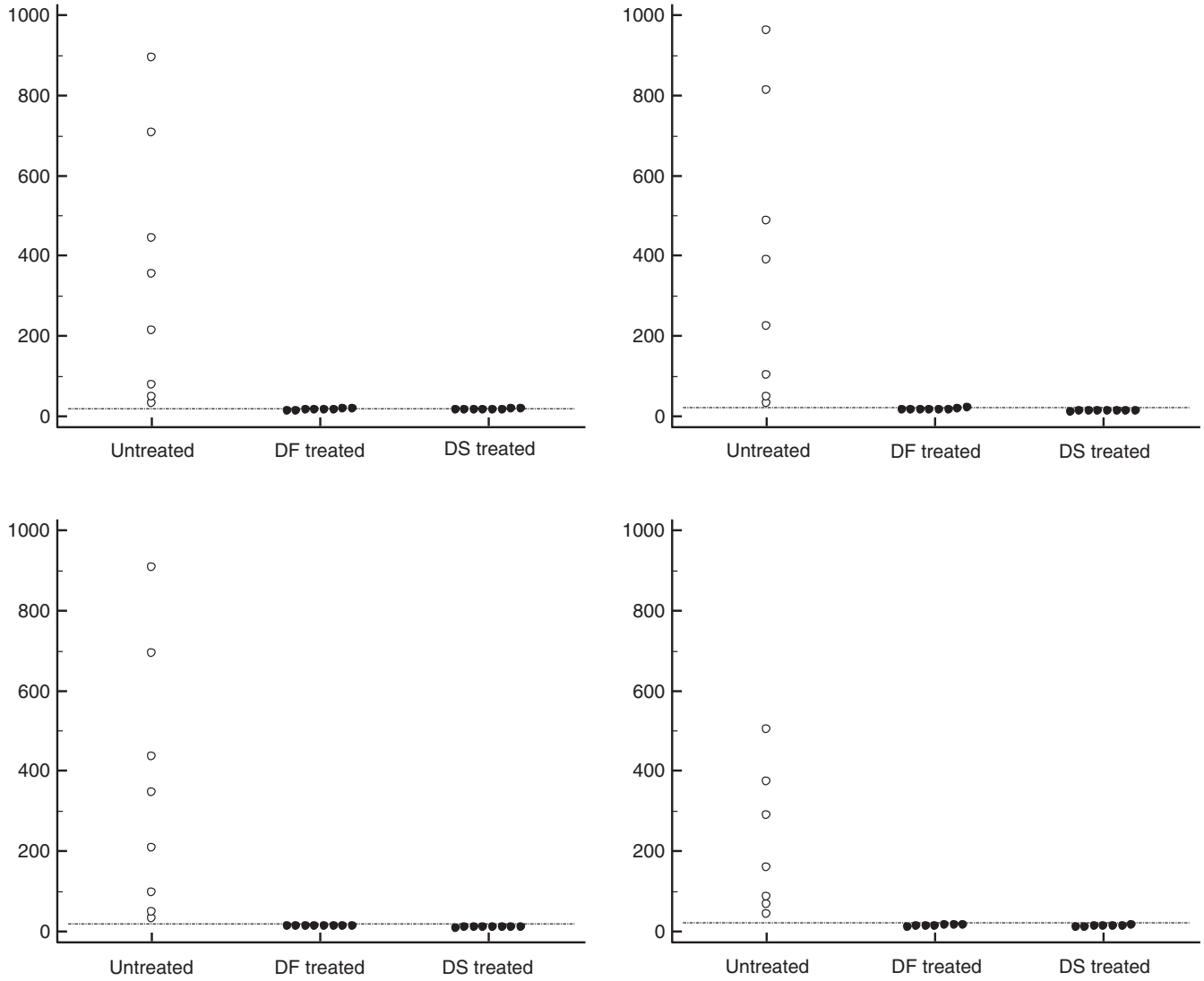

FIGURE 1 **Efficacy of DOAC removal by DOAC Filter and DOAC-Stop in spiked normal pooled plasma.** Apixaban, rivaroxban, edoxaban and dabigatran concentrations in spiked untreated normal pooled plasma (untreated) and in spiked plasma following pretreatment of 600 μL plasma with DOAC Filter (DF treated) or following pretreatment of 1 mL plasma with one DOAC-Stop minitablet (DS treated). The dotted lines represent the lower limit of quantification (LLoQ; 20 ng/mL for apixaban and edoxaban, 21 ng/mL for rivaroxaban and dabigatran). Empty circles represent DOAC concentrations above the respective LLoQ and filled black circles represent concentrations below the respective LLoQ. DF, DOAC Filter, DS, DOAC-Stop

concentration after DF treatment with a residual concentration of 20 ng/mL that equals the LLoQ.

DOAC measurement was performed on DOAC-containing samples from patients with LAC request (n = 35) and patients without LAC request (n = 21) before and after DF treatment. DOAC measurement after DS treatment was only performed on samples with LAC request (n = 35). Results are presented in Table 1. DOAC presence in untreated samples was confirmed as a DOAC concentration above the LLoQ was obtained for all samples (Table 1). After DOAC removal, by both DF and DS treatment, no DOAC concentrations above LLoQ were measured for all samples with LAC request. Among the 21 samples without LAC request, DOAC was incompletely removed in three samples after DF treatment, resulting in concentrations above the corresponding LLoQ. Two samples containing 94 and 191 ng/mL apixaban before DF treatment, contained a DOAC level of 28 and 33 ng/mL, respectively, after filtration. For one sample containing 135 ng/mL dabigatran before DF treatment, a concentration of 27 ng/mL was measured after filtration.

3.2 | DOAC interference and effect of pretreatment by DF and DS on LAC testing (spiking experiment)

3.2.1 | LAC test results in neat (untreated) NPP spiked with DOAC

In NPP spiked with increasing concentrations of DOAC (see above), a concentration-dependent increase of NCR was seen for the three dRVVT LAC steps and for the aPTT screen and mixing step. DRVVT

TABLE 1 Efficacy of DOAC removal by DOAC Filter and DOAC-Stop in patient samples

	Apixaban				
	Patient samples without LAC request		Patient samples with LAC request		
No.of samples	5		4		
Treatment	Untreated	DF treated	Untreated	DF treated	DS treated
DOAC concentration range (ng/ml)	94 – 350	<20–33	51–279	<20	<20
LLoQ (ng/ml)	20		20		
No. of samples above LLoQ	5	2	4	0	0
	Rivaroxaban				
	Patient samples without LAC request		Patient samples with LAC request		
No.of samples	5		20		
Treatment	Untreated	DF treated	Untreated	DF treated	DS treated
DOAC concentration range, ng/mL	153-449	<21	27-508	<21	<21
LLoQ, ng/mL	21		21		
No. of samples above LLoQ	5	0	20	0	0
	Edoxaban				
	Patient samples without LAC request		Patient samples with LAC request		
No. of samples	6		5		
Treatment	Untreated	DF treated	Untreated	DF treated	DS treated
DOAC concentration range (ng/ml)	131–413	<20	22–239	<20	<20
LLoQ (ng/ml)	20		20		
No. of samples above LLoQ	6	0	5	0	0
	Dabigatran				
	Patient samples without LAC request		Patient samples with LAC request		
No. of samples	5		6		
Treatment	Untreated	DF treated	Untreated	DF treated	DS treated
DOAC concentration range, ng/mL	135-441	<21–27	23-373	<21	<21
LLoQ, ng/mL	21		21		
No. of samples above LLoQ	5	1	6	0	0

Abbreviations: DF, DOAC Filter; DOAC, direct oral anticoagulant; DS, DOAC-Stop; LLoQ, lower limit of quantification.

and aPTT results of untreated spiked NPP for all four DOACs in function of the DOAC concentration are shown in Figure S1. LAC results were most affected by dabigatran, even from the lowest concentration spiked (44 ng/mL), while LAC results were least influenced by apixaban. Table S2 shows the highest DOAC concentrations in untreated NPP for which no false-positive LAC result was obtained. The dRVVT system was highly effected, with a false-positive dRVVT conclusion for samples containing >49 ng/mL rivaroxaban, >32 ng/mL edoxaban, or >21 ng/mL dabigatran. For apixaban, no false-positive dRVVT conclusion was obtained (up to the highest spiked concentration of 894 ng/mL) due to a mild effect on dRVVT screen and a stronger effect on dRVVT confirm or dRVVT confirm mix, leading to a confirmatory NCR below the cut-off. For the aPTT system end conclusion, no false-positive results were obtained since the aPTT confirmatory tests were unaffected for all DOACs. Altogether, as also shown in Table S2, false-positive LAC final conclusions were seen for rivaroxaban, edoxaban, and dabigatran in DOAC spiked NPP samples, due to false positivity in the dRVVT test system. No false-positive LAC results were obtained for NPP samples spiked with apixaban.

