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Dilute Russell's viper venom time reagents in lupus anticoagulant testing: a well-considered choice

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Abstract

Background: Lupus anticoagulant (LAC) detection represents diagnostic challenges among which the multitude of available reagents and interference by anticoagulant treatment. One of the two advised tests is the dilute Russell's viper venom time (dRVVT). However, it is currently not clear whether all dRVVT reagents may be considered equivalent. The objective of the study was to evaluate the diagnostic performance of two dRVVT reagents, with special attention to the influence of anticoagulant therapy.

Methods: STA®-Staclot® dRVV Screen/Confirm (Stago, Asnières-sur-Seine, France) and dRVT-LS/dRVTL-LR (Haematex, Hornsby, Australia) were evaluated on 443 patient samples [358 consecutive patients with LAC request including six antiphospholipid syndrome (APS) patients, 18 non-consecutively selected APS patients and 37 vitamin K antagonists (VKA)-treated and 30 direct oral anticoagulants (DOAC)-treated non-APS patients]. Additionally, pooled normal plasma (PNP) was spiked with factor deficient plasma (n=33) and DOAC calibrators (n=21) to evaluate sensitivity for factor deficiencies and false-positivity rates, respectively.

Results: A higher number of samples were defined as LAC positive by Stago vs. Haematex [11.5% (41/358) vs. 3.63% (13/358)]. Most discordances were in the VKA and DOAC group. Haematex was less prone to VKA-related factor deficiencies, explaining the absence of false-positive LAC results in VKA-treated non-APS patients compared to 10.8% with Stago. We observed no false-positive LAC ratios with Haematex in DOAC-spiked PNP and a lower number in DOAC-treated non-APS patients. However, increased specificity seemed to be at cost of a reduced sensitivity as Haematex showed less positive APS patient samples (45.8% vs. 87.5%).

Conclusions: dRVVT reagents differ in LAC sensitivity and for VKA and DOAC interference.

Keywords: dilute Russell's viper venom test; direct oral anticoagulants; lupus anticoagulant; vitamin K antagonist.

Introduction

Lupus anticoagulant (LAC) detection is a challenge for laboratories in the diagnosis of antiphospholipid syndrome (APS) due to the heterogeneous and variable spectrum of antiphospholipid antibodies (aPL) [1], multistep testing algorithm including a screening, mixing and confirmation step [2, 3], the several assay principles and multitude of available reagents [4], the interference by antithrombotic treatment and the lack of a gold standard [4–10].

To improve standardization, the Scientific Standardization Subcommittee (SSC) of the International Society of Thrombosis and Haemostasis (ISTH) 2009 guidelines limits the choice to a dilute Russell's viper venom time (dRVVT) and an activated partial thromboplastin time (aPTT) system [6]. The Clinical and Laboratory Standards Institute (CLSI) guidelines also give priority to the aPTT/dRVVT combination but without restricting other tests [4].

dRVVT is assumed to result in less false-positive results caused by factor deficiencies and is shown to be more specific and robust compared to aPTT or other tests [11, 12]. Nevertheless, sensitivity and specificity differences for dRVVT [11, 13–16] were previously reported. In addition, newly developed reagents claim to be less influenced by antithrombotic therapy [15].

False-positive LAC results are mainly caused by anticoagulation in patients at the time of testing [16]. Although LAC analysis is better postponed until treatment is ceased, clinicians often overrule this recommendation in daily practice [6, 17]. The ISTH provides guidelines on testing patients treated with unfractionated heparin

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(UFH), vitamin K antagonists (VKA) and low-molecular-weight heparin (LMWH) [6]. However, guidelines are lacking on how to deal with direct oral anticoagulants (DOAC) [18]. So far, only the CLSI guidelines briefly mention the risk of false-positive LAC interpretations in dabigatran (DAB)- and rivaroxaban (RIV)-treated patients [4]. Although several reports exist on the false-positivity in LAC testing by DOAC [19–24], susceptibility of specific reagents has not been investigated yet.

In this study, we evaluated the performance of two dRVVT Screen/Confirm reagents, with special attention to the influence of anticoagulant therapy. We aimed to acquire more information on the diagnostic performance and pitfalls in dRVVT reagents from different manufacturers.

Materials and methods

Patients samples

We collected samples from 426 consecutive patients with LAC request at the Ghent University Hospital analysed for a diagnostic work-up of hypercoagulability, pregnancy complications, auto-immune diseases or a prolonged aPTT. Four patients receiving UFH and 64 patients with lack of therapy information were excluded, resulting in a final cohort of 358 patients. Patients were divided according to the therapy group (VKA, LMWH, DOAC or no anticoagulation). Eighty-two percent (287/358) were not anticoagulated at the time of LAC analysis, 9.50% (34/358) were treated with VKA [international normalized ratio (INR) 1.21–6.94, median 2.81], 6.70% (24/358) with LMWH (aPTT 39.8–127.1 s, median 51.0 s) and 3.63% (13/358) received DOAC [DAB (n=2) and RIV (n=10); INR 1.24–3.91, median 2.13; aPTT 41.5–98.0 s, median 52.2 s].

Six consecutive patients fulfilled both laboratory and clinical criteria for APS [10]. In addition, stored plasmas from previously diagnosed definitive APS patients with persistent LAC were enrolled (n=24 APS cases, 10/24 positive in aPTT and 24/24 positive in dRVVT (previously used LA Screen/LA Confirm, Life Diagnostics, Clarkston, GA, USA). Sixteen out of 24 (66.7%) APS patients received VKA treatment (INR 0.96–5.11, median 2.56), 8.33% received DOAC (INR median 3.37; aPTT median 71.8 s) and 25.0% (6/24) were not anticoagulated. All APS patients were defined by a history of thrombotic events (n=23; deep venous thrombosis (10/24), pulmonary embolism (8/24), catheter-related thrombosis (1/24), non-specified venous (3/24)/arterial (1/24) thrombosis) or miscarriage (1/24)). Our cohort of APS patients consisted of 66.7% (16/24) patients with LAC alone and 33.3% (8/24) with more than one laboratory criterion (LAC with anticardiolipin n=6, LAC with anticardiolipin and β_2-glycoprotein-I n=2).

