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# Influence of dabigatran and rivaroxaban on routine coagulation assays

## A nationwide Belgian survey

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**Summary**

The Belgian national External Quality Assessment Scheme performed a nationwide survey using lyophilised plasma samples spiked with dabigatran or rivaroxaban to demonstrate to the Belgian clinical laboratories how these drugs affect their routine coagulation assays prothrombin time (PT), activated partial thromboplastin time (aPTT), fibrinogen and antithrombin. Virtually all Belgian laboratories performing routine coagulation testing (189/192) participated in the survey. Both, dabigatran and rivaroxaban significantly prolonged the PT and aPTT in a concentration- and reagent-dependent manner. PT reagents were more influenced by rivaroxaban than by dabigatran and aPTT reagents more influenced by dabigatran than by rivaroxaban. Among PT reagents, Neoplastin R® was the most sensitive to rivaroxaban and Innovin® and Thromborel S® the least sensitive. Converting PT results to INR only increased the variability between reagents. Among aPTT reagents, Actin FSL® was the least sensitive to dabigatran while the other aPTT reagents showed slightly higher sensitivities. The presence of dabigatran led to falsely reduced fibrinogen concentrations when measured with a low thrombin concentration reagent. The presence of dabigatran caused an overestimation of the antithrombin level when measured with a thrombin-based activity assay and the presence of rivaroxaban an overestimation of the antithrombin level when measured with a FXa-based assay. Instrument-related differences were found for all tested parameters. In conclusion, this paper provides detailed information on the effect of dabigatran and rivaroxaban on routine coagulation assays as performed with a large number of reagent/instrument combinations.

**Keywords**

Dabigatran, external quality assessment, rivaroxaban, routine coagulation assays

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## Introduction

The direct oral anticoagulants (DOAC) dabigatran etexilate (Pradaxa®, Boehringer Ingelheim International GmbH, Ingelheim am Rhein, Germany) and rivaroxaban (Xarelto®, Bayer Pharma AG, Berlin, Germany) have now been in clinical use for a few years. In Belgium, both drugs have been approved for the prevention of thromboembolic events after elective hip- or knee-replacement surgery and for the prevention of stroke and systemic embolism in patients with atrial fibrillation. Rivaroxaban has also been approved for the treatment and secondary prevention of venous thromboembolism (VTE). In contrast to the vitamin K antagonists (VKA) inhibiting the activation of several coagulation factors, these DOAC specifically target either thrombin or activated factor X (FXa). Dabigatran etexilate is an oral prodrug that is hydrolysed in the liver to dabigatran, a reversible, specific and direct inhibitor of both free and clot bound thrombin (1). Rivaroxaban is an oral direct inhibitor of free, prothrombinase bound and clot associated FXa (2). Both drugs have a rapid onset of action (1–3 hours [h]) and short half-lives (8–15 h). Compared to the VKA, there are minimal food interactions and very limited drug interactions.

For the majority of patients, these drugs are prescribed at fixed doses and do not require routine coagulation monitoring (3). However, their presence can significantly influence coagulation assays, potentially leading to incorrect interpretation of test results. The Subcommittee on Control of Anticoagulation of the Scientific and Standardization Committee of the International Society on Thrombosis and Haemostasis and the British Committee for Standards in Haematology recommend that laboratories are aware of the sensitivity of their assays to each of these drugs (4, 5). Therefore, the Belgian national External Quality Assessment Scheme (EQAS) for blood coagulation, performed a nationwide survey using dabigatran or rivaroxaban spiked pooled normal human plasma to investigate and illustrate to the Belgian clinical laboratories the impact of these drugs on their routine coagulation assays. Most published data on the effect of dabigatran and rivaroxaban on routine coagulation assays lack details regarding the instruments used, creating some limitations for application of

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these observations by clinical laboratories. This paper aims to provide information on the effect of dabigatran and rivaroxaban on routine coagulation assays as performed with a large number of reagent/instrument combinations.

## Materials and methods

### Study design: sample material and distribution

In April 2012, five lyophilised plasma samples were sent to the 192 Belgian clinical laboratories performing routine coagulation testing. The samples consisted of a plasma pool from 12 healthy volunteers spiked with dabigatran or rivaroxaban at the following final concentrations: 0 µg/l, 100 and 250 µg/l dabigatran, 120 and 290 µg/l rivaroxaban. The samples were purchased from Hyphen BioMed (Neuville sur Oise, France). They were packaged in accordance with postal regulations and distributed by overnight mail. The samples were tested negative for hepatitis B surface antigen and antibodies to hepatitis C virus and human immunodeficiency viruses 1 and 2.