3.2.2 | LAC test results in spiked NPP after treatment with DF and DS

dRVVT and aPTT results of DF- and DS-treated spiked NPP in function of the DOAC concentration are shown in Figure S1 for all four DOACs. NCR were calculated using untreated NPP and NPP pretreated with

DF. The reduction in CTs after DF as well as DS treatment in the dRVVT screen, mix, and confirmatory step showed NCR below the in-house cutoff for apixaban-, rivaroxaban-, and edoxaban-spiked NPP samples, overcoming of false-positive dRVVT conclusion, even up to the highest DOAC concentrations (Table S2). In dabigatran-spiked NPP samples, false-positive dRVVT screen, mix, and confirmatory results were observed for both DF and DS pretreated samples containing a dabigatran concentration of 67 ng/mL. Therefore, negative dRVVT screen, mix, and confirmatory results obtained for higher dabigatran concentrations within the spiking experiment were considered unreliable. Of note, a false-positive dRVVT conclusion was not obtained when applying NCR calculation with neat NPP after DF treatment. For all three steps of the aPTT system, NCRs below the in-house cutoff values were obtained after both DF or DS for all concentrations, for all DOACs (Figure S1B). No false-positive aPTT conclusions were obtained after DF or DS treatment, as was also the case in untreated spiked NPP (Table S2). A more detailed comparison of results obtained after DF and DS pretreatment of the spiked NPP samples is presented in Table S3.

3.3 | Effect of pretreatment by DF on LAC testing in NPP and patient samples

3.3.1 | Study population

In this study, we included 134 patient samples with a routine LAC request: 53.7% (n = 72) of samples originated from a LAC request in the context of thrombophilia screening (3 patients with suspected APS), 17.9% (n = 24) patients with (suspected) autoimmune disease (2 patients with suspected APS), 5.5% (n = 7) LAC requests in the context of pregnancy complications and in vitro fertilization, 3.7% (n = 5) patients with liver disease, 5.2% (n = 7) patients with a workup for prolonged aPTT, 9.7% (n = 13) patients with known APS in follow-up, and 4.5% (n = 6) patients for which no clear indication for LAC testing could be identified. Further specifications are presented in Table S4. At the time of sample collection, 68 patients (50.7%) did not receive any anticoagulant therapy, 35 (26.1%) patients were treated with DOAC (apixaban, n = 4; rivaroxaban, n = 20; edoxaban, n = 5; and dabigatran n = 6) and 31 patients

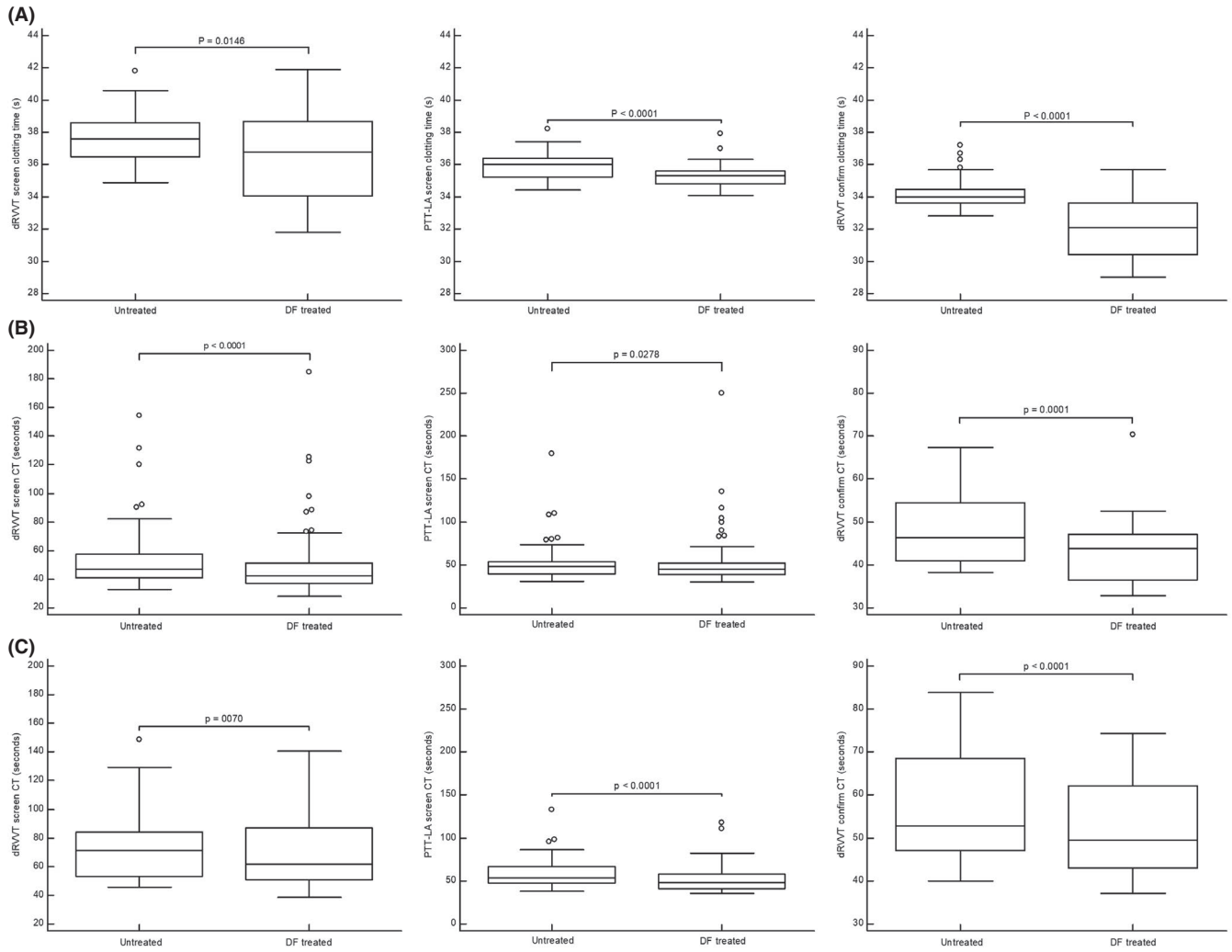

FIGURE 2 = 51 for dRVVT and PTT-LA screen; n = 47 for dRVVT confirm); (B) non–anticoagulant-containing patient samples (n=68); (C) vitamin K antagonists or heparin-containing patient samples (n = 31). *P* values obtained from Wilcoxon signed-rank test. CT, clotting time; DF, DOAC Filter; LAC, lupus anticoagulant

with other anticoagulants (LMWH, n = 9; UFH, n = 3; VKA, n = 17; LMWH + VKA, n = 2). Anti-Xa activities in heparin-containing samples ranged from 0.1 to 1.05 IU/mL and PT levels (international normalized ratios); VKA-containing samples ranged from 1.3 to 3.9. In addition, 5 LAC positive patient samples (3 patients with known APS and 2 patients APS not confirmed) were spiked with rivaroxaban (±300 ng/mL).

3.3.2 | Effect of DF pretreatment on clotting times of screening and confirmation step in LAC testing

DF treatment of NPP samples showed a statistically significant difference for CTs in dRVVT screen, PTT-LA screen and dRVVT confirm assays (Figure 2A); however, the shortenings in CTs were very limited. Data are presented in Table S4. Boxplots shown in Figure 2B and C present CTs before and after DF treatment of the patient subgroups without anticoagulant and patients on VKA, UFH, or LMWH therapy. Within the samples from nonanticoagulated patients, decrease in CTs for dRVVT screen, PTT-LA screen, and dRVVT confirm were similar to those observed in NPP (Table S5). dRVVT screen, PTT-LA screen, and dRVVT confirm results after DF treatment in the VKA/UFH/LMWH patient group also decreased significantly. Differences were more pronounced compared to those seen for NPP or nonanticoagulated patient samples (Table S5, Figure 2C). As dRVVT screen and confirm were both affected to a similar extent for VKA/UFH/LMWH-containing samples, the resulting screen/confirm ratios were not significantly different ($P = .21$). Mean percentage differences in CTs for dRVVT screen, PTT-LA screen, and dRVVT confirm in both patient subgroups were all <10%, except for PTT-LA screen within the VKA/UFH/LMWH-containing patient group (mean difference, −11.5% [−15.7% to −7.3%]). As expected, in DOAC-containing samples, DF treatment decreased the CTs for dRVVT screen, PTT-LA screen, and dRVVT confirm significantly.