Besides, we performed LAC testing on samples from VKA- (n=37) and DOAC- (n=30) anticoagulated patients treated for atrial fibrillation without LAC request. In the DOAC cohort, five patients received apixaban (API) (INR 1.06–1.97, median 1.15; aPTT 35.7–59.7 s, median 37.0 s), 11 patients received DAB (INR 1.00–2.95, median 1.32; aPTT 44.7–141 s, median 71.1 s) and 14 patients received RIV (INR 1.24–4.97, median 2.49; aPTT 32.8–87.4 s, median 52.2 s).

The study was approved by the Ethical Committee of the Ghent University Hospital.

Lupus anticoagulant testing

LAC analysis by an aPTT system (PTT-LA and Staclot-LA; Diagnostica Stago, Asnières, France) in parallel with Stago and Haematex dRVVT reagents, was performed according to the guidelines [6] on the STA-R Evolution (Stago). Cut-offs (COs) were calculated by the 99th percentile on 120 healthy donors [6] except for the Staclot LA, for which a locally validated CO (8 s) was accepted [25, 26].

LAC screening was performed with the STA®-Staclot® dRVV Screen by Stago and dRVT-LS by Haematex. Confirmation tests were performed by the corresponding phospholipid (PL)-rich dRVVT reagent (STA®-Staclot® dRVV Confirm and dRVT-LR, respectively) on all samples, in contrast to the routine work-up where we only perform confirmation tests in the case of a prolonged screen and mix result. Next to the 1:1 mixing step [1/2, one part patient plasma (PP)/total parts PP and pooled normal plasma (PNP)], we performed additional mixing steps with increasing PNP volumes (1/4, 1/8, 1/10, 1/15 and 1/20) for 15/24 APS patient samples to evaluate the sensitivity of the dRVVT reagents. Screen, mix and confirm test results were expressed as normalized ratios by dividing the PP clotting time (CT) by the PNP CT analysed in each run [6, 27, 28].

The LAC ratio (screen/confirm) performed on PP was judged in the confirmation step of the "three-step interpretation". In the case of a prolonged confirm, we additionally calculated the LAC ratio on 1:1 mixed PP:PNP, previously shown to improve diagnostic accuracy in VKA-treated patients [29, 30], which is referred to as the "alternative interpretation". A test result was considered positive if its value exceeded the respective CO (shown for all test results in the legend of Figure 1).

LAC was present according to the three-step interpretation if the screen, screen mix and LAC ratio were positive. Following the alternative interpretation, LAC was present when the screen and LAC ratio PP:PNP were positive.

Spiking experiments

Influence of DOAC: Commercially available calibrators for API (CAL4, 455 ng/mL; Stago), DAB (CAL3, 468 ng/mL; Hyphen BioMed, Neuville-sur-Oise, France) and RIV (CAL3, 525 ng/mL; Hyphen Biomed) were analysed in dilution series with PNP (seven concentrations for each DOAC) in concentrations in line with the literature-based therapeutic ranges [31–34]. The spiked samples were measured in duplicate by all dRVVT reagents. A check of the spiked concentrations was performed on the STA-R Evolution (Stago) by a chromogenic anti-Xa assay for RIV (STA-Liquid Anti-Xa, Stago) and API (DiXal kit, Hyphen Biomed) and a diluted thrombin time (Hemoclot®, Hyphen Biomed) for DAB.

Influence of factor deficiencies: VKA cause factor deficiencies for factor II (FII) and factor X (FX) which may interfere in dRVVT LAC testing. In order to explore the sensitivity of the dRVVT reagents for factor deficiencies, plasma with decreasing concentrations of FII,

