



Full Length Article

Neutralising rivaroxaban induced interference in laboratory testing for lupus anticoagulant (LA): A comparative study using DOAC Stop and andexanet alfa



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ABSTRACT

Introduction: Lupus anticoagulant (LA) investigation in patients on anticoagulant therapy is problematic. Rivaroxaban in particular causes significant interference, prolonging both LA screening and confirmation tests, and falsely raising LA screen/confirm ratios, leading to potential false identification of LA. The Russell Viper Venom Time (RVVT) assay, key to the investigation of LA, is especially sensitive to rivaroxaban.

Materials and methods: We assessed cross laboratory (n = 82) testing of four samples to investigate whether rivaroxaban induced interference in LA testing could be neutralised. Testing was performed blind to sample type. The samples comprised: (A) A pool of normal plasma (LA-negative control); (B) sample A spiked with rivaroxaban (200 ng/mL) to create rivaroxaban-induced interference (LA 'false' positive sample); (C) sample B subsequently treated with a commercial 'DOAC-neutraliser' (DOAC Stop); (D) sample B treated with andexanet alfa (200 µg/mL).

Results: As expected, the rivaroxaban-spiked sample (B) caused prolongation of most LA-tests, and also generated a falsely prolonged RVVT screen/confirm ratio (median 1.37, compared to 0.97 for sample A). The sample (C) treated with DOAC Stop evidenced a correction in LA-test clotting times, as well as neutralising the false positive LA (median RVVT screen/confirm ratio of 0.99). Although the andexanet alfa treated sample (D) also yielded a low median RVVT screen/confirm ratio of 0.88, it did not fully correct LA-test clotting times. Consistent with test findings, all laboratories interpreted samples A and C as being LA-negative. For sample B (rivaroxaban), 45.3% identified this as LA positive, and 38.7% identified LA interference. Most (61.3%) also identified sample D as LA negative, with the remainder (38.7%) identifying LA interference.

Conclusions: DOAC Stop was able to neutralise the false LA activity induced by rivaroxaban, both in terms of clot-times and LA ratios. In contrast, whilst andexanet alfa negated the rivaroxaban-prolonged LA-ratio, it did not fully correct clot-times, leaving some residual LA interference, and requiring additional testing to investigate prolonged clotting times.

1. Introduction

Anticoagulant therapy is prescribed for a variety of clinical indications, especially thrombosis treatment and prevention [1–3]. The direct oral anticoagulants (DOACs), directed against activated factor X or thrombin, are increasingly being deployed, and their use has recently been shown to exceed vitamin K antagonist (VKA; e.g., warfarin) use in

Australia [2,4]. Indeed, data suggests that DOACs are not only replacing VKA use, but are also being increasingly prescribed for anticoagulant naïve patients [2,5]. Lupus anticoagulants (LA) define a class of anti-phospholipid antibodies (aPL) associated with various adverse clinical events, in particular thrombosis and pregnancy morbidity [6,7]. DOAC use and LA testing are intricately inter-twined. LA testing comprises a major aspect of 'thrombophilia' investigation, and since patients with

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LA have an increased risk of thrombosis, and patients with thrombosis, or at risk, are treated with DOACs, there is a high chance of patients being tested for LA whilst on anticoagulant therapy [8].

Three LA testing guidelines are currently available [9–11], as recently overviewed [7]. The first, from the International Society on Thrombosis and Haemostasis (ISTH) Scientific and Standardisation Committee (SSC), was published (in 2009) prior to use of DOACs, and thus only covered testing of patients on heparin or VKA therapy [9]. The second, from the British Committee for Standards in Haematology (BCSH), was released (2012) when DOACs started to emerge [10], and again did not cover testing of LA on these patients. The most recent, from the Clinical and Laboratory Standards Institute (CLSI), was published in 2014 [11], and although it did cover such testing, the essential conclusion was that LA testing was not recommended on these patients. Unfortunately, clinicians still order ‘thrombophilia’ tests such as LA, on DOAC patients [8,12], albeit perhaps for good intentions, since the diagnosis of antiphospholipid antibody syndrome could lead to extended duration of anticoagulation to prevent recurrent thrombosis. Indeed, given the need to repeat tests such as LA for confirmation, and since thrombosis-affected patients are quickly placed on anticoagulant therapy, it is inevitable that a proportion of patients will be assessed for LA whilst on anticoagulant therapy, and despite guidelines recommending otherwise [6,7,9–11].

Several studies, including those from us [8,13–15], have previously highlighted problems with testing of LA in patients on anticoagulant therapy, especially as related to DOACs. All the current DOACs (namely dabigatran, a direct thrombin inhibitor, and apixaban, rivaroxaban and edoxaban, direct Xa inhibitors), affect a wide variety of coagulation assays [8,12–17]. Notably, these assays include those key to investigation of LA according to all the guidelines (e.g., activated partial thromboplastin time (APTT), and Russell Viper Venom Time (RVVT)) [8,9,11–17]. However, although all DOACs prolong ‘LA screen’ and ‘LA confirm’ tests, the different DOACs show dissimilar patterns of effects. For example, dabigatran affects both ‘LA screen’ and ‘LA confirm’ test results similarly, and so resulting ‘RVVT LA ratios’ (i.e., RVVT LA screen/confirm) appeared to be only moderately affected, with low ultimate risk of false LA identification [8,13]. In contrast, apixaban has less effect on RVVT clotting times as compared to dabigatran, and also appears to prolong ‘RVVT LA confirm’ more than ‘RVVT LA screen’, causing ‘RVVT LA ratios’ to fall slightly with increasing apixaban concentration, and more likely suggesting a potential for false negative LA to arise in an LA-positive patient, than of a false positive LA in an LA negative patient [8,14]. Rivaroxaban shows a different pattern again, with a relatively larger effect on RVVT clotting times compared to apixaban, and importantly with greater effect on ‘RVVT LA screen’ than ‘RVVT LA confirm’, causing the ‘RVVT LA ratio’ to rise slightly with increasing rivaroxaban concentration, and suggesting the highest potential for false positive LA among the three most widely used DOACs [8,14].

Andexanet alfa is a newly approved ‘antidote’ for anti-Xa agents, including rivaroxaban, and competitively binds to factor Xa and neutralises rivaroxaban activity *in vivo* and *in vitro* [18–20]. DOAC Stop is a recently released commercial product that has been shown to neutralise the *in vitro* activity of all the DOACs, including rivaroxaban [21]. Given this background, we were interested in investigating whether a rivaroxaban-induced false positive LA effect could be neutralised, either by

its ‘clinical antidote’ (andexanet alfa) or by the commercial product (DOAC Stop). We tested this by means of a large international cross-laboratory exercise.