3.3.3 | LAC test results in patients treated with DOAC

In 33 of 35 (94.2%) untreated DOAC-containing samples, LAC was positive, of which 30 samples were positive only in the dRVVT-system, and 3 samples were positive for both dRVVT and aPTT systems. Two untreated DOAC-containing samples, both containing apixaban (60 and 279 ng/mL), were LAC negative. Table 2 presents

TABLE 2 2 × 2 Contingency table for dRVVT- and aPTT-based LAC screening, mixing, and confirmatory tests along with conclusions in both test systems and final LAC conclusions. Results before and after DF treatment and DS treatment in patient samples containing DOACs

		DOAC					
		After DF (a)		After DF (b)		After DS (a)	
	Not treated	Positive	Negative	Positive	Negative	Positive	Negative
dRVVT							
Screening	Positive	5	30	5	30	9	26
	Negative	0	0	0	0	0	0
Mixing	Positive	5	0	5	0	8	1
	Negative	0	0	0	0	0	0
Confirmatory	Positive	5	0	5	0	8	0
	Negative	0	0	0	0	0	0
Conclusion	Positive	5	28	5	28	8	25
	Negative	0	2	0	2	0	2
aPTT							
Screening	Positive	4	16	4	16	5	15
	Negative	0	15	0	15	0	15
Mixing	Positive	3	0	2	1	4	0
	Negative	0	1	0	1	0	1
Confirmatory	Positive	2	0	2	0	3	0
	Negative	0	2	0	2	0	2
Conclusion	Positive	2	1	2	1	3	0
	Negative	0	32	0	32	2	30
LAC							
Final conclusion	Positive	5	28	5	28	9	24
	Negative	0	2	0	2	0	2

Note: Results interpreted by NCR calculated by the clotting time of neat NPP (a) and the clotting time of DF treated NPP (b).

Abbreviations: aPTT, activated thromboplastin time; DF, DOAC Filter; DOAC, direct oral anticoagulant; dRVVT, diluted Russell's viper venom time; DS, DOAC-Stop; LAC, lupus anticoagulant; NCR, normalized clotting ratio; NPP, normal pooled plasma.

the effect of DF and DS treatment on LAC interpretations in a 2 × 2 contingency table. Final LAC conclusions changed from positive to negative after DF treatment for 28 of 33 (84.8%) samples, because of the dRVVT system becoming negative in 27 samples and both systems becoming negative in 1 sample. Final LAC conclusions remained positive in 5 of 33 (15.2%) samples: 1 sample containing dabigatran (32 ng/mL before DF), 3 samples containing rivaroxaban (27, 220, and 352 ng/mL before DF), and 1 sample containing edoxaban (63.5 ng/mL before DF). Three 5 samples remained positive in the dRVVT-based system and 2 samples in both dRVVT- and aPTT-based systems. Residual DOAC concentration, measured after DF treatment, were all below the respective LLoQ. One sample originated from a patient with known APS taking rivaroxaban. The other 4 samples remaining LAC positive after filtration were from patients with a low probability for APS (no or provoked thrombosis, no pregnancy complications, a negative second LAC testing and low titers for anticardiolipin and anti-β2 glycoprotein I antibodies). In the 2 DOAC-containing samples, LAC negative before DF treatment, final LAC conclusions remained negative after filtration. Of note, within this subgroup of DOAC-containing samples, no different dRVVT-based, aPTT-based, or final LAC conclusions were obtained when calculating NCR using neat NPP versus filtered NPP (Table 2).

For all DOAC-containing samples, DF treatment as well as DS treatment was performed. Final LAC conclusions were concordant in 31 of 35 (88.6%) of the cases (Table 2). In 4 samples, dRVVT-based LAC interpretation, and consequently also the final LAC conclusion, became negative after DF treatment, whereas the dRVVT system remained positive after DS treatment (Table 3). Residual DOAC measurement was below the LLoQ after DF and DS treatment in all 4 samples. None of these samples originated from patients with APS; in fact, all patients had a very low probability for APS diagnosis (1 patient without any history of thrombosis, 3 patients with a negative second LAC testing after >12 weeks, and none of the 4 patients with positive anticardiolipin or anti-β2 glycoprotein I antibodies).

To verify not missing true LAC positives in DOAC-containing samples after DF treatment, 5 LAC positive samples were spiked with rivaroxaban. A significant increase in CT was seen in all LAC steps of both systems compared to the unspiked samples. After DF treatment, CTs returned to the values obtained before DOAC-spiking of the samples and, importantly, interpretation of dRVVT-based, aPTT-based, and final LAC conclusions remained the same in all samples, not missing any true LAC positivity.

3.3.4 | LAC test results in patients treated with VKA and heparins

Within the sample group containing VKA or heparins, final LAC conclusions were not influenced by DF treatment in 25 of 31 (80.6%) samples (Table 4). In 5 patient samples, the final LAC conclusions altered from positive to negative (detailed results in Table 5). Two LMWH-containing samples became negative in LAC conclusion due to a negative PTT-LA screen after filtration and originated from patients without APS. The other samples, 2 VKA-containing and 1 VKA/LWMH-containing sample, originated from 2 patients with known APS and 1 patient with suspected APS. In 1 VKA-containing sample from a patient with APS, the final LAC interpretation changed to negative due to the dRVVT screen result becoming negative after DF treatment. For the 2 other samples (1 VKA-containing sample from a patient with APS and 1 patient with suspected APS containing VKA/LMWH), the dRVVT mix as well as the PTT-LA screen results altered into a negative interpretation, leading to a negative final LAC conclusion after filtration (Table 5). In 1 LMWH-containing sample, the LAC end conclusion is changed from negative to positive due to a borderline positive Staclot result after DF treatment. Within this patient subgroup containing VKA/LMWH/UFH, several changes in the different steps of LAC measurement were noticed. Interpretation of dRVVT screen, mix, and confirmatory tests altered in 3.2%, 16.7%, and 5.0% of the cases, respectively. As a result, dRVVT-based LAC conclusions changed in 4 of 31 (12.9%) (Tables 4 and 5). It is noteworthythat in 2 extra samples containing VKA, the dRVVT-based conclusion after filtration and consequently also the final LAC conclusion, was influenced from a negative to positive interpretation when using NCR calculated with filtered NPP, while interpretations did not alter when using neat NPP for NCR calculation. On the other hand, final LAC interpretation of the sample of the patient with suspected APS containing VKA and LMHW changed from positive to negative after DF using neat NPP for NCR calculation, while it remained positive after DF when using filtered NPP for NCR calculation. Discordances in the aPTT-based system were more pronounced, with changes in screen, mix, and confirmatory interpretation in 35.5%, 21.2%, and 7.1% of the cases, respectively, and altered aPTT system end conclusion in 5 of 31 (16.1%) samples (Tables 4 and 5).

3.3.5 | LAC test results in nonanticoagulated patients

In the patient control group without any anticoagulants, final LAC conclusions were unchanged after DF treatment in 63 of 68 (92.6%) cases (Table 4). In 4 patients, LAC conclusions altered from positive to negative, whereas 1 negative result became positive (detailed results in Table 5). In 8 of 68 samples, dRVVT screen interpretation altered due to a shortening in CT, resulting in NCR below the in-house established cutoff (Table 4), as evidenced by the median ratios presented in Tables 6 and 7. This resulted in a negative dRVVT end conclusion for 5 samples after filtration, leading to an altered (negative) final LAC conclusion for 4 samples (Table 4). Within the aPTT system, however, conclusions changed from negative to positive after DF treatment for 4 of 68 (5.9%) patient samples, due to a slightly higher CT measured for the buffer control aPTT during the aPTT confirmation test, resulting in a higher calculated difference (Tables 6 and 7). All 4 discordances altered into a borderline positive Staclot result (Table 5). aPTT screening results altered in 13 of 68 (19.1%) cases, with 12 resulting in a negative aPTT screen due

TABLE 3 Discrepancies in LAC results for patient samples containing DOAC. Comparing samples treated with DF to samples treated with DS