Dabigatran and rivaroxaban concentrations were only revealed to the laboratories after receipt of the results.

### Study design: sample analysis

Participants were requested to reconstitute the lyophilised plasmas with 1.0 ml distilled water and to incubate them with gentle mixing for at least 15 minutes at room temperature before analysis. They were asked to determine the following coagulation assays: prothrombin time (PT), activated partial thromboplastin time (aPTT), fibrinogen and antithrombin. They were also required to mention the reagent and analyser used for testing and were asked to process the samples according to their usual procedures.

Participants could submit their results via a secured website. Results had to be returned within 2 weeks.

### Study design: reporting of results to participants

A full report including the results of the survey as well as comments and recommendations from the advisory board was sent to the laboratories in December 2012.

### Statistical analysis

Method-specific medians and robust standard deviations were calculated for all methods with ≥4 reported results (interquartile-range based method of Tukey). Differences between methods were assessed by means of non-parametric statistics (Wilcoxon test with subsequent Bonferroni correction of the p-values). P-values less than 0.05 were considered to be statistically significant.

**Table 1: Effect of dabigatran and rivaroxaban on PT results.** Results are expressed as PT ratio, i.e. the ratio of the clotting time measurement of the sample spiked with dabigatran or rivaroxaban compared with the clotting time of the plasma pool. The 1st column shows the reagents used, the 2nd column the number of results and the 3rd to the 6th column the method-specific median PT ratios obtained for the samples containing 100 and 250 µg/l dabigatran (100D and 250D) and 120 and 290 µg/l rivaroxaban (120R and 290R), respectively. Method-specific medians and coefficients of variation (between brackets) are shown for all methods with ≥4 reported results.

| Reagent | N | 100D | 250D | 120R | 290R |
|---|---|---|---|---|---|
| Innovin | 53 | 1.14 (1.3%) | 1.33 (3.1%) | 1.15 (1.5%) | 1.34 (2.8%) |
| Neoplastin CI Plus | 55 | 1.23 (1.9%) | 1.47 (1.7%) | 1.37 (2.3%) | 1.88 (3.3%) |
| Neoplastin R | 20 | 1.26 (1.3%) | 1.52 (1.4%) | 1.56 (3.5%) | 2.35 (4.2%) |
| RecombiPlasTin 2G | 45 | 1.17 (1.2%) | 1.35 (1.7%) | 1.37 (1.2%) | 1.95 (1.4%) |
| Thromborel S | 13 | 1.20 (1.5%) | 1.43 (1.4%) | 1.14 (1.6%) | 1.36 (3.5%) |
| **Global result** | **189** | **1.18 (4.5%)** | **1.39 (7.2%)** | **1.35 (12.3%)** | **1.87 (23.3%)** |

## Results

Of the 192 Belgian clinical laboratories performing routine coagulation testing, 189 participated in the survey (response rate of 98.3%). All participating laboratories returned results for PT and aPTT, 183 laboratories returned results for fibrinogen and 69 for antithrombin.

### Effect of dabigatran and rivaroxaban on PT results

Six different reagents were used, which were all Quick type methods (6, 7). About two thirds of the participants (62.4%) used a recombinant human thromboplastin: Innovin® (Siemens Healthcare Diagnostics, Marburg, Germany, 28.0% of the participants), RecombiPlasTin 2G® (Instrumentation Laboratory, Milan, Italy, 23.8%) or Neoplastin R® (Diagnostica Stago, Asnières, France, 10.6%). The remaining participants utilised the reagents Neoplastin CI Plus® (rabbit brain thromboplastin, Diagnostica Stago, 29.1%), Thromborel S® (human placental thromboplastin, Siemens Healthcare Diagnostics, 6.9%) or TriniCLOT PT Excel S® (rabbit brain thromboplastin, Diagnostica Stago, only three laboratories).

►Table 1 summarises the influence of dabigatran and rivaroxaban on the PT results. Both, dabigatran and rivaroxaban significantly prolonged the PT in a concentration- and reagent-dependent manner but the sensitivity to dabigatran was low: 100 and 250 µg/l dabigatran prolonged the PT only 1.2 and 1.4 times the basal value, respectively. The PT was more sensitive to rivaroxaban: 120 and 290 µg/l rivaroxaban prolonged the PT 1.4 and 1.9 times the basal value. At 120 µg/l rivaroxaban, all PT results were prolonged.