2. Materials and methods

For this international cross-laboratory exercise, we prepared four different samples as follows:

- (A) A pool of normal plasma was prepared by pooling 13 packs of fresh frozen plasma (as obtained from the NSW Red Cross Blood Bank). After pooling, an approximate quarter aliquot was quarantined to form the study control (or baseline) sample (= ‘A’; LA negative sample, as confirmed by local testing in the ICPMR laboratory).
- (B) A larger (approximate $\frac{3}{4}$) aliquot of this pool was treated by spiking with rivaroxaban, with the aim to reach a rivaroxaban level of 200 ng/mL. This target concentration was chosen based on earlier studies that showed this to be a feasible and clinically relevant *ex vivo* rivaroxaban concentration, and also one that raises the LA-ratio (RVVT screen/confirm) to above 1.3 [14], which is a weak positive LA finding in most laboratories in our geographic area [22]. This rivaroxaban spiked sample was then split into three parts. An approximate 1/3 aliquot was quarantined to form the study treatment (or ‘rivaroxaban’) sample (= ‘B’; false LA positive status and rivaroxaban concentration was also confirmed by local testing in the ICPMR laboratory) (Supplementary Fig. 1).
- (C) A second (approximate 1/3) aliquot (of the rivaroxaban spiked sample, B) was subsequently treated with the commercial product ‘DOAC Stop’, which has been identified by the manufacturer to neutralise the *in vitro* activity of all the DOACs [21]. The commercial product was kindly donated by the manufacturer (Haematex, Sydney Australia), and was used as per manufacturer’s recommendations, at approximately one-tablet per mL of plasma. This sample forms the study treatment for ‘rivaroxaban + DOAC-Stop’ (= ‘C’). The ICPMR laboratory confirmed the rivaroxaban neutralisation prior to sample lyophilisation and cross-laboratory testing (Supplementary Fig. 1).
- (D) A final 1/3 aliquot of the rivaroxaban spiked sample (B) was instead treated with andexanet alfa, as kindly donated by the manufacturer Portola Pharmaceuticals Inc. (CA, USA). Andexanet alfa was used at a concentration of 200 µg/mL final, as selected based on several considerations. First, this concentration is expectedly close to that which neutralised rivaroxaban *in vivo* in reported clinical trials [18,19]. Second, a series of local titrations performed at both the ICPMR and WACTH (Western Australian Centre for Thrombosis and Haemostasis) showed this concentration effectively neutralised most of the rivaroxaban (e.g., as assessed by a specific chromogenic anti-factor Xa assay), as well as neutralising most of the false (rivaroxaban induced) LA-activity (ICPMR data shown in Supplementary Fig. 1). Higher concentrations of andexanet alfa had marginal incremental neutralising effect on rivaroxaban, and counterintuitively actually increased RVVT clotting times (Supplementary Fig. 1). This final sample formed the last

Table 1
Summary of study samples.

Sample	Represents
A	A pool of normal plasma (= LA-negative control or ‘baseline’)
B	Sample A spiked with rivaroxaban (target 200 ng/mL) to induce rivaroxaban-induced interference (‘false’ LA positive sample)
C	Sample B subsequently treated with a commercial ‘DOAC-neutraliser’ (DOAC Stop) to assess potential to neutralise rivaroxaban and its LA-test interference
D	Sample B subsequently treated with andexanet alfa (target 200 µg/mL) to assess potential to neutralise rivaroxaban and its LA-test interference

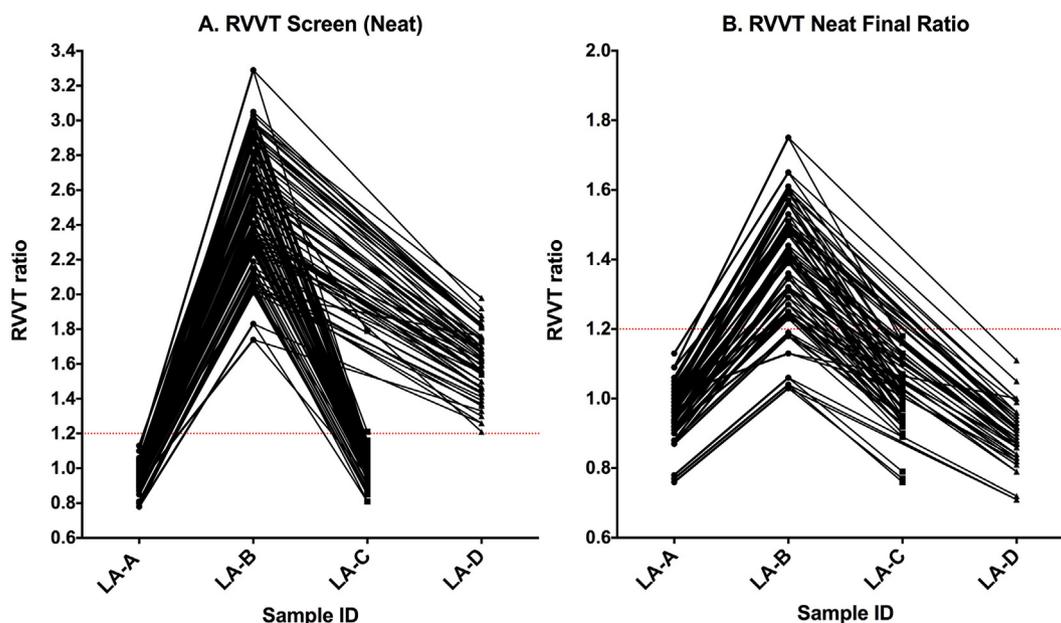


Fig. 1. Sample data presented as before-after plot to highlight general consistency of participant laboratory data. A: RVVT screen as performed on neat plasma and shown as a ratio of test sample to laboratory normal result; B: RVVT final ratios (screen/confirm) on samples tested neat. To improve visual assessment, data entries with missing data points for some samples, as well as an occasional gross outlier, have been removed – please refer to Figs. 2–6 for all data presentations (where no outliers have been removed). The dotted horizontal line at 1.2 indicates the standard positive vs negative cut-off used for RVVT ratios by most laboratories in our geographical region.

study treatment (or ‘rivaroxaban + andexanet alfa’) sample (= ‘D’).

Table 1 provides a summary of the 4 study samples. Samples were prepared for lyophilisation by addition of standard excipients, frozen in bulk, and later commercially lyophilised in 2 mL aliquots. Samples sets comprising all 4 lyophilised samples were later dispatched to participant laboratories by post, together with a set of instructions. These laboratories derived from those who were already enrolled in the Lupus Anticoagulant module of the RCPAQAP (Royal College of Pathologists of Australasia Quality Assurance Program) [23]. The sample set as sent to participants was simply identified as a ‘Supplementary Exercise’ for LA testing. Laboratories were asked to undertake their usual LA testing approach, and a data sheet was provided for completion. The specific nature of the samples was never disclosed to laboratories, and so all testing was performed blind to sample type. Laboratories were only informed that “The aim of this study is to help understand how different clinical scenarios may influence Lupus Anticoagulant testing.” The data sheet requested details on laboratory test methodology as well as results of testing. Laboratories were also asked to interpret their own overall test data, using the following options: Negative (not LA), Moderate Positive LA, Borderline LA, Strong Positive LA, Weak Positive LA, Positive LA (no grading), Interference by Inhibitor-like activity but not LA, Other (please specify). As the data sheet looked like a standard datasheet for the RCPAQAP LA module, and as participants are used to such datasheets, it was anticipated that participants would interpret test findings similarly to that of the standard LA module (*i.e.*, that the process for this special exercise would not specifically identify the nature of the samples as being a rivaroxaban ‘neutralisation’ study and so this would preserve the blind-testing nature of the process). Returned data was collated on an Excel worksheet and then analysed blind to the participant identities, being identified only by a study number.