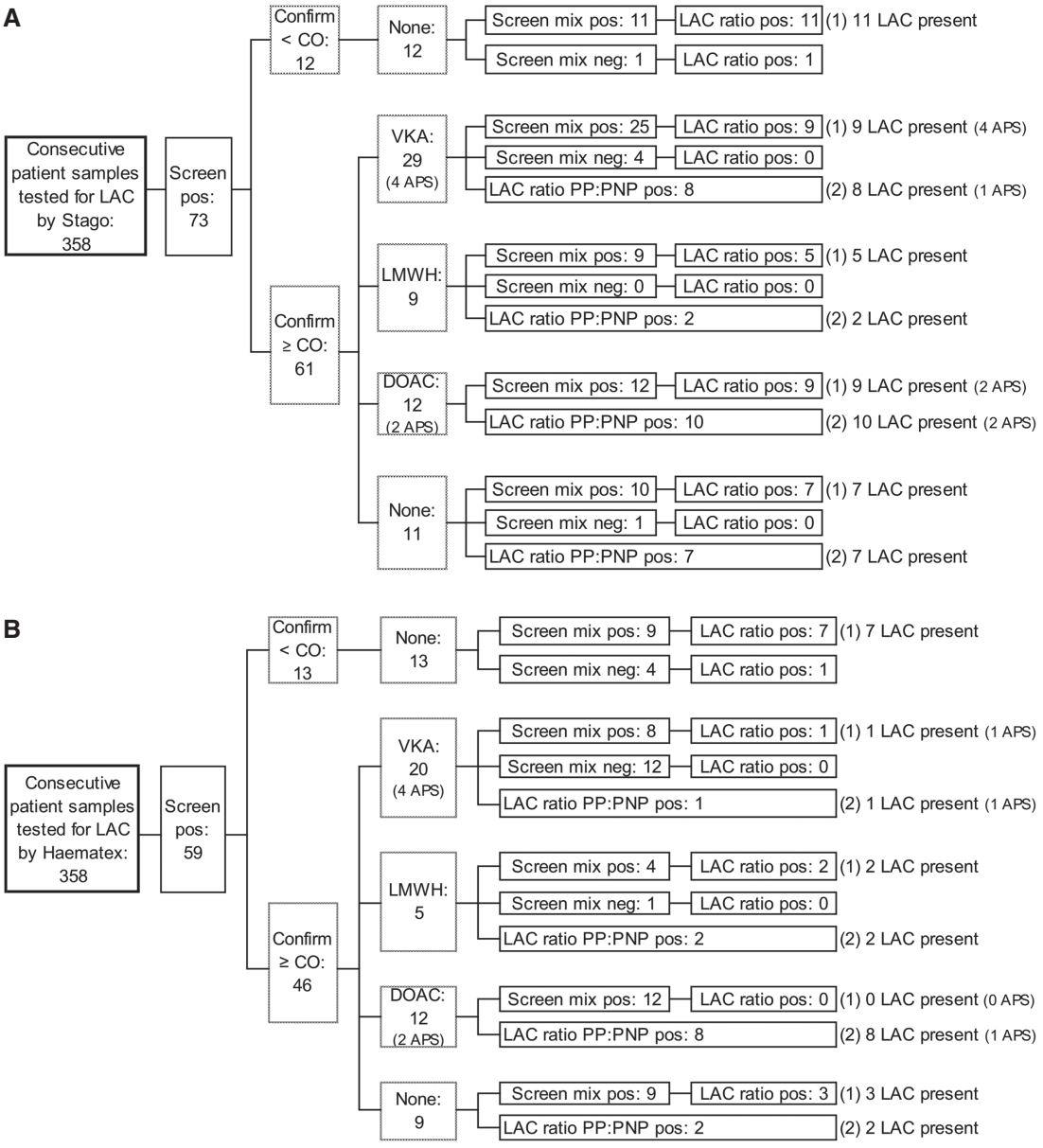

Figure 1: LAC analysis by Stago (A) and Haematex (B) dRVVT reagent on 358 consecutive patient samples.
Screen: normalized ratio of the screening test on neat PP (Stago CO 1.53, Haematex CO 1.44); confirm: normalized ratio of the confirmation test on neat PP (Stago CO 1.21, Haematex 1.29); LAC ratio: ratio screen/confirm (Stago CO 1.25, Haematex CO 1.28); screen mix: normalized ratio of the screening test on 1:1 mixed PP:PNP (Stago CO 1.18, Haematex CO 1.20); confirm Mix: normalized ratio of the confirmation test on 1:1 mixed PP:PNP (Stago CO 1.11, Haematex 1.12); LAC ratio PP:PNP: ratio screen mix/confirm mix (Stago CO 1.11, Haematex CO 1.13). Final conclusion LAC presence according to (1) screen pos, screen mix pos and LAC ratio pos (=three-step interpretation) or (2) screen pos and LAC ratio PP:PNP pos (=alternative interpretation). LAC, lupus anticoagulant; APS, antiphospholipid syndrome; PP, patient plasma; PNP, pooled normal plasma; VKA, vitamin K antagonists; DOAC, direct oral anticoagulants; LMWH, low-molecular-weight heparin; none, no anticoagulation; CO, cut-off; pos, above CO; neg, below CO.

Factor V (FV) and FX were measured. Commercial STA-Immunodeficient (STA-ID) (FII) or STA-deficient (STA-D) plasma (FV, FX) (all by Stago) were mixed with increasing volumes of PNP. FII, FV and FX levels were determined in duplicate on the undiluted PNP (100% factor level), the undiluted factor deficient plasma (1% factor level) and nine dilutions with factor levels between 10% and 90% by one-stage clotting assays by Neoplastine CI plus reagent on the STA-R Evolution (Stago) [35].

Statistical analysis

Statistic calculations were performed using SPSS statistics 23 and MedCalc for Windows, version 12.3 (Mariakerke, Belgium). Tukey and the right-sided Grubbs' test were used to remove outliers prior to CO calculation. The Spearman's rank correlation coefficient (ρ) was calculated as a non-parametric hypothesis test to measure associations. A p-value <0.01 was considered statistically significant.

Results

LAC dRVVT analysis on patient samples

In the consecutive patient cohort (n=358), the Stago Screen reagent showed 20.4% prolongations (73/358) of which 68.5% originated from anticoagulated patients (Figure 1A). Haematex showed a prolonged screen in 16.5% of the cases (59/358) of which 62.7% received anticoagulants (Figure 1B). Figure 1A and 1B illustrate for all samples with a prolonged screen, in respect to the therapy group, the number of positive and negative mixing steps with corresponding LAC ratios, and LAC ratios PP:PNP if a prolonged confirm was observed. Consequently to the lower number of screen positives, Haematex showed a lower number of screen mix positives (71.2%, 42/59) and LAC ratio positives (22.0%, 13/59) compared to Stago (91.8%, 67/73 and 56.2%, 41/73, respectively). Correlation plots are shown in Figure 2A–E for Stago vs. Haematex for the screen, confirm, screen mix, confirm mix and LAC ratio test results regarding all samples (n=358). Correlation was lower regarding the LAC ratio, especially among the samples with a prolonged screen and confirm (n=61 Stago, n=46 Haematex), indicative for antithrombotic therapy (Figure 2F, ρ=0.558). Equally, the LAC ratio PP:PNP was positive in 44.3% (27/61) of the samples by Stago and 28.3% (13/46) of the samples by Haematex (Figure 2G, ρ=0.755).