The agreement between laboratories using the same reagent was good, with coefficients of variation (CVs) ranging between 1.2 and 4.2%, but there was a wide variation in responsiveness be-


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tween reagents. The reagents Innovin® and Thromborel S® were the least sensitive to rivaroxaban with 290 µg/l prolonging the PT clotting time only 1.3 fold. Neoplastin R® was the most sensitive with 290 µg/l rivaroxaban prolonging the PT 2.4 fold.

►Table 2 compares, for the rivaroxaban-spiked samples, the PT results expressed as ratio with the international normalised ratios (INR) reported by the participants. For the low rivaroxaban concentration, the overall CVs for PT ratio and INR were 12.3% and 14.6%, respectively, and for the high rivaroxaban concentration, 23.3 and 32.0%, respectively, indicating that the INR results showed a wider spread of values, especially at the high rivaroxaban concentration. Indeed, 290 µg/l rivaroxaban yielded a median INR of 1.6 with the reagents Innovin® and Thromborel S® compared to a median INR of 2.5 with the reagents Neoplastin CI Plus® and Neoplastin R®.

## Effect of dabigatran and rivaroxaban on aPTT results

In total, 10 different reagents were used and the following were employed by at least four participants: Actin FS® (soya bean phospholipids and ellagic acid as activator, Siemens Healthcare Diagnostics, 23.8% of the participants), STA-PTT A® (cephalin from rabbit brain and silica as activator, Diagnostica Stago, 16.4%), STA-Cephascreen® (cephalin from rabbit brain and polyphenolic activator, Diagnostica Stago, 16.4%), SynthASil® (synthetic phospholipids and micronised silica, Instrumentation Laboratory, 11.6%), APTT-SP® (synthetic phospholipids and micronised silica, Instrumentation Laboratory, 11.1%), Actin FSL® (purified soy and rabbit brain phosphatides and ellagic acid as activator, Siemens Healthcare Diagnostics, 11.1%), STA-CK Prest® (cephalin from rabbit brain and kaolin as activator, Diagnostica Stago, 4.2%) and TriniCLOT aPTT HS® (porcine and chicken phospholipids and micronised silica, Diagnostica Stago, 2.6%).

►Table 3 shows the influence of dabigatran and rivaroxaban on the aPTT results. Both, dabigatran and rivaroxaban significantly prolonged the aPTT in a concentration- and reagent-dependent manner. The aPTT was more influenced by dabigatran than by rivaroxaban: the global aPTT ratio amounted to 2.0 for the high dabigatran concentration and to 1.5 for the high rivaroxaban concentration. At 100 µg/l dabigatran, all aPTT results were prolonged.

Again, results varied in function of the reagent used. Actin FSL® was the least sensitive to dabigatran with a median aPTT ratio of 1.7 at 250 µg/l while the other aPTT reagents showed slightly higher sensitivities with ratios ranging between 2 and 2.15. At 290 µg/l rivaroxaban, median aPTT ratios ranged between 1.4 and 1.7.

## Effect of dabigatran and rivaroxaban on fibrinogen results

Most of the participants (86.3%) used for fibrinogen determination a Clauss type clotting assay based on the addition of an excess of thrombin to plasma. The following methods were employed by at least four participants: STA-Fibrinogen® (containing ~80 National Institutes of Health [NIH] units/ml of thrombin, Diagnostica Stago, 39.3% of the participants), Thrombin Reagent® (containing ~100 NIH units/ml of thrombin, Siemens Healthcare Diagnostics, 26.8%), Fibrinogen C® (containing ~35 NIH units/ml of thrombin, Instrumentation Laboratory, 12.6%), Multifibren U® (containing ~50 NIH units/ml of thrombin, Siemens Healthcare

**Table 2: Comparison of the PT results of the rivaroxaban-spiked samples expressed as ratio and as INR.** The 1st column shows the reagents used, the 2nd column the number of results, the 3rd and 5th column the method-specific median PT ratios and the 4th and 6th column the method-specific median INR results reported by the participants for the samples containing 120 and 290 µg/l rivaroxaban (120R and 290R), respectively. Method-specific medians and coefficients of variation (between brackets) are shown for all methods with ≥4 reported results.