3. Results

The regular RCPAQAP LA module normally comprises some 96 laboratories. In the current exercise, 82 of these laboratories (85.4%)

returned test data, although returned data was occasionally incomplete. As typical for the LA test module, the test processes employed by laboratories were identified to be generally disparate, although most laboratories (in keeping with all the LA test guidelines [7,9–11]) performed both APTT and RVVT based testing (Supplementary Table 1). However, a wide variety of instrumentation and commercial test reagents were used by different laboratories (Supplementary Table 2). The participants also derived from a wide geographical area, including Australia ($n = 44$; 53.7%), Hong Kong (9, 11.0%), Malaysia (8, 9.8%), New Zealand (9, 11.0%), South Africa (8, 9.8%), other (India and Brunei; 4, 4.9%).

Despite the dispersed geography and the heterogeneity of instruments and reagents in use, the reported test data for each sample was in general pleasingly consistent. An example of this consistency, using data for RVVT screen ratio and for RVVT final screen/confirm ratios is shown as before-after plots in Fig. 1. Reported data is otherwise presented here as box-plots in Figs. 2–6, respectively for APTT and RVVT screen (neat plasma method), APTT and RVVT screen (mix plasma method), APTT and RVVT confirm (neat plasma method), APTT and RVVT confirm (mix plasma method), and finally APTT and RVVT screen/confirm final ratios (both neat and mix plasma methods). Reported interpretations from participating laboratories are shown in Fig. 7.

As shown, except for occasional outlier data, sample A yielded normal APTT and RVVT clot times, as well as normal assay ratios (Figs. 2–6), consistent with an absence of LA in this pooled (‘baseline’) sample. Moreover, *all* participating laboratories reported this sample to be LA negative (Fig. 7). In contrast, sample B (‘+ rivaroxaban’) yielded prolonged APTT and RVVT clot times, as well as prolonged assay ratios (Figs. 2–6), consistent with the presence of (false) LA in this sample, and with the anticipated rivaroxaban-induced interference. Indeed, 45.3% laboratories identified this sample to be LA positive, whilst 38.7% identified LA interference (Fig. 7). Of interest, laboratories identified as based in the public hospital system were more likely to identify LA interference than LA positivity. In contrast, laboratories identified as based in private pathology were more likely to identify LA positivity than LA interference. This differential may reflect greater

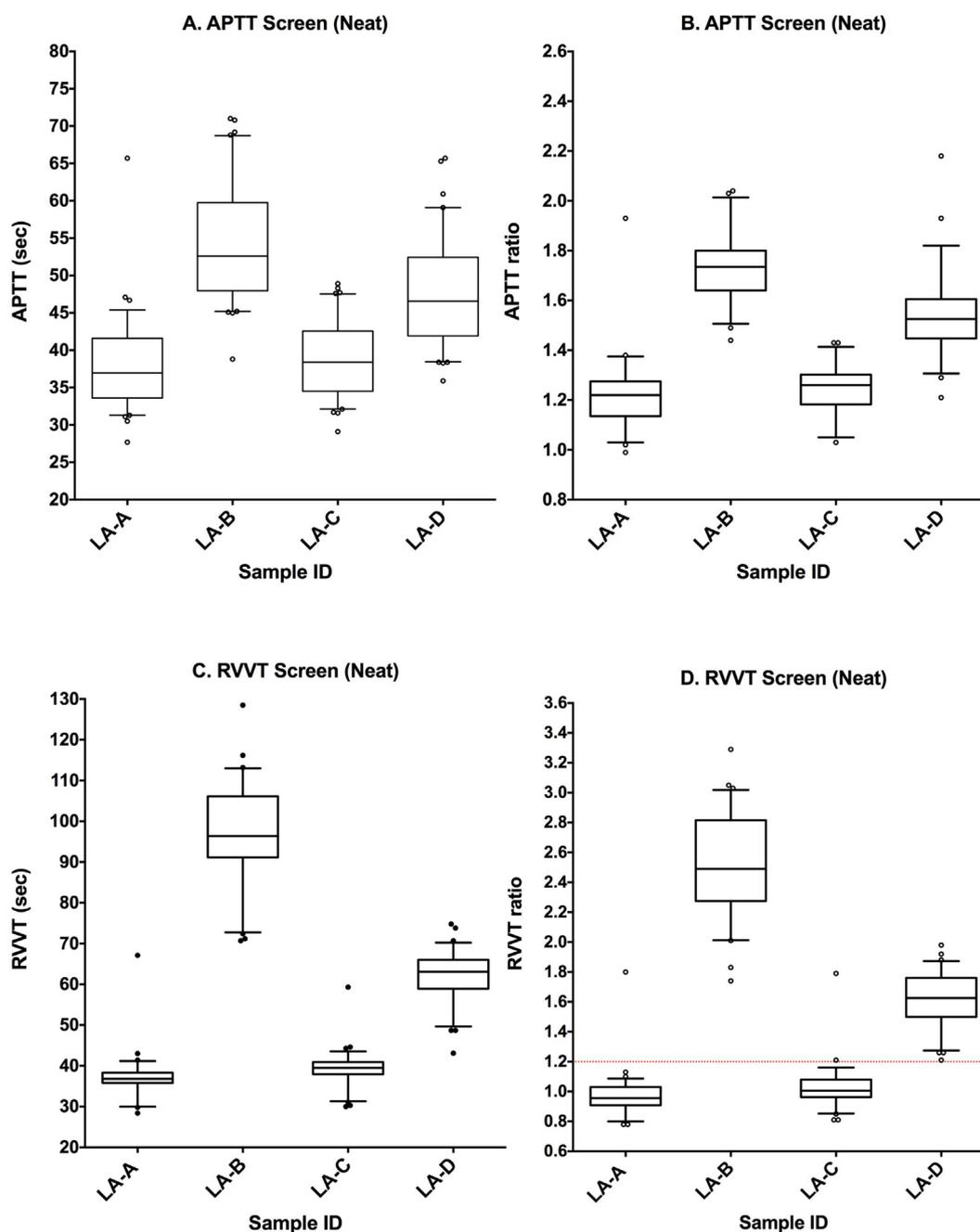


Fig. 2. Laboratory data for APTT and RVVT screen as returned by participant laboratories for tests performed on neat plasma. Data shown as box plots of 5th–95th percentile, with outliers shown as dot points. APTT screen shown as a clotting time (Fig. A) or ratio of test sample clotting time to laboratory normal result (Fig. B). RVVT screen shown as a clotting time (Fig. C) or ratio of test sample clotting time to laboratory normal result (Fig. D). Note that: (i) rivaroxaban (Sample B) prolonged both clotting times and ratios; (ii) DOAC Stop (Sample C) as added to the rivaroxaban sample was able to normalise both clotting times and ratios; (iii) Andexanet alfa (Sample D) as added to the rivaroxaban sample was only partially able to correct clotting times and ratios, but these remained generally ‘abnormal’. The dotted horizontal line at 1.2 for Fig. D indicates the standard positive vs negative cut-off used for RVVT ratios by most laboratories in our geographical region.

knowledge in public hospital systems of DOACs and their capacity to induce haemostasis interference, with these facilities more likely to treat acutely ill patients on DOAC therapy and/or also undertaking DOAC testing.