Comparison of the LAC conclusion by Stago according to the alternative vs. three-step interpretation for samples with a prolonged confirm (n=61) showed a comparable number of LAC positives (27 vs. 30, respectively, Figure 1A). The number of LAC positives by Haematex (n=46) doubled using the alternative vs. three-step interpretation (13 vs. 6, respectively), mainly due to a different interpretation in DOAC-treated patients (8/12 vs. 0/12, respectively).

In total, based on the three-step interpretation, 11.5% (41/358) patient samples were defined as LAC positive by Stago compared to 3.63% (13/358) by Haematex. Most discordances were observed in the VKA [22.0% (9/41) vs. 7.69% (1/13)] and DOAC [22.0% (9/41) vs. 0% (0/13)] group. All six APS samples were found positive by Stago but only 1/6 by Haematex. Likewise, the alternative interpretation also yielded a higher positivity rate by Stago (44.3%, 27/61) compared to Haematex (28.3%, 13/46).

In a cohort of 24 LAC positive APS patients (Figure 3A and 3B), serial dilutions with increasing PNP volumes (1/2, 1/4, 1/8, 1/10, 1/15, 1/20) were performed until a negative LAC ratio PP:PNP was obtained. Stago analysis yielded a prolonged LAC ratio PP:PNP for 87.5% (21/24) APS patients in the 1/2 dilution of which only 15 could be included for further mixing due to limited sample volume (Figure 3A). Positivity was maintained until the 1/20 dilution. With Haematex, 45.8% APS samples (11/24) showed a positive LAC ratio PP:PNP in the 1/2 dilution, of which six were included for further mixing (Figure 3B) and all samples rendered LAC negative at the 1/8 dilution.

Influence of DOAC on dRVVT Screen and Confirm reagents

We performed dRVVT on plasma from DOAC-treated patients non-suspicious for APS (n=30). Screen and screen mix results showed a high false-positivity rate by both Stago (86.7%, 26/30) and Haematex (83.3%, 25/30 and 80.0%, 24/30 respectively) (Figure 4A). The three-step interpretation excluded LAC positivity by Haematex and showed 30.0% (9/30) false-positive LAC results by Stago which all originated from RIV-treated patients (121–380 ng/mL). The alternative interpretation increased the number of false-positives in both dRVVT systems (43.3%, 13/30 for Stago and 30.0%, 9/30 for Haematex).

To explore the influence of API, DAB and RIV on dRVVT LAC analysis separately, PNP was spiked with increasing volumes of DOAC calibrators in order to obtain three series of seven samples with DOAC concentrations between 0 and 500 ng/mL. All three DOAC caused a concentration-dependent prolongation of screen, screen mix, confirm and confirm mix, comparable for both dRVVT reagents (Table 1). DAB falsely prolonged test results from 20 ng/mL on, which is lower than the therapeutic range for peak and trough levels in high-dosed patients [36]. False-prolonged screen and confirm by RIV occurred at lower concentrations for Stago (65 ng/mL) compared to Haematex (115 ng/mL). API caused a minor interference with both screen reagents (310–412 ng/mL), which was more pronounced for the confirm reagents (99–193 ng/mL).

Stago LAC ratios in RIV-spiked PNP were positive starting from 115 ng/mL. By the alternative interpretation, DAB and RIV caused false-positive LAC conclusions by Stago reagents starting from 70 ng/mL and 65 ng/mL, respectively.

Sensitivity of dRVVT reagents for factor deficiencies

LAC screening on plasma from VKA-treated patients non-suspicious for APS (n=37) by Stago resulted into a remarkable higher positivity rate vs. Haematex (89.2%, 33/37 vs.