Sample	Patient samples: DOAC																			
	Not treated										After DF (a)									
	dRVVT-based				aPTT-based				LAC	DOAC	dRVVT-based				aPTT-based				LAC	DOAC
	Scr	Mix	Conf	Conc	Scr	Mix	Conf	Conc	Conc	ng/ml	Scr	Mix	Conf	Conc	Scr	Mix	Conf	Conc	Conc	ng/ml
Cut-off	1.39	1.10	1.10		1.33	1.12	8 s				1.39	1.10	1.10		1.33	1.12	8 s			
Apix	Pos 2.07	pos 1.43	pos 1.07[a]	pos	neg	–	–	neg	pos	147.5	neg 1.20	–	–	neg	neg	–	–	neg	neg	16.5
Rivar	pos 1.92	pos 1.44	pos 1.43	pos	neg	–	–	neg	pos	59	neg 1.28	–	–	neg	neg	–	–	neg	neg	6
Rivar	pos	pos	pos	pos	pos 1.57	pos 1.16	pos 15.9 s	pos	pos	26.5	pos	pos	pos	Pos	neg 1.26	–	–	neg	pos	16
Dabig	pos 2.20	pos 1.42	pos 1.20 [a]	pos	neg	–	–	neg	pos	22.5	neg 0.88	–	–	neg	neg	–	–	neg	neg	19
Edox	pos 2.11	pos 1.45	pos 1.19 [a]	pos	pos	pos	neg	neg	pos	21.5	neg 1.38	–	–	neg	pos 1.38	pos 1.14	neg	neg	neg	11
Edox	pos 2.22	pos 2.39	pos 1.66	pos	pos	pos	pos	pos	pos	63.5	pos 1.41	pos 1.54	pos 1.70	pos	pos	pos	pos	pos	pos	13

Sample	Patient samples: DOAC																			
	After DF (b)										After DS (a)									
	dRVVT-based				aPTT-based				LAC		dRVVT-based				aPTT-based				LAC	DOAC
	Scr	Mix	Conf	Conc	Scr	Mix	Conf	Conc	Conc		Scr	Mix	Conf	Conc	Scr	Mix	Conf	Conc	Conc	ng/ml
Cut-off	1.39	1.10	1.10		1.33	1.12	8 s				1.39	1.10	1.10		1.33	1.12	8 s			
Apix	neg 1.11	–	–	neg	neg	–	–	neg	neg		pos 1.49	pos 1.25	pos 1.27	pos	neg	–	–	neg	pos	16
Rivar	neg 1.24	–	–	neg	neg	–	–	neg	neg		pos 1.40	pos 1.18	pos 1.41	pos	neg	–	–	neg	pos	3.5
Rivar	pos	pos	pos	pos	neg 1.24	–	–	neg	pos		pos	pos	pos	pos	pos 1.58	pos 1.19	pos 16.7 s	pos	pos	13
Dabig	neg 0.87	–	–	neg	neg	–	–	neg	neg		pos 1.58	pos 1.25	pos 1.15	pos	neg	–	–	neg	pos	1

(Continues)

TABLE 3 (Continued)

Sample	Patient samples: DOAC																	
	After DF (b)								After DS (a)									
	dRVVT-based				aPTT-based			LAC	dRVVT-based				aPTT-based				LAC	DOAC
	Scr	Mix	Conf	Conc	Scr	Mix	Conf	Conc	Scr	Mix	Conf	Conc	Scr	Mix	Conf	Conc	Conc	ng/ml
Cut-off	1.39	1.10	1.10		1.33	1.12	8 s		1.39	1.10	1.10		1.33	1.12	8 s			
Edox	neg 1.38	–	–	neg	pos 1.36	neg 1.12	neg	neg	pos 1.73	pos 1.33	pos 1.31	pos	pos 1.41	pos 1.16	neg	neg	pos	9
Edox	pos 1.40	pos 1.52	pos 1.63	pos	pos	Pos	pos	pos	neg 1.27	–	–	neg	pos	Pos	pos	pos	pos	14

Note: dRVVT- and aPTT-based LAC screening, mixing. Confirmatory and conclusion test results expressed as positive or negative based on in-house established cutoff values. DOAC measurements for apixaban, rivaroxaban, dabigatran, and edoxaban presented in nanograms per milliliter. Results interpreted by NCR calculated by the clotting time of neat NPP (a) and the clotting time of DF treated NPP (b). Results in red indicate discordant findings comparing results obtained for DF treated versus DS treated samples.

Abbreviations: –, test was not performed, as previous step within the three-step LAC procedure was negative (following ISTH-SSC recommendations for LAC detection); Apix, apixaban; aPTT, activated thromboplastin time; Conc, conclusion; Conf, confirmatory; Dabig, dabigatran; DF, DOAC-Filter; DOAC, direct oral anticoagulant; dRVVT, diluted Russell's viper venom time; DS, DOAC-Stop; Edox, edoxaban; LAC, lupus anticoagulant; Mix, mixing; NCR, normalized clotting time ratio; Neg, negative; Pos, positive; Rivar, rivaroxaban; Scr, screening.

[a]Interpretation dRVVT confirmatory step by dRVVT screen mix/dRVVT confirm mix ratio greater than in-house cutoff (0.92).

to a decreased NCR (below the in-house cutoff) (Tables 6 and 7), but not changing the aPTT end conclusion due to negative following steps before DF treatment. Of the 4 samples with an altered final LAC conclusion into negative after DF treatment, only 1 patient was suspected for APS diagnosis but not confirmed. Of note, differences in dRVVT conclusion, aPTT conclusion, or end LAC conclusion when calculated NCR using neat NPP versus filtered NPP were not observed.

3.4 | Effect of DF on routine and specialized coagulation assays

To further examine the shortened CTs of the LAC assays after DF treatment and to verify the sample integrity after filtration, PT, aPTT, TT, fibrinogen, coagulation factors, VWF:Ag and VWF:GPIbR, TG, and TFPI were measured before and after DF treatment on 20 LAC-negative patient samples not containing any anticoagulant. Results are shown in Table 8, presenting median values before and after filtration and mean percentage differences.

PT, aPTT, and TT showed a statistically significant difference; however, all mean differences (<10%) were not clinically relevant. For factors II, X, and XII an increase in clotting activity with mean differences of >10% was seen (Table 8), which can cause a procoagulant effect, possibly explaining the shortened CTs seen in the LAC assays. Although for all other coagulation factors, a statistically significant difference is observed, the differences seem to be clinically irrelevant (mean differences of <10%). VWF:Ag measurements before and after DF did not show a statistical or clinical significant difference. In contrast, VWF:GPIbR showed a significant increase, with a mean difference of 11.9%. A procoagulant effect after filtration was also seen in the TG assay. Results are expressed as normalized ratio (Table 8). Normalization of TG parameters was performed by an untreated NPP analyzed in every run.[38] The thrombogram showed a significantly higher peak height (PH) and velocity index (VI) and a lower time to peak (TTP). In addition, a statistically significant but very limited increase in endogen thrombin potential (ETP) was observed, while a significant shortening of the lag time (LT) could not be detected. In all 20 samples, a significant decrease in TFPI concentration was observed, with a mean difference of −47.3%.

4 | DISCUSSION

Interference of DOACs (apixaban, rivaroxaban, dabigatran, and edoxaban) on dRVVT- and aPTT-based LAC testing, resulting in false-positive LAC interpretation, is well known within the hemostasis laboratories.[4,6,9,16,17,30,39] Our observations on DOAC influence in functional LAC clotting assays are in line with previous published findings.[1-3,16,17,30,39] We showed interference of rivaroxaban, dabigatran, and edoxaban resulting in false-positive dRVVT LAC conclusions starting from concentrations above 49, 21, and 32 ng/mL, respectively. Apixaban showed a prolongation of CT

TABLE 4 2 × 2 Contingency table for dilute dRVVT- and aPTT-based LAC screening, mixing and confirmatory tests along with conclusions in both test systems and final LAC conclusions. Results before and after DF treatment in patient samples without any anticoagulants and patient samples containing VKAs or heparins

		No anticoagulants				VKA/LMWH/UFH			
		After DF (a)		After DF (b)		After DF (a)		After DF (b)	
	Not treated	Positive	Negative	Positive	Negative	Positive	Negative	Positive	Negative
dRVVT									
Screening	Positive	17	8	17	8	23	1	23	1
	Negative	0	43	0	43	0	7	1	6
Mixing	Positive	17	0	17	0	18	4	20	2
	Negative	0	0	0	0	0	2	1	1
Confirmatory	Positive	17	0	17	0	18	1	19	1
	Negative	0	0	0	0	0	1	1	0
Conclusion	Positive	17	5	17	5	18	4	19	3
	Negative	0	46	0	46	0	9	2	7
aPTT									
Screening	Positive	26	12	28	10	15	9	17	7
	Negative	1	29	1	29	2	5	2	5
Mixing	Positive	23	3	25	1	10	3	11	2
	Negative	0	5	2	3	1	5	1	5
Confirmatory	Positive	7	0	7	0	6	0	6	0
	Negative	4	21	4	21	1	7	1	7
Conclusion	Positive	7	0	7	0	6	4	6	4
	Negative	4	57	4	57	1	20	1	20
LAC									
Final conclusion	Positive	19	4	19	4	19	5	20	4
	Negative	1	44	1	44	1	6	3	4

Note: Results interpreted by NCR calculated by the clotting time of neat NPP (a) and the clotting time of DF treated NPP (b).