Sample C (‘rivaroxaban + DOAC Stop’) yielded normal APTT and RVVT clot times, as well as normal LA-ratios (Figs. 2–6), consistent with the absence of LA in this sample, and representative of the ‘neutralisation’ of the rivaroxaban-induced interference/false LA-positive effect. Interestingly, sample D (‘rivaroxaban + andexanet alfa’) still yielded prolonged APTT and RVVT clot times (Figs. 2–5), but normal final screen/confirm ratios (Fig. 6). Thus, although findings with

sample D were consistent with the absence of LA (normal final screen/confirm ratios), and thus somewhat representative of the ‘neutralisation’ of the rivaroxaban-induced false LA-positive effect, the prolonged clot times are likely to be a result of competitive binding of factor Xa by andexanet in the APTT and RVVT assays. This is likely to explain the partial but not full correction of the APTT and RVVT clot times from rivaroxaban. The prolongation of clotting times but normalisation of the ratio led to 61.3% laboratories identifying sample D as LA negative, with the remainder (38.7%) identifying LA interference (Fig. 7). Also interesting was that andexanet alfa generally yielded final APTT and RVVT screen/confirm ratios that were lower than that of sample A (Fig. 6).

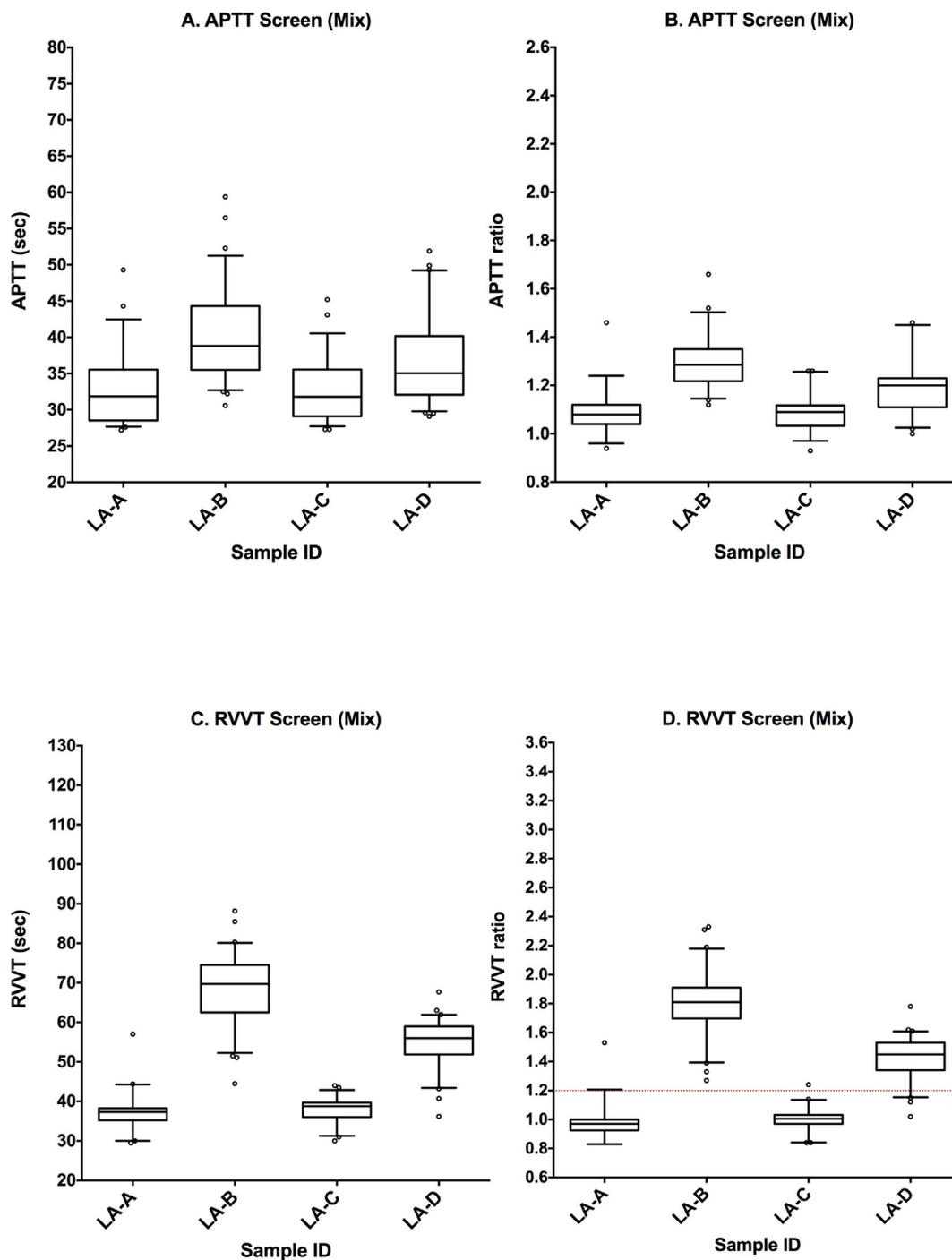


Fig. 3. Laboratory data for APTT and RVVT screen as returned by participant laboratories for tests performed as mixing tests with normal plasma. Data shown as box plots of 5th–95th percentile, with outliers shown as dot points. APTT screen shown as a clotting time (Fig. A) or ratio of test sample clotting time to laboratory normal result (Fig. B). RVVT screen shown as a clotting time (Fig. C) or ratio of test sample clotting time to laboratory normal result (Fig. D). Note that: (i) rivaroxaban (Sample B) prolonged both clotting times and ratios (albeit to lesser extent than tests performed on neat plasmas – compare to Fig. 2); (ii) DOAC Stop (Sample C) as added to the rivaroxaban sample was again able to normalise both clotting times and ratios; (iii) Andexanet alfa (Sample D) as added to the rivaroxaban sample was again only partially able to correct clotting times and ratios, but these remained generally ‘abnormal’. The dotted horizontal line at 1.2 for Fig. D indicates the standard positive vs negative cut-off used for RVVT ratios by most laboratories in our geographical region.

Finally, laboratories need to perform an increased number of confirm and mixing tests on this sample in order to investigate the raised screen test times (Supplementary Table 1).