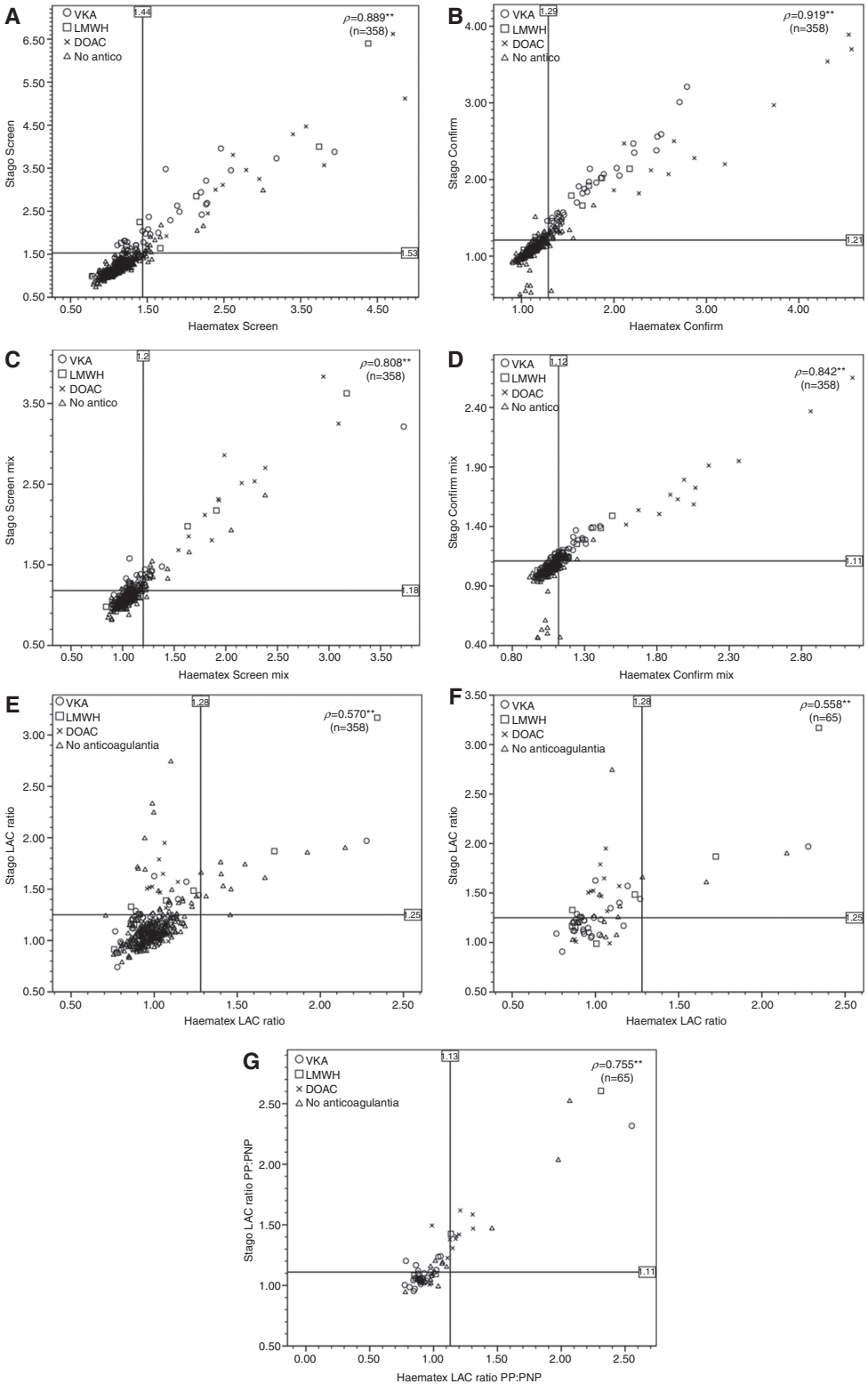

Figure 2: Correlation plots of the Stago and Haematex test results.
Screen (A), confirm (b), screen mix (c), confirm mix (D) and LAC ratio (E) performed on all samples (n=358) or limited to the samples with prolonged screen and confirm ratios (n=65) for LAC ratio (F) and LAC PP:PNP ratio (G). The corresponding formulas to the different test results are described in the legend of Figure 1. The ρ-values and amount of samples (n) are depicted in every right-hand corner. Correlation is significant at the 0.01 level (**). The respective COs are indicated in each plot on a full line on the top (Haematex) and right side (Stago). LAC, lupus anticoagulant; PP, patient plasma; PNP, pooled normal plasma; VKA, vitamin K antagonists; DOAC, direct oral anticoagulants; LMWH, low-molecular-weight heparin; ρ, Spearman's rank correlation coefficient, CO, cut-off.