| Reagent | N | PT ratio 120R | PT INR 120R | PT ratio 290R | PT INR 290R |
|---|---|---|---|---|---|
| Innovin | 53 | 1.15 (1.5%) | 1.32 (2.8%) | 1.34 (2.8%) | 1.55 (4.8%) |
| Neoplastin CI Plus | 55 | 1.37 (2.3%) | 1.64 (4.5%) | 1.88 (3.3%) | 2.52 (5.3%) |
| Neoplastin R | 20 | 1.56 (3.5%) | 1.67 (4.5%) | 2.35 (4.2%) | 2.52 (2.5%) |
| RecombiPlasTin 2G | 45 | 1.37 (1.2%) | 1.43 (4.9%) | 1.95 (1.4%) | 2.00 (4.8%) |
| Thromborel S | 13 | 1.14 (1.6%) | 1.35 (3.3%) | 1.36 (3.5%) | 1.61 (3.7%) |
| **Global result** | **189** | **1.35 (12.3%)** | **1.45 (14.6%)** | **1.87 (23.3%)** | **2.03 (32.0%)** |

**Table 3: Effect of dabigatran and rivaroxaban on aPTT results.** Results are expressed as aPTT ratio, i.e. the ratio of the clotting time measurement of the sample spiked with dabigatran or rivaroxaban compared with the clotting time of the plasma pool. Data are presented as in Table 1.

| Reagent | N | 100D | 250D | 120R | 290R |
|---|---|---|---|---|---|
| Actin FS | 45 | 1.64 (3.2%) | 2.06 (3.4%) | 1.32 (1.8%) | 1.66 (2.8%) |
| Actin FSL | 21 | 1.43 (1.2%) | 1.71 (1.3%) | 1.23 (1.7%) | 1.44 (2.1%) |
| APTT-SP | 21 | 1.63 (2.7%) | 2.05 (2.1%) | 1.22 (2.8%) | 1.44 (2.0%) |
| STA-CK Prest | 8 | 1.71 (1.4%) | 2.15 (1.6%) | 1.34 (1.5%) | 1.63 (2.4%) |
| STA-Cephascreen | 31 | 1.61 (1.2%) | 2.02 (1.2%) | 1.26 (1.3%) | 1.49 (1.5%) |
| STA-PTT A | 31 | 1.64 (1.4%) | 2.04 (1.9%) | 1.24 (1.5%) | 1.45 (1.1%) |
| SynthASil | 22 | 1.67 (1.8%) | 2.10 (2.4%) | 1.33 (2.8%) | 1.57 (2.8%) |
| TriniCLOT aPTT HS | 5 | 1.67 (3.7%) | 2.02 (1.7%) | 1.24 (1.4%) | 1.44 (1.8%) |
| **Global result** | **189** | **1.63 (2.7%)** | **2.04 (3.4%)** | **1.27 (4.8%)** | **1.50 (9.0%)** |

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| Reagent | N | No drug | 100D | 250D | 120R | 290R |
|---|---|---|---|---|---|---|
| Clauss | 158 | 2.21 (11.8 %) | 2.16 (14.8 %) | 2.13 (16.0 %) | 2.15 (12.1 %) | 2.16 (12.4 %) |
| Fibrinogen C | 23 | 2.10 (5.3 %) | 1.75 (4.2 %) | 1.33 (5.6 %) | 2.05 (4.0 %) | 1.97 (9.2 %) |
| Multifibren U | 6 | 2.11 5.6 % | 2.10 (5.7 %) | 2.10 (3.8 %) | 2.08 (2.1 %) | 2.09 (3.6 %) |
| QFA Thrombin | 4 | 2.15 (8.9 %) | 2.04 (9.7 %) | 2.04 (6.9 %) | 2.11 (5.8 %) | 2.04 (4.6 %) |
| STA-Fibrinogen | 72 | 2.42 (4.7 %) | 2.39 (4.3 %) | 2.39 (6.2 %) | 2.40 (5.3 %) | 2.38 (4.7 %) |
| Thrombin Reagent | 49 | 2.05 (4.7 %) | 2.03 (6.2 %) | 2.01 (5.5 %) | 2.01 (6.3 %) | 2.04 (5.1 %) |
| PT derived | 25 | 2.02 (6.6 %) | 2.08 (8.6 %) | 2.22 (7.3 %) | 2.12 (6.6 %) | 2.10 (18.4 %) |
| RecombiPlasTin 2G | 19 | 2.03 (5.3 %) | 2.14 (5.9 %) | 2.25 (5.8 %) | 2.19 (3.6 %) | 2.18 (19.4 %) |
| Innovin | 4 | 1.96 (4.4 %) | 2.10 (2.6 %) | 1.96 (2.6 %) | 2.12 (5.4 %) | 1.98 (6.7 %) |
| **Global result** | **183** | **2.18 (12.6 %)** | **2.14 (14.9 %)** | **2.15 (14.1 %)** | **2.15 (11.4 %)** | **2.15 (12.4 %)** |