4. Discussion

This study has uncovered several interesting findings. First, the data

has confirmed previous findings related to rivaroxaban-induced interference in LA testing, such that both LA screen and confirm assays become prolonged [8,14], but because screen assays tend to be more prolonged than confirm assays, the final screen/confirm ratios also prolong, and thus identification of a false LA is likely. In any case, even if LA interference is identified instead of the false LA-positivity, laboratories are still required to increase the level of testing by confirm

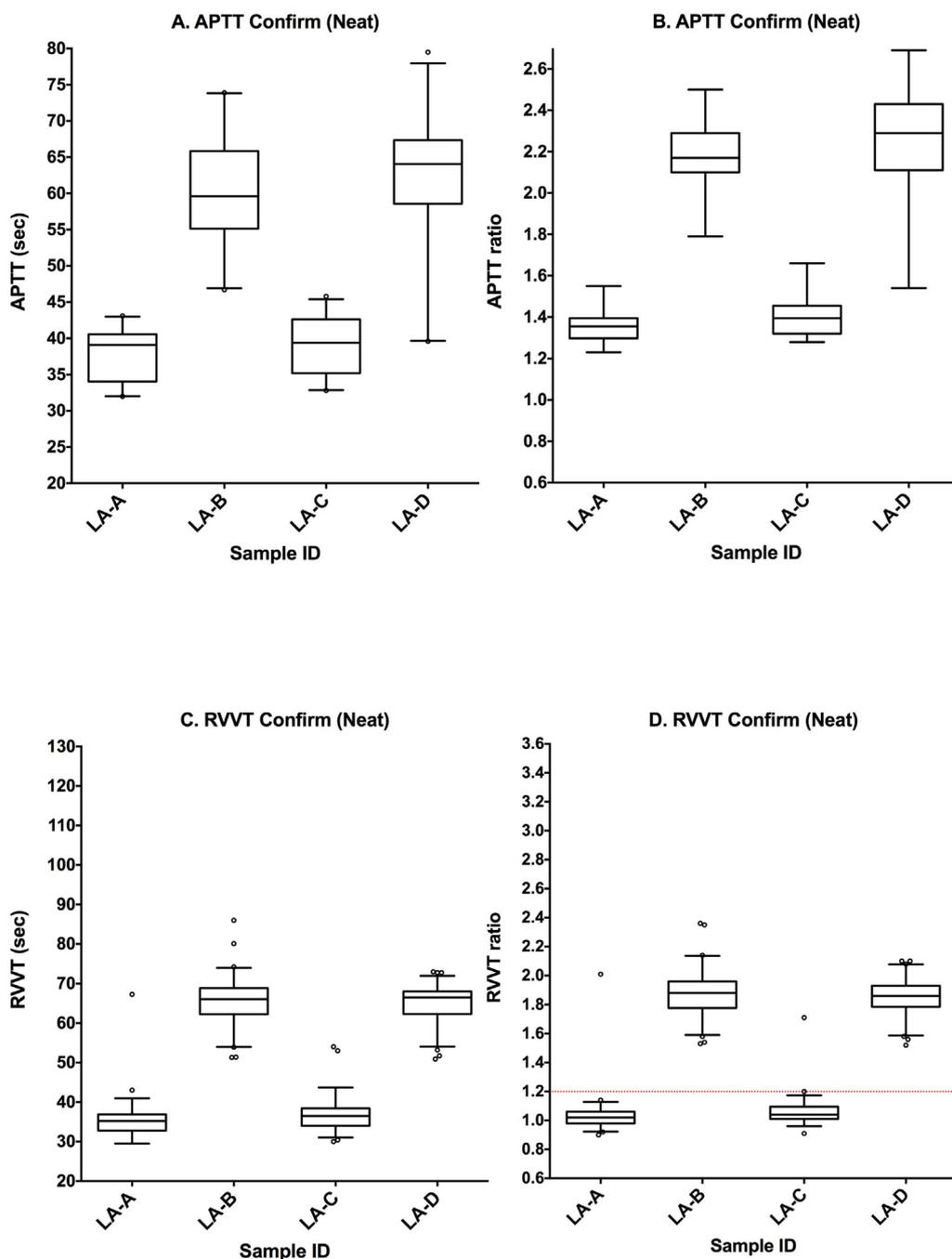


Fig. 4. Laboratory data for APTT and RVVT confirm assays as returned by participant laboratories for tests performed on neat plasma. Data shown as box plots of 5th–95th percentile, with outliers shown as dot points. APTT screen shown as a clotting time (Fig. A) or ratio of test sample clotting time to laboratory normal result (Fig. B). RVVT screen shown as a clotting time (Fig. C) or ratio of test sample clotting time to laboratory normal result (Fig. D). Note that: (i) rivaroxaban (Sample B) prolonged both clotting times and ratios; (ii) DOAC Stop (Sample C) as added to the rivaroxaban sample was able to normalise both clotting times and ratios; (iii) Andexanet alfa (Sample D) as added to the rivaroxaban sample was not able to correct clotting times and ratios, with these remaining generally ‘abnormal’ (APTT based testing results were often higher than for the rivaroxaban sample). The dotted horizontal line at 1.2 for Fig. D indicates the standard positive vs negative cut-off used for RVVT ratios by most laboratories in our geographical region.

and mixing tests (Supplementary Table 1) in order to correctly characterise the sample. DOAC Stop was able to fully neutralise the rivaroxaban effect, normalising LA test clotting times as well as screen/confirm ratios, and abrogating the need for additional confirm and mixing tests. Of ultimate importance, *all* participating laboratories correctly identified both samples A and C as being LA-negative (Fig. 7). This was despite the great variety of test methods, instruments and reagents used by different laboratories (Supplementary Tables 1 and 2). Although andexanet alfa normalised LA screen/confirm ratios, the prolonged LA clotting test times caused by the anti-Xa effect of andexanet produced diagnostic uncertainty, which resulted in additional testing to clarify LA interference. Moreover, andexanet produces consistently lower LA screen/confirm ratios than those of the baseline sample (A), and suggesting the possibility of a false LA negative finding (should an LA-positive patient be treated with rivaroxaban, thereby yielding falsely higher LA positive result, and then the patient or sample

be treated with andexanet alfa). Andexanet alfa use was also associated with a high need to perform confirm and mixing tests (Supplementary Table 1) in order to identify the interference.

To our knowledge, previous published reports on the effect of DOAC Stop are limited [21,24–28]. The manufacturer reported the initial study and showed evidence that DOAC Stop could remove all types of DOACs (including dabigatran, apixaban, rivaroxaban and edoxaban) from test plasmas, with minimal effect on any of the (mainly clotting) tests investigated in that study, which included APTT and RVVT assays [21]. More recently, Jacquemin and colleagues compared the effectiveness of DOAC Stop against idarucizumab (a humanized antibody fragment that binds and neutralises dabigatran) in a range of assays, and reporting DOAC Stop to be as effective *in vitro* to neutralise the dabigatran induced prolongation of these assays [24]. Kopatz and co-workers also investigated DOAC Stop using a thrombin generation assay (calibrated automated thrombography; CAT) and similarly reported