Abbreviations: aPTT, activated thromboplastin time; DF, DOAC Filter; DOAC, direct oral anticoagulants; dRVVT, diluted Russell's viper venom time; LAC, lupus anticoagulant; NCR, normalized clotting time ratio; NPP, normal pooled plasma; VKA, vitamin K antagonist.

but to a much lesser extent, not resulting in false-positive LAC interpretations up to a concentration of 849 ng/mL. Concordant with our previous findings,[30] the aPTT-based system is less affected by DOAC, not obtaining any false-positive aPTT conclusions in the spiking experiment.

Many different strategies have been proposed to eliminate DOAC interferences in coagulation assays, but all have their limitations.[4,9,14,40] As DOAC prescription has increased significantly over the past few years,[41,42] clinical laboratories will receive more DOAC-containing samples and have to choose a strategy on how to cope with this to obtain reliable test results. DOAC-removing agents will be used more commonly by laboratories to eliminate DOAC interference on several coagulation assays.[40] The effects of DS, DOAC-Remove, and active charcoal have been evaluated in multiple studies, giving interesting results on elimination capacity and sample integrity.[23-30,43,44] ISTH guidelines, however, still recommend to interpret results after using a DOAC-removing agent with caution.[14,15] In this study, we evaluated a new device, DOAC Filter, on its efficacy to remove DOACs even at high concentrations and eliminate their interference encountered in LAC testing. Furthermore, we verified the sample integrity after filtration by evaluating multiple coagulation parameters. To the best of our knowledge, this study included the largest study population and control group to evaluate DF to date.

In accordance with the study of Sevenet et al,[34] including DOAC concentrations up to 300 ng/mL, we demonstrated an effective trapping of the tested DOACs by DF. In our study, even supratherapeutic concentrations were effectively removed for all four DOACs (Figure 1). In a communicated preliminary study,[45] the efficacy of DOAC removal by DS, DP-Filter (Universite De Namur, Belgium) and the DF used in our study was investigated in spiked NPP with concentrations of rivaroxaban, dabigatran, and apixaban up to 500 ng/mL. The three DOAC-removing techniques reduced all rivaroxaban and dabigatran concentrations to below the LLoQ. For apixaban, however, DF was unable to eliminate a concentration >250 ng/mL.[45] Measuring rivaroxaban and apixaban concentrations by high-performance liquid chromatography–tandem mass spectrometry with a LLoQ of 2 ng/mL, Farkh and colleagues[46]

TABLE 5 Discrepancies in LAC results for patient samples not containing any anticoagulants and patient samples containing vitamin K antagonists or heparins

Sample	Patient samples: no anticoagulants												
	Not treated									After DF (a)			
	dRVVT-based				aPTT-based				LAC	dRVVT-based			
	Scr	Mix	Conf	Concl	Scr	Mix	Conf	Concl	Concl	Scr	Mix	Conf	Concl
Cut-off	**1.39**	**1.10**	**1.10**		**1.33**	**1.12**	**8 s**			**1.39**	**1.10**	**1.10**	
APS	pos	pos	Pos	pos	pos	pos	neg 3.4 s	neg	pos	pos	pos	pos	pos
LAC pos	pos 1.49	pos	Pos	pos	pos	pos	neg	neg 4.4 s	pos	neg 1.32	–	–	neg
APS	pos	pos	Pos	pos	pos	pos	neg 5.5 s	neg	pos	pos	pos	pos	pos
LAC pos	pos 1.40	pos	Pos	pos	pos	pos	neg	neg	pos	neg 1.33	–	–	neg
LAC neg	neg	–	–	neg	pos	pos	neg 6.7 s	neg	neg	neg	–	–	neg
LAC pos	pos 1.52	pos	Pos	pos	neg	–	–	neg	pos	neg 1.36	–	–	neg
Suspected APS	pos 1.48	pos	Pos	pos	pos 1.34	pos	neg	neg	pos	neg 1.22	–	–	neg
LAC pos	pos 1.44	pos	Pos	pos	neg	–	–	neg	pos	neg 1.25	–	–	neg
Patient samples: AVK/UFH/LMWH													
LMWH	neg	–	–	neg	pos 1.64	pos	pos	pos	pos	neg	–	–	neg
VKA	pos	neg 1.06	–	neg	neg	–	–	neg	neg	pos	neg 1.02	–	neg
APS (VKA)	pos 2.01	pos	Pos	pos	neg	–	–	neg	pos	neg 1.25	–	–	neg
Suspected APS (UFH)	pos	pos 1.23	Pos	pos	pos	pos	pos	pos	pos	pos	neg 1.07	–	neg
LMWH	pos	pos	Pos	pos	pos 1.57	pos	pos	pos	pos	pos	pos	pos	pos
LMWH	neg	–	/	neg	pos 1.35	pos	pos	pos	pos	neg	––	–	neg
Suspected APS (LMWH +VKA)	pos	pos 1.20	Pos	pos	pos 1.85	pos	neg	neg	pos	pos	neg 1.10	–	neg
LMWH	neg	–	–	neg	pos	pos	neg 5.9	neg	neg	neg	–	–	neg
VKA	pos	pos 1.13	neg 1.10[a]	neg	neg	–	–	neg	neg	pos	neg 1.08	–	neg
VKA + LMWH	pos	pos	Pos	pos	pos 1.67	pos	pos	pos	pos	pos	pos	pos	pos
APS (VKA)	pos	pos 1.21	Pos	pos	pos 1.35	neg	–	neg	pos	pos	neg 1.07	–	neg

TABLE 5 (Continued)

Sample	Patient samples: no anticoagulants													
	After DF (a)					After DF (b)								
	aPTT-based				LAC	dRVVT-based				aPTT-based				LAC
	Scr	Mix	Conf	Concl	Concl	Scr	Mix	Conf	Concl	Scr	Mix	Conf	Concl	Concl
Cut-off	1.33	1.12	8 s			1.39	1.10	1.10		1.33	1.12	8 s		
APS	pos	pos	pos 11.5 s	pos	pos	pos	pos	pos	pos	pos	pos	pos 11.5 s	pos	pos
LAC pos	pos	pos	pos 19.0 s	pos	pos	neg 1.18	–	–	neg	pos	pos	pos 19.0 s	pos	pos
APS	pos	pos	pos 12.1 s	pos	pos	pos	pos	pos	pos	pos	pos	pos 12.1 s	pos	pos
LAC pos	pos	pos	neg	neg	neg	neg 1.22	–	–	neg	pos	pos	Neg	neg	neg
LAC neg	pos	pos	pos 9.2 s	pos	pos	neg	–	–	neg	pos	pos	pos 9.2 s	pos	pos
LAC pos	neg	–	–	neg	neg	neg 1.25	–	–	neg	neg	–	–	neg	neg
Suspected APS	neg 1.29	–	–	neg	neg	neg 1.23	–	–	neg	neg 1.29	–	–	neg	neg
LAC pos	neg	–	–	neg	neg	neg 1.26	–	–	neg	neg	–	–	neg	neg
Patient samples: AVK/UFH/LMWH														
LMWH	neg 1.24	–	–	neg	neg	neg	–	–	neg	neg 1.28	–	–	neg	neg
VKA	neg	–	–	neg	neg	pos	pos 1.12	pos	pos	neg	–	–	neg	pos
APS (VKA)	neg	–	–	neg	neg	neg 1.37	–	–	neg	neg	–	–	neg	neg
Suspected APS (UFH)	pos	pos	pos	pos	pos	pos	neg 1.08	–	neg	pos	pos	Pos	pos	pos
LMWH	neg 1.21	–	–	neg	pos	pos	pos	pos	pos	neg 1.15	–	–	neg	pos
LMWH	neg 1.13	–	–	neg	neg	neg	–	–	neg	neg 1.18	–	–	neg	neg
Suspected APS (LMWH +VKA)	neg 1.32	–	–	neg	neg	pos	pos 1.30	pos	pos	pos 1.39	pos	Neg	neg	pos
LMWH	pos	pos	pos 8.8	pos	pos	neg	–	–	neg	pos	pos	pos 8.8	pos	pos
VKA	neg	–	–	neg	neg	pos	pos 1.11	pos 0.93[a]	pos	neg	–	–	neg	pos
VKA + LMWH	neg 1.11	–	–	neg	pos	pos	pos	pos	pos	neg 1.09	–	–	neg	pos
APS (VKA)	neg 1.26	–	–	neg	neg	pos	neg 1.05	–	neg	neg 1.24	–	–	neg	neg

Note: Comparing untreated samples to samples treated with DF. dRVVT- and aPTT-based LAC screening, mixing, confirmatory, and conclusion test results expressed as positive or negative based on in-house established cutoff values. Results interpreted by NCR calculated by the clotting time of neat NPP (a) and the clotting time of DF treated NPP (b). Results in red indicate discordant results obtained for DF-treated samples compared to untreated samples.