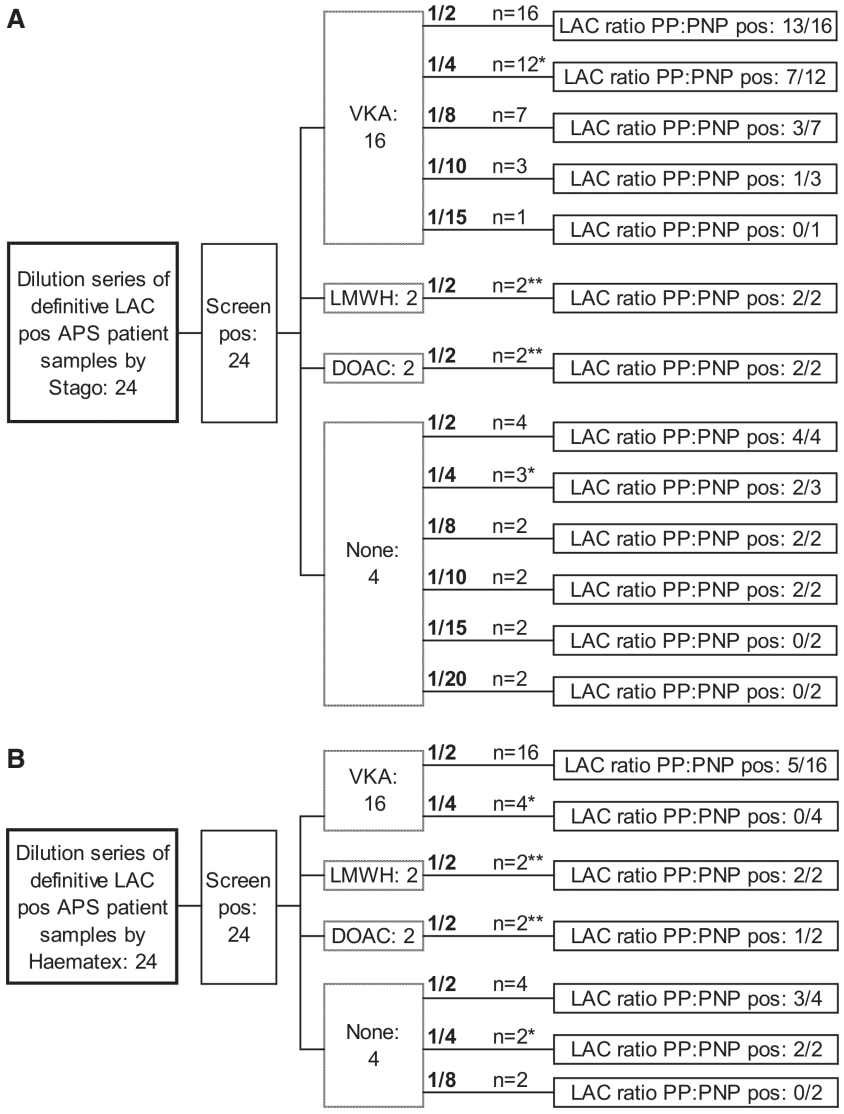

Figure 3: LAC analysis by Stago (A) and Haematex (B) dRVVT reagent on dilution series of 24 definitive LAC positive APS patient samples. The corresponding formulas and respective COs to screen and LAC ratio PP:PNP test results are described in the legend of Figure 1. Samples with insufficient volume for further dilutions are indicated by *(one sample insufficient volume) or **(two samples insufficient volume). The respective dilution (one part PP/total parts PP and PNP) and number of samples (n) are indicated between the therapy group (VKA, LMWH, DOAC or None) and the LAC PP:PNP ratio. LAC, lupus anticoagulant; APS, antiphospholipid syndrome; PP, patient plasma; PNP, pooled normal plasma; VKA, vitamin K antagonists; DOAC, direct oral anticoagulants; LMWH, low-molecular-weight heparin; none, no anticoagulation; CO, cut-off; pos, above CO; neg, below CO.

32.4%, 12/37) (Figure 4B). Mixing excluded LAC positivity in most cases by Haematex in contrast to Stago. Following the three-step and alternative interpretation, Stago analysis led to 18.9% (7/37) and 10.8% (4/37) false-positives, respectively, whilst false-positive LAC results were absent with Haematex.

Additionally, we investigated the sensitivity of both dRVVT Screen/Confirm reagents for VKA-related (FII, FX) and FV factor deficiencies using 95th percentile COs for screen and confirm interpretations [35]. FV decrease was detected by Stago Screen from a level below 62% (Figure 5A) and Haematex Screen (Figure 5C) prolongation started from a level of 40% on. Confirm prolongations started from 73% FV for both dRVVT reagents. Screen (Figure 5A) and Confirm (Figure 5B) determined by Stago prolonged for moderately decreased FII and FX levels (46%–57%) whereas the Haematex Screen (Figure 5C) and Confirm (Figure 5D) reagents prolonged from <1% FII, and FX deficiencies of 24% and 36%, respectively.

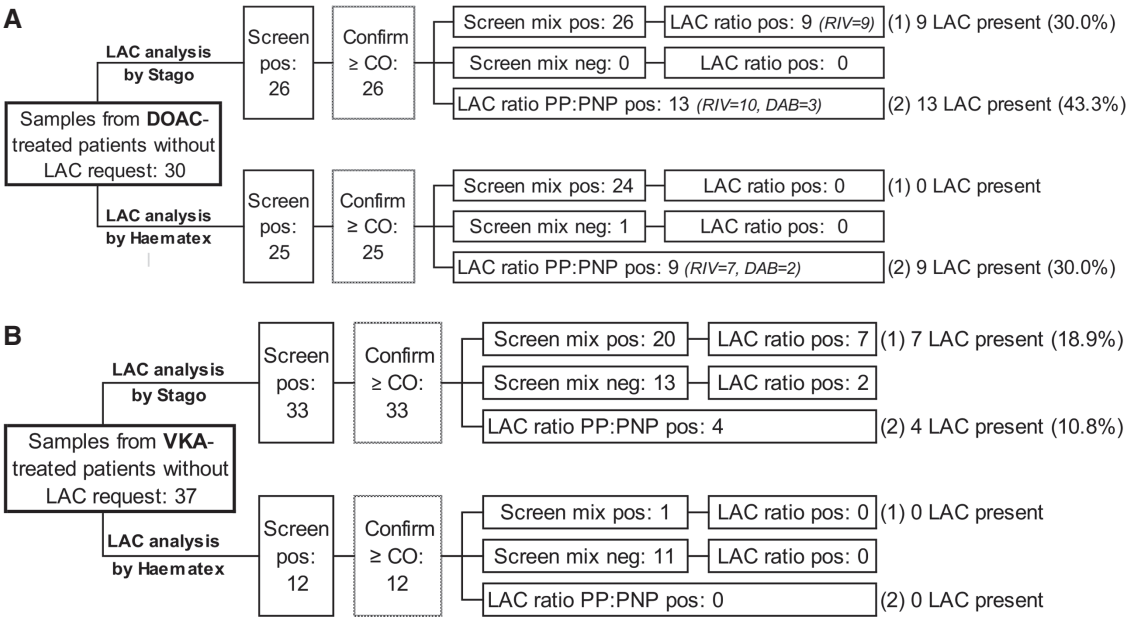

Figure 4: LAC analysis by Stago and Haematex dRVVT reagents on 30 DOAC-treated patients (A) and 37 VKA-treated patients (B) without LAC request.
The corresponding formulas and respective COs to the different test results together with the final LAC interpretations (1=three-step interpretation and 2=alternative interpretation) are described in the legend of Figure 1. LAC, lupus anticoagulant; PP, patient plasma; PNP, pooled normal plasma; VKA, vitamin K antagonists; DOAC, direct oral anticoagulants; CO, cut-off; pos, above CO; neg, below CO; RIV, rivaroxaban; DAB, dabigatran.

Discussion and conclusion

We showed that different dRVVT reagents may lead to opposing LAC conclusions. By the three-step interpretation, Haematex dRVVT reagents showed a lower positivity rate (3.63%, 13/358) compared to Stago (11.5%, 41/358) in a consecutive patient cohort. Discordances were mainly observed in the VKA- and DOAC-treated patients. For the samples with a prolonged confirm test result (n=61 Stago, n=46 Haematex), LAC presence by Stago was comparable between the alternative interpretation and the three-step interpretation (27 vs. 30, respectively), but differed remarkably for Haematex, especially in DOAC-treated patients (8 vs. 0, respectively).