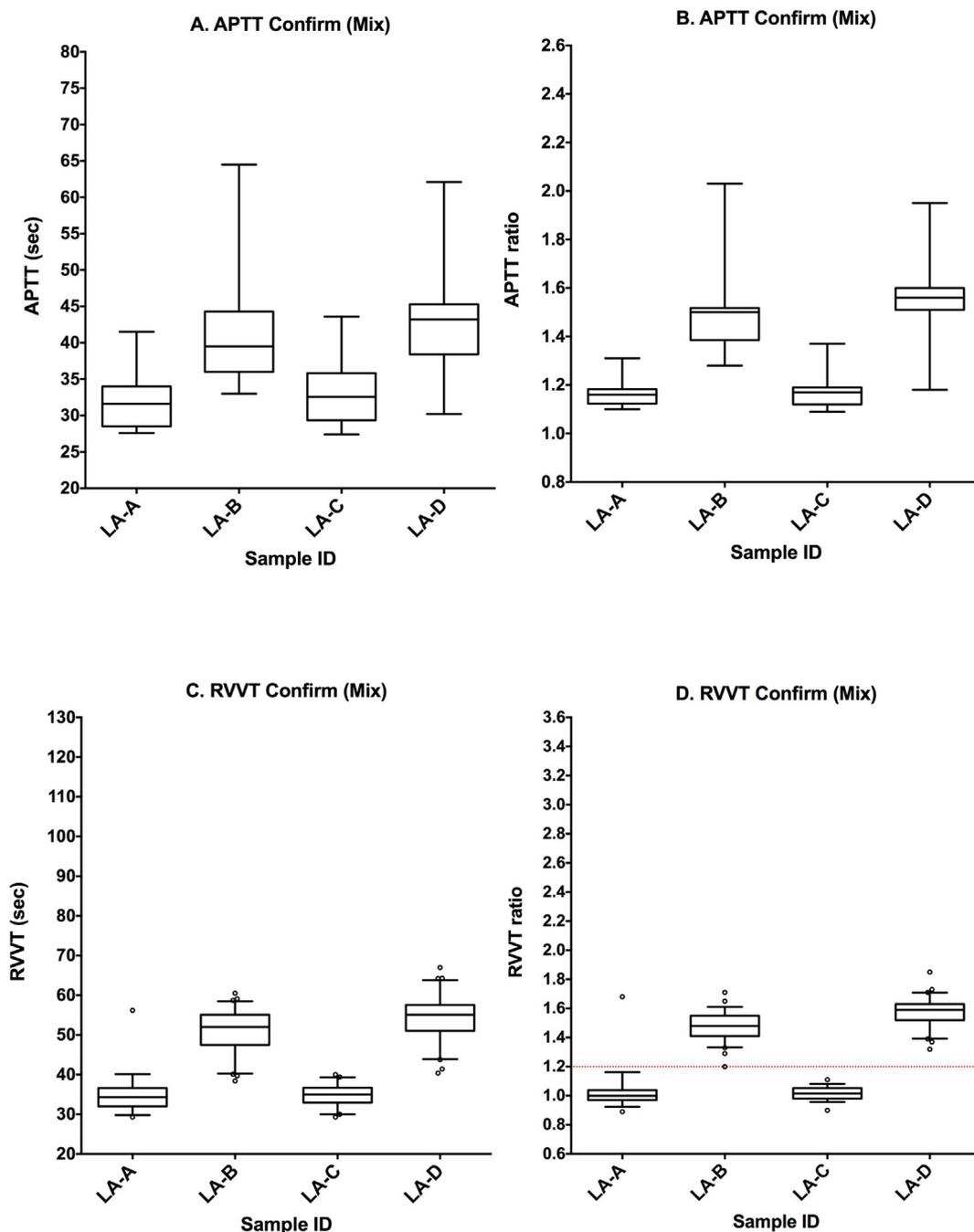


Fig. 5. Laboratory data for APTT and RVVT confirm assays as returned by participant laboratories for tests performed as mixing tests with normal plasma. Data shown as box plots of 5th–95th percentile, with outliers shown as dot points. APTT screen shown as a clotting time (Fig. A) or ratio of test sample clotting time to laboratory normal result (Fig. B). RVVT screen shown as a clotting time (Fig. C) or ratio of test sample clotting time to laboratory normal result (Fig. D). Note that: (i) rivaroxaban (Sample B) prolonged both clotting times and ratios (albeit to lesser extent than tests performed on neat plasmas – compare to Fig. 4); (ii) DOAC Stop (Sample C) as added to the rivaroxaban sample was again able to normalise both clotting times and ratios; (iii) Andexanet alfa (Sample D) as added to the rivaroxaban sample was not able to correct clotting times and ratios, with these remaining generally ‘abnormal’ (APTT based testing results were often higher than for the rivaroxaban sample). The dotted horizontal line at 1.2 for Fig. D indicates the standard positive vs negative cut-off used for RVVT ratios by most laboratories in our geographical region.

that DOAC Stop effectively neutralised apixaban, dabigatran, edoxaban and rivaroxaban [25]. Interestingly, the manufacturers of DOAC Stop have recently extended the anticoagulants assessed against DOAC Stop for associated neutralisation [26]. Two additional reports on the utility of this reagent to neutralise DOACs in patient samples for a variety of assays, including LA testing, have also recently emerged [27,28]. Nevertheless, to our knowledge, there has never been any previously published study to assess the effect of DOAC Stop in a cross laboratory

or external quality assessment setting. Also to our knowledge, there has never been any published study assessing andexanet alfa in the context of correcting DOAC induced interference in clotting assays such as APTT and RVVT, or in LA investigation.

Nonetheless, we acknowledge that this study has several limitations. First, this data reflects the findings of an *in vitro* rivaroxaban spiked sample study, although based on previous experience with *ex vivo* samples, *ex vivo* data is likely to provide for similar conclusions. Second,