Abbreviations: –, test was not performed, as previous step within the three-step LAC procedure was negative (following ISTH-SSC recommendations for LAC detection); Apix, apixaban; APS, antiphospholipid syndrome; aPTT, activated thromboplastin time; Conc, conclusion; Conf, confirmatory; Dabig, dabigatran; DF, DOAC-Filter; DOAC, direct oral anticoagulant; dRVVT, diluted Russell's viper venom time; Edox, edoxaban; LAC, lupus anticoagulant; LMWH, low-molecular-weight heparins; Mix, mixing; NCR, normalized clotting time ratio; Neg, negative; Pos, positive; Rivar, rivaroxaban; Scr, screening; UFH, unfractinated heparins; VKA, vitamin K antagonist.

[a]Interpretation dRVVT confirmatory step by dRVVT screen mix/dRVVT confirm mix ratio greater than in-house cut-off (0.92).

TABLE 6 dRVVT- and aPTT-based LAC screening, mixing, and confirmatory test results expressed as NCR in patient samples containing DOACs before and after DF and DS treatment expressed as median (range) along with mean % differences (95% CI) between results before and after DF treatment

	DOAC										
	Not treated		After DF (a)			After DF (b)			After DS (a)		
	n	Median (range)	n	Median (range)	mean % difference (95% CI)	n	Median (range)	mean % difference (95% CI)	n	Median (range)	mean % difference (95% CI)
dRVVT											
Screening (NCR)	35	2.64 (1.65 to 7.56)	35	1.11 (0.85 to 2.82)	−78.8 (−88.1 to −69.5)	35	1.11 (0.87 to 2.79)	−75.7 (−84.5 to −66.9)	35	1.22 (0.92 to 3.46)	−69.2 (−79.0 to −59.5)
Mixing (NCR)	35	1.90 (1.12 to 4.75)	5	1.36 (1.23 to 2.25)	−47.6 (−87.3 to −7.9)	5	1.36 (1.33 to 2.22)	−46.3 (−86.5 to −6.0)	9	1.28 (1.13 to 2.24)	−28.6 (−49.0 to −8.3)
Confirm (NCR)	35	1.93 (1.34 to 3.27)	5	1.26 (0.83 to 1.60)	−49.0 (−83.4 to −14.7)	5	1.30 (0.86 to 1.65)	−45.2 (−78.2 to −12.2)	8	1.39 (0.99 to 1.74)	27.9 (9.7 to 46.1)
Confirm mix (NCR)	35	1.49 (1.18 to 2.35)	5	1.16 (1.00 to 1.31)	−24.2 (−47.6 to −0.8)	5	1.23 (1.03 to 1.35)	−20.1 (−41.2 to 0.1)	8	1.19 (0.98 to 1.33)	15.6 (2.1 to 29.1)
Screen/confirm (NCR)	35	1.38 (0.67 to 3.24)	5	1.40 (0.95 to 2.50)	−18.0 (−42.8 to 6.8)	5	1.47 (0.91 to 2.40)	−20.7 (−49.4 to −8.1)	8	1.26 (1.08 to 2.19)	−16.1 (−28.7 to −3.6)
Screen mix/confirm mix (NCR)	35	1.21 (0.87 to 2.93)	5	1.14 (1.04 to 1.89)	−24.9 (−45.3 to −4.5)	5	1.13 (1.00 to 1.81)	−27.6 (−51.3 to −3.8)	8	1.14 (1.04 to 1.92)	−13.9 (−27.9 to 0.2)
aPTT											
aPTT Screen (NCR)	35	1.36 (0.88 to 3.39)	35	0.99 (0.80 to 2.31)	−28.1 (−35.0 to −21.2)	35	1.00 (0.81 to 2.28)	−26.6 (−33.3 to −19.9)	35	1.02 (0.84 to 2.56)	−25.2 (−31.4 to −19.0)
aPTT Mix (NCR)	20	1.29 (1.06 to 2.17)	4	1.44 (0.99 to 1.81)	−5.7 (−24.9 to 13.5)	4	1.42 (0.99 to 1.78)	−6.8 (−25.9 to 12.4)	7	1.19 (1.01 to 1.79)	−3.2 (−16.2 to −9.8)
Confirmatory (Staclot)	20	−0.4 (−69.2 to 31.4)	4	16.4 (3.4 to 31.9)	38.8 (−18.4 to 88.0)	4	16.4 (3.4 to 31.9)	38.8 (−18.4 to 88.0)	5	15.4 (3.0 to 21.1)	6.7 (−29.7 to 43.1)

Note: Results interpreted by NCR calculated by the clotting time of neat NPP (a) and the clotting time of DF-treated NPP (b).

Abbreviations: aPTT, activated thromboplastin time; CI, confidence interval; DF, DOAC Filter; DOAC, direct oral anticoagulant; dRVVT, diluted Russell's viper venom time; DS, DOAC-Stop; LAC, lupus anticoagulant; NCR, normalized clotting time ratio.

observed noncomplete DOAC adsorption following DF treatment in 17 of 41 apixaban-containing patient samples. In our study, we also confirmed the capacity of DF to trap DOACs on patient samples. Residual DOAC measurements greater than LLoQ were observed for two apixaban-containing patient samples and one sample containing dabigatran (Table 1). Initial concentrations of these samples were below the highest concentration spiked, indicating that the trapping capacity of DF is not the limiting factor. As the studies of Sevenet et al,[34] Bouvy et al,[45] and Farkh et al[46] also observed residual apixaban concentrations, and the number of apixaban-containing samples (n = 9) in this study is rather limited, further investigation on the removal consistency of apixaban may be needed. It is noteworthy that the residual DOAC levels measured here were below the highest concentration not causing false-positive LAC results (even for the LAC screening steps) in spiked plasma, except for dabigatran (27 ng/mL). Residual DOAC levels after the use of DOAC-removing agents are reported in several studies, some with even higher left-over concentrations.[24-26,28-30,44] With this observation and following the recommendation of the ISTH guidelines,[14,15] a residual DOAC concentration measurement should be performed after DF treatment to ensure complete DOAC removal, before interpretation of the obtained LAC results.

Within the DOAC-containing sample population initially being LAC positive, DF treatment eliminated false-positive LAC results in 84.8% of the cases. Prior studies indicated similar elimination rates of false-positive LAC results using other DOAC-removing agents, such as DS,[23,25,26,30,44] DOAC-Remove,[28,29] or activated charcoal (AC).[24] In our study, application of DF appeared to be an effective way to overcome DOAC influence in LAC detection. By eliminating false-positive LAC results using this new device, unnecessary

repeat LAC testing and prolongation or switch of anticoagulant therapy may be avoided. In four samples becoming negative after DF treatment, a discordant final LAC result was obtained after DS, as they remained positive. All four patients had a very low probability of APS diagnosis, suggesting indeed to have obtained a false-positive LAC result due to DOAC influence. A prolongation of CTs by pretreatment of DS has been described in several studies[30,31] and may be an explanation of the LAC results remaining falsely positive after DS treatment in this study. Two samples containing apixaban obtained negative initial LAC results, in concordance with the observations of our spiking experiment where apixaban-containing samples did not lead to falsely positive final LAC conclusions. Both apixaban-containing samples remained negative after DF as well as DS treatment, suggesting not to be a false-negative LAC result by

TABLE 7 dRVVT- and aPTT-based LAC screening, mixing, and confirmatory test results expressed as NCR in patient samples without anticoagulant and patient samples containing vitamin K antagonists or heparins before and after DF treatment expressed as median (range) along with mean % differences (95% CI) between results before and after DF treatment