These results were confirmed in an additional DOAC cohort (n=30) whereas for both interpretation methods (three-step vs. alternative), Haematex showed a higher specificity (100% vs. 70.0%, respectively) compared to Stago (70.0% vs. 56.7%, respectively). In the additional VKA cohort (n=37), both interpretations showed comparable false-positive LAC conclusions for Stago (10.8% vs. 18.9%) and Haematex (0%). Spiking experiments showed that Haematex Screen/Confirm reagents were less prone to VKA-related factor deficiencies (FII<1%, FX 24%/36%, respectively) compared to Stago Screen/Confirm (FII 48%/46%, FX 57%, respectively), which might explain the absence of false-positive LAC results in VKA-treated patients compared to Stago.

However, the increased specificity of Haematex seemed to be at cost of a reduced sensitivity. Haematex showed in the definitive APS patient samples a remarkable lower positivity rate (11/24, 45.8%) compared to Stago (21/24, 87.5%). The 13 missed APS cases by Haematex included 12 APS patients with LAC present alone and one APS patient with more than one laboratory criterion. Patients showed thrombosis (12/13) or miscarriage (1/13). Although Haematex mainly missed APS patients with LAC alone, and did detect 7/8 APS patients with more than one laboratory criterion, this does not imply that Haematex detects 'more relevant' LAC, as 29.3% of the genuine APS patient are reported to be only LAC positive [37]. Five out of the six consecutive APS patients, also positive in the aPTT system, were missed by Haematex [LAC ratios (0.99–1.27), median 0.99] but found positive by Stago [LAC ratios (1.25–1.79), median 1.44]. Also, 1/6 non-consecutive APS patient samples, positive with Stago but negative with Haematex, showed a positive aPTT LAC result.

Although there is no gold standard for LAC testing and therefore no clear identification of which reagent actually reflects 'true LAC', we assume that the samples

Table 1: Correlation between Stago (S) and Haematex (H) dRVVT test results and spiked DOAC concentrations in PNP.

Type of DOAC	Parameter	Screen		Confirm		Screen mix		Confirm mix		LAC ratio		LAC ratio PP:PNP	
		H CO 1.44	S CO 1.53	H CO 1.29	S CO 1.21	H CO 1.20	S CO 1.18	H CO 1.12	S CO 1.11	H CO 1.28	S CO 1.25	H CO 1.13	S CO 1.11
False positive dRVVT LAC results introduced by DOAC													
APIX	[DOAC]>CO, ng/mL	412	310	193	99	310	193	193	99	>500	>500	>500	>500
	Spearman's ρ	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	−0.775 (p=0.041)	0.964	−0.873 (p = 0.01)	−0.964
DAB	[DOAC]>CO, ng/mL	20	20	20	20	20	20	20	20	>500	>500	>500	70
	Spearman's ρ	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	0.893	0.955	0.946	0.883
RIV	[DOAC]>CO, ng/mL	115	65	115	65	115	65	65	65	>500	115	>500	65
	Spearman's ρ	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	−0.126 (p=0.788)	1.000	0.874 (p=0.016)	1.000

For each DOAC, the lowest mean DOAC concentration ([DOAC]) causing a prolongation of the mean test result above the CO and corresponding Spearman's rho correlation coefficients between test result and [DOAC] are shown. All measurements were performed in duplicate. Correlation was significant at the 0.01 level unless otherwise specified (p-value only mentioned between brackets if ≥0.01 level). The corresponding formulas to the different test results (Screen, Confirm, Screen mix, Confirm mix, LAC ratio and LAC PP:PNP ratio) are described in the legend of Figure 1, COs are described below each test result. DOAC, direct oral anticoagulants; APIX, apixaban; DAB, dabigatran; RIV, rivaroxaban; CO, cut-off; H, Haematex; S, Stago; PP, patient plasma; PNP, pooled normal plasma.