**Table 4: Effect of dabigatran and rivaroxaban on fibrinogen results.** Results are expressed in g/l. The upper part of the table indicates the fibrinogen results obtained with Clauss type clotting assays and the lower part the derived fibrinogen results extrapolated from clot kinetics during PT testing. The 1st column shows the reagents used, the 2nd column the number of results and the 3rd to the 7th column the method-specific median fibrinogen concentration obtained for the samples containing no drug, 100 and 250 µg/l dabigatran (100D and 250D) and 120 and 290 µg/l rivaroxaban (120R and 290R), respectively. Method-specific medians and coefficients of variation (between brackets) are shown for all methods with ≥4 reported results.

Diagnostics, 3.3 %) and QFA Thrombin® (containing ~100 NIH units/ml of thrombin, Instrumentation Laboratory, 2.2 %). Derived fibrinogen results extrapolated from clot kinetics during PT testing were reported by 13.7 % of the participants and were mainly obtained with the RecombiPlasTin 2G® reagent (Instrumentation Laboratory, 10.4 %).

►Table 4 displays the impact of dabigatran and rivaroxaban on the fibrinogen results. The Clauss fibrinogen assay Fibrinogen C® significantly underestimated the fibrinogen level of the dabigatran-spiked samples. The median fibrinogen concentration obtained with this reagent amounted to 2.4 g/l for the sample containing no dabigatran and decreased to 1.8 g/l and 1.3 g/l for the samples with 100 and 250 µg/l dabigatran, respectively. This underestimation of the fibrinogen concentration also had an impact on the interpretation of the results. Indeed, 65.2 % of the users of the Fibrinogen C® reagent considered the plasma pool as normal while respectively 30 % and none of them still considered the samples with 100 and 250 µg/l dabigatran as normal.

| Reagent | N | No drug | 100D | 250D | 120R | 290R |
|---|---|---|---|---|---|---|
| Activity IIa | 39 | 84.0 (6.6 %) | 92.0 (6.3 %) | 100.0 (4.6 %) | 85.0 (7.0 %) | 85.0 (7.0 %) |
| AT Cobas c | 5 | 85.6 (3.5 %) | 94.3 (2.4 %) | 104.0 (2.2 %) | 83.0 (4.0 %) | 84.0 (3.8 %) |
| Berichrom Antithrombin III | 7 | 87.0 (7.4 %) | 92.0 (11.3 %) | 98.4 (13.5 %) | 86.1 (13.8 %) | 86.9 (9.2 %) |
| Stachrom AT III | 27 | 83.0 (5.8 %) | 90.7 (4.5 %) | 100.0 (3.4 %) | 85.0 (6.1 %) | 84.0 (6.6 %) |
| Activity Xa | 29 | 82.0 (2.7 %) | 80.2 (5.5 %) | 83.0 (5.4 %) | 93.0 (4.9 %) | 108.0 (8.2 %) |
| Coamatic Antithrombin | 6 | 81.5 (0.9 %) | 80.5 (10.1 %) | 81.5 (6.4 %) | 91.0 (3.3 %) | 104.5 (10.6 %) |
| Innovance Antithrombin | 5 | 89.6 (3.3 %) | 84.7 (0.9 %) | 86.0 (2.3 %) | 102.0 (0.2 %) | 127.0 (2.0 %) |
| Liquid Antithrombin | 17 | 81.0 (3.7 %) | 80.0 (3.7 %) | 80.0 (5.6 %) | 90.0 (4.1 %) | 107.9 (5.5 %) |

**Table 5: Effect of dabigatran and rivaroxaban on antithrombin results.** Results are expressed in %. The upper part of the table indicates the antithrombin results obtained with thrombin-based antithrombin activity assays and the lower part the results obtained with FXa-based antithrombin activity assays. Data are presented as in Table 4.