	No anticoagulants									
	Not treated		After DF (a)				After DF (b)			
	n	Median (range)	n	Median (range)	mean % difference (95% CI)	*P* value*	n	Median (range)	mean % difference (95% CI)	*P* value*
dRVVT										
Screening (NCR)	68	1.26 (0.84 to 3.98)	68	1.11 (0.73 to 4.77)	−8.7 (−10.8 to −6.6)	<0.0001	68	1.14 (0.75 to 4.87)	−5.9 (−8.1 to −3.7)	<.0001
Mixing (NCR)	25	1.32 (1.05 to 3.05)	17	1.50 (1.16 to 3.24)	−1.7 (−4.4 to 1.0)	0.4543	17	1.63 (1.19 to 3.31)	4.4 (1.4 to 7.3)	.005
Confirm (NCR)	24	1.30 (1.01 to 1.91)	17	1.26 (0.98 to 2.00)	−10.1 (−13.1 to −7.2)	0.0001	17	1.33 (1.03 to 2.00)	−5.3 (−7.8 to −2.8)	.0013
Confirm mix (NCR)	24	1.16 (1.03 to 1.48)	17	1.14 (1.00 to 1.23)	−2.4 (−8.7 to −2.0)	0.0004	17	1.18 (1.06 to 1.33)	−0.6 (−4.7 to 3.6)	.78
Screen/confirm (NCR)	24	1.40 (0.94 to 2.20)	17	1.51 (0.99 to 2.43)	6.4 (3.4 to 9.4)	0.0001	17	1.52 (0.93 to 2.61)	7.6 (3.6 to 11.6)	.002
Screenmix/confirmmix (NCR)	24	1.23 (0.95 to 2.06)	17	1.31 (1.02 to 2.86)	3.7 (−0.7 to 8.1)	0.0386	17	1.28 (0.95 to 2.92)	4.9 (−0.1 to 9.9)	.08
aPTT										
Screening (NCR)	68	1.36 (0.86 to 4.90)	68	1.27 (1.17 to 1.31)	−2.0 (−4.6 to 0.7)	0.0243	68	1.29 (0.86 to 7.08)	−0.2 (−2.9 to 2.4)	.52
Mixing (NCR)	38	1.20 (0.99 to 3.25)	35	1.18 (1.07 to 4.16)	1.8 (−0.7 to 4.3)	0.3082	35	1.20 (1.08 to 4.31)	3.1 (0.4 to 5.7)	.0327
Confirmatory (Staclot. s)	34	1.0 (−5.1 to 100.4)	34	4.05 (−5.0 to 121.6)	50.5 (−8.6 to 109.5)	0.0017	34	4.05 (−5.0 to 121.6)	50.5 (−8.6 to 109.5)	.002
	AVK/UFH/LMWH									
	Not treated		After DF (a)				After DF (b)			
	n	Median (range)	n	Median (range)	Mean % difference (95% CI)	*P* value*	n	Median (range)	Mean % difference (95% CI)	*P* value*
dRVVT										
Screening (NCR)	31	1.89 (1.17 to 4.26)	31	1.67 (1.00 to 4.03)	−6.9 (−11.5 to −2.2)	0.0068	31	1.71 (0.92 to 4.14)	−3.0 (−8.4 to 2.5)	0.394
Mixing (NCR)	24	1.28 (1.06 to 2.59)	24	1.19 (1.02 to 2.62)	−5.0 (−7.1 to −2.9)	0.0005	24	1.29 (1.05 to 3.11)	−3.1 (−4.3 to 3.2)	0.7103
Confirm (NCR)	23	1.58 (1.18 to 2.55)	23	1.46 (1.10 to 2.27)	−9.6 (−12.3 to −6.9)	<0.0001	23	1.30 (0.91 to 2.29)	−3.1 (−6.6 to 0.3)	0.0897
Confirm mix (NCR)	23	1.16 (1.08 to 1.40)	23	1.12 (1.04 to 1.42)	−2.8 (−4.1 to −1.5)	0.0002	23	1.23 (1.07 to 1.49)	3.5 (1.1 to 5.8)	0.3038
Screen/confirm (NCR)	23	1.32 (0.93 to 2.00)	23	1.33 (0.97 to 2.14)	3.3 (−1.7 to 8.3)	0.2113	23	1.30 (0.91 to 2.29)	1.5 (−4.1 to 7.2)	0.4455

(Continues)

TABLE 7 (Continued)

	AVK/UFH/LMWH									
	Not treated		After DF (a)				After DF (b)			
	n	Median (range)	n	Median (range)	Mean % difference (95% CI)	*P* value*	n	Median (range)	Mean % difference (95% CI)	*P* value*
Screenmix/confirmmix (NCR)	23	1.08 (0.97 to 2.09)	23	1.05 (0.93 to 2.11)	−1.8 (−4.4 to 0.7)	0.1564	23	−6.70 (−24.30 to 14.40)	−3.6 (−8.2 to 1.0)	0.2288
aPTT										
Screening (NCR)	31	1.51 (1.06 to 3.78)	31	1.33 (0.98 to 3.35)	−11.5 (−15.7 to −7.3)	<0.0001	31	1.36 (1.03 to 3.39)	−10.0 (−14.4 to −5.6)	0.0002
Mixing (NCR)	24	1.19 (1.01 to 2.91)	21	1.19 (1.01 to 3.49)	−0.3 (−2.8 to 2.1)	0.2645	21	1.20 (1.02 to 3.66)	1.3 (−1.6 to 4.3)	0.5217
Confirmatory (Staclot. s)	22	5.1 (−5.7 to 61.5)	16	2.2 (−5.8 to 95.2)	11.0 (−2.0 to 24.0)	0.0906	16	2.2 (−5.8 to 95.2)	11.0 (−2.0 to 24.0)	0.0906

Note: Results interpreted by NCR calculated by the clotting time of neat NPP (a) and the clotting time of DF treated NPP (b).

Abbreviations: aPTT, activated thromboplastin time; CI, confidence interval; DF, DOAC Filter; dRVVT, diluted Russell's viper venom time; LAC, lupus anticoagulant; LMWH, low-molecular-weight heparins; NCR, normalized clotting time ratio; UFH, unfractionated heparins; VKA, vitamin K antagonists.

**P* values obtained from Wilcoxon signed-rank test.

the presence of DOAC, as was reported by Bonar et al[7] for apixaban due to a more extensive effect on dRVVT confirm than screen, leading to falsely lowered dRVVT confirmatory results. On the other hand, for two other patient samples containing apixaban, initial LAC results were positive and changed to negative after DF, illustrating that results of the spiking experiment cannot fully be extrapolated to patient samples.

Five samples remained positive after DF as well as DS treatment, with residual DOAC levels below the LLoQ. Four samples originated from patients with a low probability of APS diagnosis, suggesting to be transient LAC results, and one sample originated from a patient with known APS receiving rivaroxaban. In addition, positive LAC results in five rivaroxaban-spiked LAC-positive samples after DF treatment indicate that DF allows a reliable detection of true LAC positives in DOAC-containing samples. In contrast, in the VKA/heparin patient group, two patients with known APS and one patient suspected for APS obtained a negative final LAC conclusion after DF treatment. These postfiltration LAC results were obtained by a shortening in CT for dRVVT screen or PTT-LA screen, leading to NCR below the in-house cutoff value (Table 5). Discordances within the nonanticoagulated patient group, with final LAC results becoming negative after filtration, mostly originated from a shorting in dRVVT screen CT. Shorter postfiltration CTs of the LAC assays, however clinically nonsignificant, were also observed by Sevenet et al.[34] In addition, the recently published work of Farkh et al[46] on the effect of DF on LAC testing reported no statistically significant differences for dRVVT and silica clotting time (SCT) screen or screen/confirm ratios in a control group of 68 nonanticoagulated patient samples. However, 4 of 37 dRVVT and 5 of 15 SCT (weakly) positive screen ratios changed to negative after filtration. In our study, we showed a shortening in CT for dRVVT screen, PTT-LA screen, and dRVVT confirm after filtration. This decrease in CT could suggest a procoagulant effect occurring through the use of DF, which was confirmed by TG assays. A significant increase of PH and VI and a decrease of TTP was seen in LAC-negative samples after filtration. A dose-dependent procoagulant effect was also described for DS and DOAC-Remove by several studies using TG obtaining increased results for PH and VI and a decreasing LT.[32,33,47] In the studies of Monteyne et al[32] and Riva et al[47] also, a significant increase in ETP and a slight shortening in LT was seen for both DS and DOAC-Remove. A small but significant reduction in free TFPI was considered as the cause of the procoagulant effect after DS or DOAC-Remove treatment.[32,33] Accordingly, we also observed a significant reduction in free TFPI levels after filtration, explaining partly the shortening in CTs and the observed procoagulant effect. Apart from trapping small amounts of free TFPI, we also observed a significant increase of factors II, X, XII, and VWF:GPIbR suggesting a limited activation of the coagulation pathways by pushing the plasma through the hydrophobic-hydrophilic solid phase, also causing a procoagulant effect. Only few studies evaluated the effect of DOAC-removing agents on coagulation factors. After DS treatment, Jacquemin et al[22] did not find any changes in factors X, VII, and VIII levels in normal plasma, which was confirmed by Platton et al[25] for factor VIII in nonanticoagulated patient samples. In contrast, Riva et al[47] observed a reduction of factors VIII, IX, X, XI, and XII after DS treatment of normal plasma.