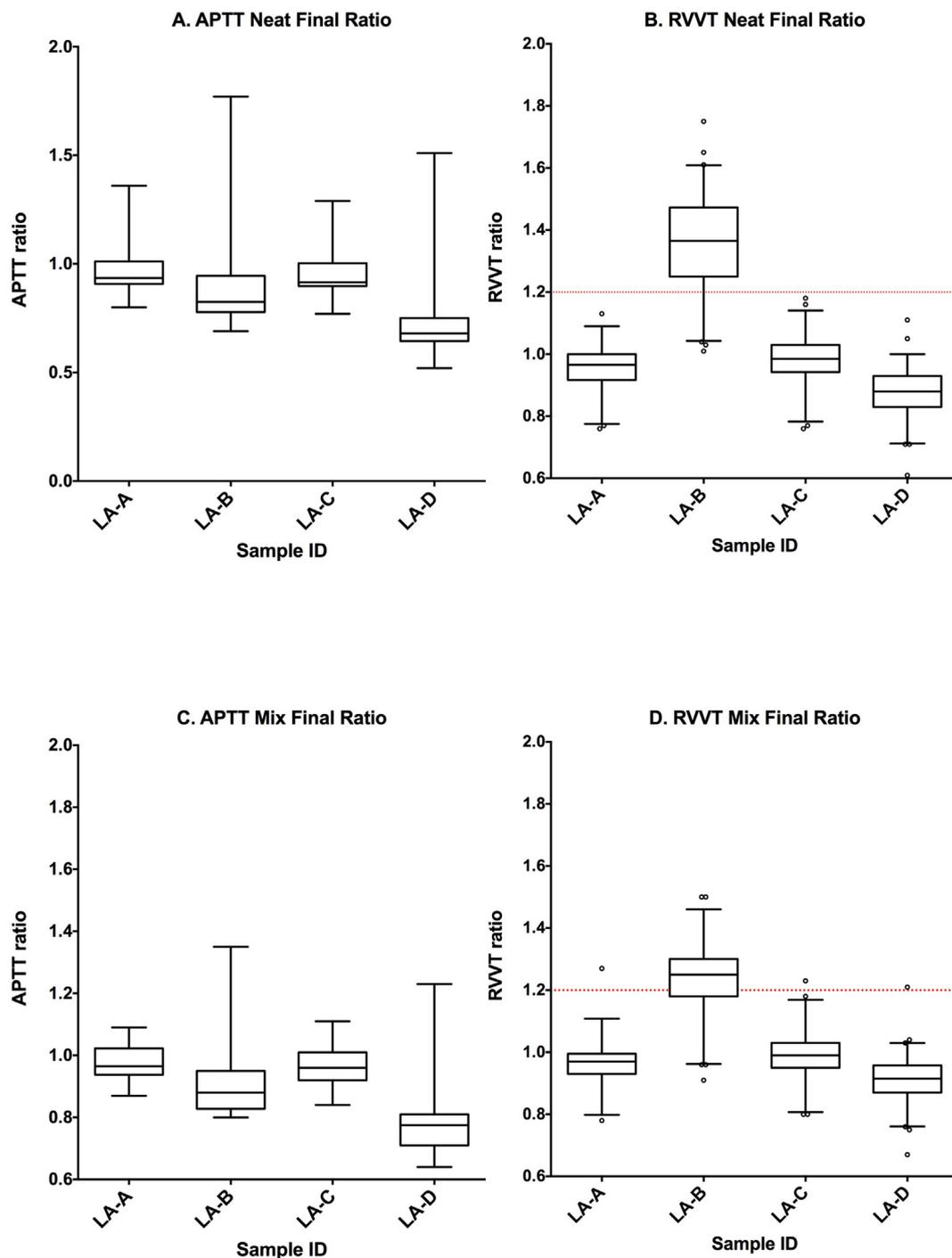


Fig. 6. Laboratory data for APTT and RVVT final ratios (*i.e.*, screen/confirm) as returned by participant laboratories for tests performed using either neat plasma or mixing tests with normal plasma. Data shown as box plots of 5th–95th percentile, with outliers shown as dot points. (A) APTT final ratio neat plasma; (B) RVVT final ratio neat plasma; (C) APTT final ratio mixed plasma; (D) RVVT final ratio mixed plasma. Note that: (i) rivaroxaban (Sample B) prolonged the RVVT ratios to yield a false LA positive result, but did not generally yield falsely raised APTT ratios; (ii) DOAC Stop (Sample C) as added to the rivaroxaban sample was able to normalise the RVVT ratio; (iii) Andexanet alfa (Sample D) as added to the rivaroxaban sample was also able to normalise the RVVT ratio; however, ratios with sample D were often much lower than those of the baseline sample (A). The dotted horizontal line at 1.2 for Fig. D indicates the standard positive vs negative cut-off used for RVVT ratios by most laboratories in our geographical region.

this study has selected a single, albeit *in vivo* clinically relevant, rivaroxaban concentration [14]. Third, this study has selected a single, albeit we believe *in vivo* feasible, andexanet alfa concentration [18,19]. Finally, this data, reflecting the rivaroxaban effect, cannot be extrapolated to the other DOACs. However, prior data suggests a lower potential for dabigatran to cause a false positive LA [8,13], given that prolongation of LA screen and confirm assays is typically similar in

magnitude, and thus does not generally lead to prolonged screen/confirm ratios. Prior data also suggests that apixaban may instead lead to a false negative LA, as it affects the LA confirm assays more than screen, and thus yields lower screen/confirm ratios [8,14]. Thus, of the three major DOACs in current use, rivaroxaban is that which may most likely lead to a false LA-positive diagnosis.

In conclusion, rivaroxaban can lead to prolongation of LA screen,

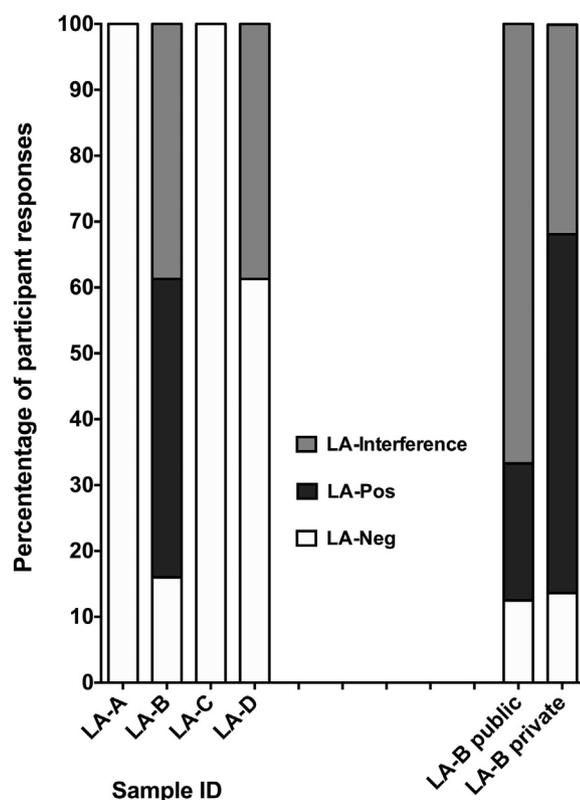


Fig. 7. Participant interpretations reported based on their own test data, and expressed as a percentage of responses received. All participants identified samples A (baseline normal) and C (rivaroxaban + DOAC Stop) as LA negative. For sample B (rivaroxaban), 45.3% identified this as LA positive, and 38.7% identified LA interference. Most (61.3%) also identified sample D as LA negative, with the remainder (38.7%) identifying LA interference. Laboratories identified as based in the public hospital system were more likely to identify LA interference than LA positivity in sample B, whereas those identified as based in private pathology were more likely to identify LA positivity than LA interference.

necessitating performance of LA confirm and mixing tests; moreover, rivaroxaban prolongs the LA screen more than the confirm, and can thus yield a false positive LA diagnosis. DOAC Stop was able to neutralise the rivaroxaban effect, normalising LA screen times and ratios, LA confirm times and ratios and final LA screen/confirm ratios. With DOAC Stop, *all* participants in this study effectively identified the sample as LA negative, despite the wide variety of instruments, methodologies and reagents in use. Andexanet alfa normalised LA screen/confirm ratios, and thus abrogated the false LA diagnosis; however, andexanet alfa did not fully neutralise the rivaroxaban effect of clot times because of its *in vitro* anti-Xa effect. The potential ‘over-correction’ by andexanet alfa in terms of normalised LA screen/confirm ratio, could thereby feasibly lead to a false negative LA finding should a true (mildly) LA positive patient be tested whilst on rivaroxaban therapy and concomitant with application of andexanet alfa (either *in vitro*, or even potentially *in vivo*).