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The other Clauss type clotting assays were not significantly affected by dabigatran or rivaroxaban at the concentrations tested.

## Effect of dabigatran and rivaroxaban on antithrombin results

More than half of the laboratories (56.5%) used an antithrombin activity assay based on the inhibition of thrombin: Stachrom AT III® (Diagnostica Stago, 39.1% of the participants), Berichrom Antithrombin III® (Siemens Healthcare Diagnostics, 10.1%) or AT Cobas c® (Roche, Mannheim, Germany, 7.2%). One laboratory determined the antithrombin concentration with an immunological method (Immage AT3® (Beckman Coulter, Fullerton, CA, USA). The other participants (42.0%) used an antithrombin activity assay based on the inhibition of FXa: HemosIL Liquid Antithrombin® (Instrumentation Laboratory, 24.6% of the participants), Coamatic Antithrombin® (Chromogenix, Milan, Italy, 8.7%), Innovance Antithrombin® (Siemens Healthcare Diagnostics, 7.2%) and Biophen AT® (Hyphen BioMed, 1 participant).

►Table 5 shows the impact of dabigatran and rivaroxaban on the antithrombin results. The median antithrombin activity of the plasma pool obtained with the thrombin-based methods was 84% (range: 72–91%).The median antithrombin activity obtained with the FXa-based assays was 82% (range: 72–92%). Of the 63 participants providing an interpretation, 77.8% considered the antithrombin level of this sample as normal, 12.7% as borderline and 9.5% as low.

The thrombin-based antithrombin activity assays significantly overestimated the antithrombin level of the dabigatran-spiked samples. At 250 µg/l dabigatran, the median increase in antithrombin level was 15% when measured with the Berichrom Antithrombin III® reagent and 21% when measured with the Stachrom AT III® or AT Cobas c® reagent. The FXa-based antithrombin activity assays significantly overestimated the antithrombin level of the rivaroxaban-spiked samples. At 290 µg/l rivaroxaban, the median increase in antithrombin level was 29% when measured with the Coamatic Antithrombin® reagent, 33% with the Liquid Antithrombin® reagent and 44% with the Innovance Antithrombin® reagent.

As expected, the FXa-based antithrombin activity assays were unaffected by the presence of dabigatran and the thrombin-based antithrombin activity assays unaffected by the presence of rivaroxaban.

The overestimation of the antithrombin activity in the presence of dabigatran or rivaroxaban also had an impact on the interpretation of the results. Of the users of a thrombin-based antithrombin activity assay, 74.3% considered the sample without dabigatran as normal while all of them considered the sample with 250 µg/l dabigatran as normal. Of the users of a FXa- based antithrombin

**Table 6: Influence of instrument on PT results.** Results are expressed as PT ratio, i. e. the ratio of the clotting time measurement of the sample spiked with dabigatran or rivaroxaban compared with the clotting time of the plasma pool. The 1st column shows the reagents and the 3rd column the instruments used. The 2nd and 4th column indicate the number of results. The last four columns show the method-specific median PT ratios obtained for the samples containing 100 and 250 µg/l dabigatran (100D and 250D) and 120 and 290 µg/l rivaroxaban (120R and 290R), respectively. Method-specific medians and coefficients of variation (between brackets) are shown for all methods with ≥4 reported results. Similar instruments from the same manufacturer are grouped into a single category.