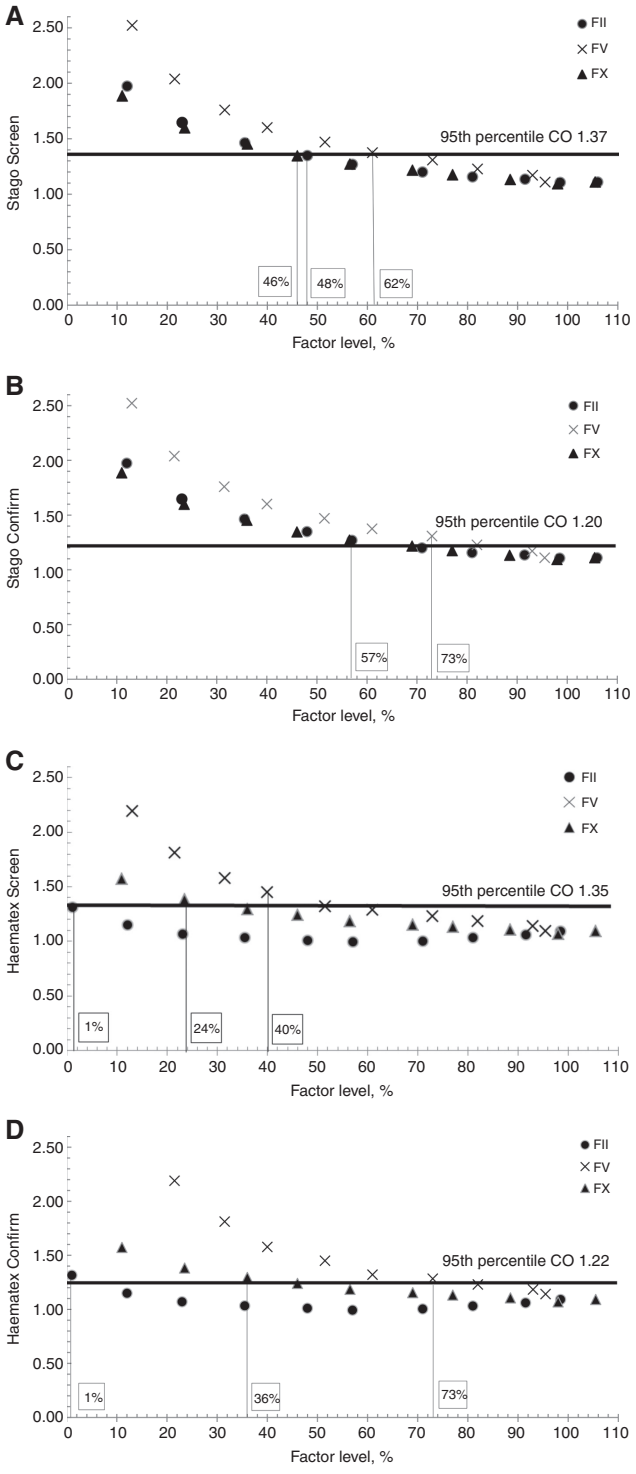

Figure 5: Stago and Haematex dRVVT Screen (A and C) and Confirm (B and D) reagent sensitivity for FII, FV and FX deficiency.

The formulas for the screen and confirm test result are described in the legend of Figure 1. The mean normalized ratio was plotted on the y-axis, the mean FII, FV and FX level on the x-axis (duplicate measurements). The 95th percentile COs are indicated by a full line on the y-axis. Factor sensitivities were deduced graphically and indicated on the x-axis. FII, factor II; FV, factor V; FX, factor X; CO, cut-off.

previously identified as positive are true LAC. Recently, different results between six dRVVT Screen/Confirm kits tested in healthy donors and LAC-positive patients were reported [11]. In another study, the number of LAC positives determined by two different dRVVTs was slightly different for APS-suspected patients and remarkably different for anticoagulated patients with a low APS probability [14].

We illustrated that positive LAC falsely introduced by DOAC depend on the type of dRVVT reagent, the type of DOAC and the DOAC concentration in both APS and non-APS patients. Both Stago and Haematex Screen/Confirm reagents showed a concentration-dependent prolongation by API, DAB and RIV in spiked PNP. However, prolongation of the screen and confirm determined by Haematex was comparable, resulting into a negative LAC ratio, excluding false-positive LAC conclusions. In contrast, for Stago, false-positive LAC ratios in DOAC-spiked PNP occurred at RIV concentrations of 115 ng/mL on, and false-positive LAC ratio PP:PNP resulted from RIV (70 ng/mL) and DAB (65 ng/mL) interference.

However, in the DOAC-treated non-APS patient samples (n=30), positive LAC conclusions with Stago and Haematex reagents was 43.3% and 30.0% according to the alternative interpretation procedure and 30.0% and 0% according to the three-step procedure, respectively. As the confirm was prolonged in all DOAC-spiked PNP and DOAC-treated patients, hence, the alternative interpretation should be applied. All together, we observed no false-positive LAC ratios with the Haematex reagent in DOAC-spiked PNP in contrast to the Stago reagent. This finding is in line with the results of the study of Exner et al. comparing four dRVVT reagents (Screen or Confirm) on DOAC-spiked PNP [38]. Although the evaluated dRVVT reagents were all sensitive to DOAC, different CTs were obtained and the Confirm reagents were considered to be less affected [38]. Regarding DOAC-treated patients in our study, both reagents resulted in false positive LAC.

False positivity in RIV-treated patients was illustrated previously [21–24]. Martinuzzo et al. found that most RIV-treated patients presented prolonged dRVVT screen results without correction with PNP and mostly LAC ratios above the CO [21]. Arachillage et al. determined that false-positive LAC results were obtained starting from 250 ng/mL on using two dRVVT systems [22]. Merriman et al. performed LAC analysis on 21 RIV-treated non-APS patients, which yielded a prolonged screen, mix and LAC ratio. Retesting after RIV cessation revealed a true LAC negative result in 92% of the cases [23]. Therefore, LAC analysis should be postponed until at least 24 h after RIV discontinuation, previously shown to correct false-positive LAC interpretations in dRVVT systems [24]. Although RIV and API both target FX, spiking API in PNP up to 466 ng/mL did not render false-positive LAC conclusions.

Here, DAB-spiked PNP showed false-positive LAC results by Stago analysis with an increased LAC ratio PP:PNP from 70 ng/mL on. These results are in agreement with those reported by Halbmeyer et al. [39]. In this multicentre study, they observed false-positive LAC ratios starting from 50 ng/mL. Postponing LAC analysis also in DAB-treated patients should be considered.

Although the venom differs (Haematex: Daboia siamensis; Stago: Russell viper), both reagents contain similar procoagulant enzymes and therefore, the venom is most likely not responsible for the observed discordances [15]. In this study, only two reagents were studied which may be considered as a possible limitation to fully explore the differences between dRVVT reagents. It was previously shown that aPL interfere in INR measurements depending on the thromboplastin's PL origin and content [40, 41]. We hypothesize that distinct PL composition and concentration in dRVVT Screen and Confirm reagents might influence the CT differently and hence the LAC interpretation. Both Haematex and Stago do not specify the PL type or concentration in the package insert [15].

In summary, we showed that dRVVT reagents differ in sensitivity for LAC detection and for VKA and DOAC interference in both APS and non-APS patients. It is advisable for clinical laboratories to investigate the gaps and pitfalls of dRVVT reagents before implementation, as reagents from different manufacturers may not considered to be equal and may lead to opposing LAC conclusions.

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