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.thromres.2019.05.013>.

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Declaration of competing interest

The authors disclose no conflicts of interest in relation to this report.

References

- [1] E.J. Favaloro, Anticoagulant therapy: present and future, *Semin. Thromb. Hemost.* 41 (2015) 109–112.
- [2] E.J. Favaloro, L. Pasalic, G. Lippi, Replacing warfarin therapy with the newer direct oral anticoagulants, or simply a growth in anticoagulation therapy? Implications for pathology testing, *Pathology* 49 (2017) 639–643.
- [3] G.Y.H. Lip, A. Banerjee, G. Boriani, et al., Antithrombotic therapy for atrial fibrillation: CHEST guideline and expert panel report, *Chest* 154 (2018) 1121–1201 Nov.
- [4] E.J. Favaloro, G.J. McCaughan, S. Mohammed, L. Pasalic, Anticoagulation therapy in Australia, *Ann. Blood* 3 (2018) 48.
- [5] B.A. Steinberg, P. Shrader, L. Thomas, et al., Off-label dosing of non-vitamin K antagonist oral anticoagulants and adverse outcomes: the ORBIT-AF II registry, *J. Am. Coll. Cardiol.* 68 (2016) 2597–2604.
- [6] S. Miyakis, M.D. Lockshin, T. Atsumi, et al., International consensus statement on an update of the classification criteria for definite antiphospholipid syndrome (APS), *J. Thromb. Haemost.* 4 (2006) 295–306.
- [7] E.J. Favaloro, R.C.W. Wong, Antiphospholipid antibody testing for the antiphospholipid syndrome: a synopsis of challenges and recent guidelines, *Pathology* 46 (2014) 481–495.
- [8] E.J. Favaloro, S. Mohammed, J. Curnow, L. Pasalic, Laboratory testing for lupus anticoagulant (LA) in patients taking direct oral anticoagulants (DOACs): potential for false positives and false negatives, *Pathology* 51 (3) (2019) 292–300. Apr.
- [9] V. Pengo, A. Tripodi, G. Reber, et al., Update of the guidelines for lupus anticoagulant detection, *J. Thromb. Haemost.* 7 (2009) 1737–1740.
- [10] D. Keeling, I. Mackie, G.W. Moore, I.A. Greer, M. Greaves, British Committee for Standards in Haematology, Guidelines on the investigation and management of antiphospholipid syndrome, *Br. J. Haematol.* 157 (2012) 47–58.
- [11] Clinical and Laboratory Standards Institute (CLSI), Laboratory Testing for the Lupus Anticoagulant; Approved Guideline. CLSI Document H60-A, CLSI, Wayne, PA, 2014.
- [12] E.J. Favaloro, Danger of false negative (exclusion) or false positive (diagnosis) for ‘congenital thrombophilia’ in the age of anticoagulants, *Clin. Chem. Lab. Med. (CCLM)* (2018), <https://doi.org/10.1515/cclm-2018-1041> in press.
- [13] R. Bonar, E.J. Favaloro, S. Mohammed, L. Pasalic, J. Sioufi, K. Marsden, The effect of dabigatran on haemostasis tests: a comprehensive assessment using in-vitro and ex-vivo samples, *Pathology* 47 (2015) 355–364.
- [14] R. Bonar, E.J. Favaloro, S. Mohammed, M. Ahuja, L. Pasalic, J. Sioufi, K. Marsden, The effect of the direct factor Xa inhibitors apixaban and rivaroxaban on haemostasis tests: a comprehensive assessment using in vitro and ex vivo samples, *Pathology* 48 (2016) 60–71.
- [15] E.J. Favaloro, L. Pasalic, J. Curnow, G. Lippi, Laboratory monitoring or measurement of direct oral anticoagulants (DOACs): advantages, limitations and future challenges, *Curr. Drug Metab.* 18 (2017) 598–608.
- [16] R.C. Gosselin, D.M. Adcock, S.M. Bates, et al., International Council for Standardization in Haematology (ICSH) recommendations for laboratory measurement of direct oral anticoagulants, *Thromb. Haemost.* 118 (2018) 437–450.
- [17] J. Douxfils, R.C. Gosselin, Laboratory assessment of direct oral anticoagulants, *Semin. Thromb. Hemost.* 43 (2017) 277–290.
- [18] S.J. Connolly, T.J. Milling Jr., J.W. Eikelboom, et al., ANNEXA-4 Investigators, Andexanet alfa for acute major bleeding associated with factor Xa inhibitors, *N. Engl. J. Med.* 375 (2016) 1131–1141.
- [19] D.M. Siegal, J.T. Curnutte, S.J. Connolly, et al., Andexanet alfa for the reversal of factor Xa inhibitor activity, *N. Engl. J. Med.* 373 (2015) 2413–2424.
- [20] M.T. Kalathottukaren, A.L. Creagh, S. Abbina, et al., Comparison of reversal activity and mechanism of action of UHRA, andexanet, and PER977 on heparin and oral FXa inhibitors, *Blood Adv.* 2 (2018) 2104–2114.
- [21] T. Exner, N. Michalopoulos, J. Pearce, R. Xavier, M. Ahuja, Simple method for removing DOACs from plasma samples, *Thromb. Res.* 163 (2018) 117–122.
- [22] E.J. Favaloro, R. Bonar, K. Marsden, Internal quality control and external quality assurance in testing for antiphospholipid antibodies: part II – lupus anticoagulant, *Semin. Thromb. Hemost.* 38 (2012) 404–411.
- [23] RCPAQAP website, <https://rcpaqap.com.au/>, Accessed date: 16 November 2018.
- [24] M. Jacquemin, J. Toelen, L. Feyen, et al., The adsorption of dabigatran is as efficient as addition of idarucizumab to neutralize the drug in routine coagulation assays, *Int. J. Lab. Hematol.* 40 (2018) 442–447.
- [25] W.F. Kopatz, H.J.M. Brinkman, J.C.M. Meijers, Use of DOAC Stop for elimination of

- anticoagulants in the thrombin generation assay, *Thromb. Res.* 170 (2018) 97–101.
- [26] T. Exner, M. Ahuja, L. Ellwood, Effect of an activated charcoal product (DOAC Stop™) intended for extracting DOACs on various other APTT-prolonging anticoagulants, *Clin. Chem. Lab. Med.* 57 (2019) 690–696.
- [27] M. Ząbczyk, M. Kopytek, J. Natowska, A. Undas, The effect of DOAC-Stop on lupus anticoagulant testing in plasma samples of venous thromboembolism patients receiving direct oral anticoagulants, *Clin. Chem. Lab. Med.* (2019), <https://doi.org/10.1515/cclm-2018-1197> Feb 14.
- [28] S. Platton, C. Hunt, Influence of DOAC Stop on coagulation assays in samples from patients on rivaroxaban or apixaban, *Int. J. Lab. Hematol.* 41 (2) (2019) 227–233, <https://doi.org/10.1111/ijlh.12950> Apr.