| Reagent | N | Instrument | N | 100D | 250D | 120R | 290R |
|---|---|---|---|---|---|---|---|
| Innovin | 54 | Sysmex CA-1500, 7000 | 33 | 1.15 (1.2%) | 1.33 (2.6%) | 1.15 (1.3%) | 1.33 (2.0%) |
| | | Sysmex CS2100i | 10 | 1.14 (0.8%) | 1.31 (1.6%) | 1.15 (1.2%) | 1.36 (1.4%) |
| | | Sysmex CA-540, 560 | 6 | 1.15 (1.0%) | 1.32 (0.4%) | 1.15 (0.9%) | 1.33 (1.4%) |
| Neoplastin CI Plus | 55 | STA Compact | 29 | 1.23 (1.7%) | 1.47 (1.4%) | 1.36 (2.4%) | 1.86 (3.5%) |
| | | STA-R Evolution | 22 | 1.22 (1.3%) | 1.47 (1.2%) | 1.37 (2.2%) | 1.88 (2.8%) |
| Neoplastin R | 20 | STA-R Evolution | 10 | 1.26 (0.7%) | 1.52 (0.7%) | 1.57 (2.0%) | 2.36 (2.3%) |
| | | STA Compact | 10 | 1.26 (4.1%) | 1.52 (3.3%) | 1.53 (3.7%) | 2.27 (5.8%) |
| RecombiPlasTin 2G | 45 | ACL Top, | 24 | 1.17 (1.1%) | 1.35 (1.4%) | 1.38 (1.1%) | 1.95 (1.3%) |
| | | ACL 9000, Elite Pro | 12 | 1.16 (0.7%) | 1.33 (1.9%) | 1.37 (1.2%) | 1.96 (1.8%) |
| | | ACL Advance/Futura | 7 | 1.17 (0.9%) | 1.34 (1.4%) | 1.38 (1.1%) | 1.93 (1.0%) |
| Thromborel S | 13 | Sysmex CA-1500, 7000 | 6 | 1.22 (1.3%) | 1.45 (2.6%) | 1.16 (1.4%) | 1.41[1] (3.2%) |
| | | BCS, BCS XP | 4 | 1.19 (1.9%) | 1.39 (3.0%) | 1.13 (1.4%) | 1.32[1] (2.8%) |

[1]p=0.04.

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activity assay, 81.5% considered the sample without rivaroxaban as normal while all of them considered the sample with 290 µg/l rivaroxaban as normal.

## Effect of instrument on test results

Altogether 23 different coagulometers from five different manufacturers were used. One third of the participants (38.6%) used a coagulometer with mechanical clot detection (STA-R Evolution® (20.6%) or STA Compact® (18.0%), both Diagnostica Stago). The remaining participants used coagulometers with photo-optical clot detection mainly from Siemens Healthcare Diagnostics (33.9%, CA-1500® and CA-7000® (20.1%), CS-2100i® (6.3%), Sysmex CA-540® and CA-560® (4.2%), BCS® and BCS XP® (3.2%)) or Instrumentation Laboratory (24.3%, ACL TOP® series (13.8%), ACL 9000® and ACL Elite Pro® (6.9%), ACL Advance® and ACL Futura® (3.7%)). ►Tables 6, 7, 8 and 9 show the influence of the instrumentation on the PT, aPTT, fibrinogen and antithrombin results, respectively. Similar instruments from the same manufacturer were grouped into a single category.

PT ratios obtained with Thromborel S® were higher when determined on a Sysmex CA-1500® or CA-7000® instrument than when determined on a BCS® or BCS XP®. The difference reached statistical significance only at the high rivaroxaban concentration.

aPTT ratios obtained with Actin FS® were systematically and significantly lower when determined on a Sysmex CA-1500® or CA-7000® instrument than when determined on a CS2100i® or an instrument of the ACL Top® series.

aPTT ratios obtained with SynthASil® were significantly higher when determined on an ACL Elite Pro® or ACL 9000® than when determined on an instrument of the ACL Top® series (all Instrumentation Laboratory but different measuring principle).

In the presence of dabigatran or rivaroxaban, PT derived fibrinogen results obtained with RecombiPlasTin 2G® were falsely elevated when determined on an ACL Elite Pro®, ACL 9000®, ACL Futura® or ACL Advance® but not when determined on an instrument of the ACL Top® series. Differences were significant for the high dabigatran and both rivaroxaban concentrations. At 290 µg/l rivaroxaban, the median increase in PT-derived fibrinogen concentration was 29% when measured on an ACL Elite Pro® or ACL 9000® and 26% when measured on an ACL Futura® or ACL Advance®.

At 250 µg/l dabigatran, the antithrombin activity obtained with Stachrom AT III® was significantly higher when determined on a STA-R Evolution® than when determined on a STA Compact® but the difference was not clinically relevant.