Observing the shortening in CTs for dRVVT screen, confirm, and PTT-LA screen after filtration, NCR of the LAC assays of DF-treated samples were calculated using DF-treated NPP and compared to the NCR calculated by neat NPP. No differences in final LAC interpretation were observed in the nonanticoagulated and DOAC-containing patient population and only minor alterations were seen in the VKA/

TABLE 8 Influence of DOAC Filter on routine coagulation parameters, coagulation factors, von Willebrand parameters and thrombin generation parameters. Median levels with minimum and maximum range and mean % deviation with 95% CIs

Parameter	n	Median untreated samples (min-max)	Median DF treated samples (min–max)	Mean % deviation (95% CI)	*P* value*
Routine coagulation parameters					
aPTT, s	20	36.0 (29.8 to 43.1)	36.6 (28.4 to 40.8)	−2.4 (−3.9 to −0.9)	**.003**
PT, s	20	13.3 (11.3 to 17.3)	13.2 (10.9 to 16.9)	−1.8 (−2.9 to −0.7)	**.003**
PT, %	20	102.0 (68.0 to 134.0)	103.5 (70.0 to 144.0)	2.7 (0.9 to 4.4)	**.005**
Fibrinogen, mg/dL	20	290.5 (171.0 to 497.0)	284.0 (157.0 to 524.0)	−0.7 (−2.6 to 1.2)	.73
TT, s	20	17.5 (15.8 to 26.4)	18.3 (16.3 to 37.6)	5.7 (3.0 to 8.4)	**<.0001**
Intrinsic factors, %					
VIII	20	82.2 (46.8 to 222.2)	78.9 (41.1 to 22202)	−9.9 (−12.9 to −6.8)	**<.0001**
IX	20	92.4 (59.8 to 121.9)	93.7 (59.8 to 128.0)	2.4 (0.7 to 4.2)	**.007**
XI	20	113.9 (80.9 to 172.8)	115.0 (73.0 to 112.7)	−5.0 (−8.2 to −1.8)	**.003**
XII	20	89.2 (38.0 to 144.5)	103.4 (46.5 to 176.7)	**16.3 (14.0 to 18.0)**	**<.0001**
Extrinsic factors, %					
II	20	100.8 (76.3 to 136.0)	118.4 (85.0 to 198.9)	**14.1 (12.0 to 16.3)**	**<.0001**
V	20	91.0 (51.1 to 142.5)	81.0 (47.6 to 135.1)	−6.7 (−10.3 to −3.0)	**.0008**
VII	20	121.0 (53.9 to 225.4)	127.0 (57.8 to 237.4)	5.3 (3.6 to 7.1)	**<.0001**
X	20	105.0 (53.4 to 145.5)	120.2 (61.6 to 195.0)	**15.2 (12.9 to 17.5)**	**<.0001**
von Willebrand Factor, %					
VWF:Ag	20	109.4 (57.5 to 220.5)	120.5 (70.3 to 225.3)	6.3 (1.0 to 11.6)	.06
VWF:GPIbR	19	110.1 (61.9 to 222.9)	117.8 (72.7 to 271.7)	**11.9 (9.1 to 14.8)**	**<.0001**
Thrombin generation assay					
PH normalized ratio	19	0.7 (0.4 to 0.9)	0.9 (0.6 to 1.2)	**32.2 (20.5 to 43.8)**	**<.0001**
VI normalized ratio	19	0.5 (0.2 to 1.1)	0.9 (0.4 to 1.2)	**40.9 (32.4 to 49.5)**	**<.0001**
TTP normalized ratio	19	1.3 (0.9 to 1.7)	1.1 (0.9 to 1.4)	**−18.2 (−24.0 to −12.4)**	**<.0001**
LT normalized ratio	19	1.2 (0.9 to 1.6)	1.2 (1.0 to 1.7)	1.7 (−2.6 to 6.0)	.26
ETP normalized ratio	19	0.9 (0.7 to 1.2)	0.9 (0.7 to 1.5)	4.3 (0.1 to 8.42)	.03
TFPI					
TFPI, ng/mL	20	15.4 (7.7–42.3)	8.4 (4.2 to 17.7)	**−47.3 (−52.3 to −42.3)**	**<.0001**

Note: Numbers in bold indicate a statistically significant difference if *P* value <.05 or a clinically significant difference if the mean deviations is >10%.

Abbreviations: aPTT, activated partial thromboplastin time; CI, confidence interval; DF, DOAC Filter; ETP, endogen thrombin potential; LT, lag time; max, maximum; min, minimum; n, number of samples; PH, peak height; PT, prothrombin time; TFPI, tissue factor pathway inhibitor; TT, thrombin time; TTP, time to peak; VI, velocity index; VWF:Ag, von Willebrand factor antigen; VWF:GPIbR, von Willebrand factor activity.

**P* values obtained from the Wilcoxon signed-rank test.

heparin-containing samples. Taking these results into account, we imply that using DF-treated NPP for NCR calculation is not necessary to obtain reliable LAC results for DOAC-containing samples.

Within the nonanticoagulated and VKA/heparin subgroup, minor alterations in LAC results were observed frequently, sometimes leading to changed LAC interpretation within the dRVVT or aPTT end conclusion. Final LAC conclusions altered in 7.4% and 19.4% for nonanticoagulated and VKA/heparin-containing patient populations, respectively. In addition, in 4 of 17 patients with APS or suspected APS within the "no anticoagulant" or "VKA/heparin" patient group, final LAC results changed from positive to negative after DF treatment, leading to misdiagnosis. Because of these alterations, applying DF in non–DOAC-containing samples may lead to erroneous LAC results and a different LAC interpretation. Therefore, DF treatment should only be applied in samples from patients on documented ongoing DOAC therapy, following the recent ISTH guidance on LAC detection in anticoagulated patients.[14,15]

The use of DOAC removing agents will rise with the increase of LAC testing on DOAC-containing samples. In practice, the 15-minute one-step DF procedure may be preferred to a 20-minute two-step (incubation and centrifugation) procedure of DS. However, a 22.6% volume loss after filtration with a mean recovered volume of 465 μL, in line with observations of Sevenet et al[34] and Farkh et al,[46] could be a limiting factor in clinical practice. In this regard, a full three-step LAC testing in two systems may need the use of 2 DFs, which is economically less interesting and may lead to insufficient sample volume (2 × 600 μL PPP). As all DOAC removing agents have their own mechanism of action and different impact on coagulation assays, interpretation of LAC assays will get more complicated. The conclusions on DF treatment made in this study

apply only for the reagents and analyzer used and cannot fully be extrapolated to other reagents for LAC measurement or other coagulation parameters.

5 | CONCLUSION

This study shows the ability of DF to efficiently trap DOACs (apixaban, rivaroxaban, edoxaban, and dabigatran) out of citrated plasma, even at supratherapeutic levels, and to eliminate DOAC interference during LAC testing. Observing some samples with an incomplete DOAC removal, even at therapeutic DOAC levels, a DOAC measurement should be performed after DF treatment to allow reliable interpretation of LAC results, following ISTH recommendations. During evaluation of the sample integrity, a postfiltration procoagulant effect was seen by TG, explaining the shortening in CTs observed during LAC testing in DF-treated samples. Minor alterations in LAC assay results were seen within the patient control groups (nonanticoagulated or VKA/heparin-containing samples). We confirm the recent ISTH guidelines not to use any DOAC-removing agents or devices in samples not containing DOAC. Whenever a DOAC-removing agent/device is used to eliminate DOAC interference during LAC testing, the LAC result should be reported with a comment and results should be interpreted with caution.

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RELATIONSHIP DISCLOSURE

The authors declare no conflict of interest.

AUTHOR CONTRIBUTIONS

KD designed the study. EL and KD analyzed the data. EL performed part of the experiments and wrote the manuscript. PDK helped with the spiking experiments. KD and PDK critically reviewed and adapted the manuscript. All authors approved the final version of the manuscript.

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SUPPORTING INFORMATION

Additional supporting information may be found in the online version of the article at the publisher's website.

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