| Reagent | N | Instrument | N | 100D | 250D | 120R | 290R |
|---|---|---|---|---|---|---|---|
| Innovin | 54 | Sysmex CA-1500, 7000 | 33 | 1.15 (1.2%) | 1.33 (2.6%) | 1.15 (1.3%) | 1.33 (2.0%) |
| | | Sysmex CS2100i | 10 | 1.14 (0.8%) | 1.31 (1.6%) | 1.15 (1.2%) | 1.36 (1.4%) |
| | | Sysmex CA-540, 560 | 6 | 1.15 (1.0%) | 1.32 (0.4%) | 1.15 (0.9%) | 1.33 (1.4%) |
| Neoplastin CI Plus | 55 | STA Compact | 29 | 1.23 (1.7%) | 1.47 (1.4%) | 1.36 (2.4%) | 1.86 (3.5%) |
| | | STA-R Evolution | 22 | 1.22 (1.3%) | 1.47 (1.2%) | 1.37 (2.2%) | 1.88 (2.8%) |
| Neoplastin R | 20 | STA-R Evolution | 10 | 1.26 (0.7%) | 1.52 (0.7%) | 1.57 (2.0%) | 2.36 (2.3%) |
| | | STA Compact | 10 | 1.26 (4.1%) | 1.52 (3.3%) | 1.53 (3.7%) | 2.27 (5.8%) |
| RecombiPlasTin 2G | 45 | ACL Top, | 24 | 1.17 (1.1%) | 1.35 (1.4%) | 1.38 (1.1%) | 1.95 (1.3%) |
| | | ACL 9000, Elite Pro | 12 | 1.16 (0.7%) | 1.33 (1.9%) | 1.37 (1.2%) | 1.96 (1.8%) |
| | | ACL Advance/Futura | 7 | 1.17 (0.9%) | 1.34 (1.4%) | 1.38 (1.1%) | 1.93 (1.0%) |
| Thromborel S | 13 | Sysmex CA-1500, 7000 | 6 | 1.22 (1.3%) | 1.45 (2.6%) | 1.16 (1.4%) | 1.41[1] (3.2%) |
| | | BCS, BCS XP | 4 | 1.19 (1.9%) | 1.39 (3.0%) | 1.13 (1.4%) | 1.32[1] (2.8%) |

[1]p=0.04.

**Table 7: Influence of instrument on aPTT results.** Results are expressed as aPTT ratio, i.e. the ratio of the clotting time measurement of the sample spiked with dabigatran or rivaroxaban compared with the clotting time of the plasma pool. Data are presented as in Table 6.


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**Table 8: Influence of instrument on fibrinogen results.** Results are expressed as ratio, i. e. the ratio of the fibrinogen concentration of the sample spiked with dabigatran or rivaroxaban compared with the fibrinogen concentration of the plasma pool. Data are presented as in Table 6.

| Reagent | N | Instrument | N | 100D | 250D | 120R | 290R |
|---|---|---|---|---|---|---|---|
| **Clauss** | **158** | | | | | | |
| Fibrinogen C | 23 | ACL Top series | 14 | 0.84 (3.7 %) | 0.64 (4.6 %) | 0.98 (6.0 %) | 0.94[1] (5.8 %) |
| | | ACL 9000, Elite Pro | 7 | 0.86 (1.3 %) | 0.62 (5.8 %) | 0.99 (1.5 %) | 1.00[1] (2.2 %) |
| STA-Fibrinogen | 72 | STA Compact | 37 | 0.99 (4.5 %) | 0.98 (3.2 %) | 0.98 (3.5 %) | 0.99 (3.8 %) |
| | | STA-R Evolution | 33 | 0.99 (2.8 %) | 0.97 (3.8 %) | 0.99 (2.9 %) | 0.98 (3.0 %) |
| Thrombin reagent | 49 | Sysmex CA-1500, 7000 | 32 | 0.98 (2.5 %) | 0.98 (2.5 %) | 0.99 2.9 %) | 0.99 (3.4 %) |
| | | Sysmex CS2100i | 10 | 0.96 (3.3 %) | 0.99 (1.0 %) | 0.99 (2.3 %) | 1.00 (2.2 %) |
| | | Sysmex CA-540, 560 | 7 | 0.96 (6.2 %) | 0.98 (2.6 %) | 0.98 (7.4 %) | 0.98 (2.8 %) |
| **PT derived** | **25** | | | | | | |
| RecombiPlasTin 2G | 19 | ACL Top series | 10 | 1.02 (2.1 %) | 1.06[2,5] (1.7 %) | 1.00[3,6] (1.2 %) | 0.99[4,7] (1.9 %) |
| | | ACL 9000, Elite Pro | 5 | 1.02 (1.7 %) | 1.12[2] (1.3 %) | 1.10[3] (3.4 %) | 1.29[4] (3.9 %) |
| | | ACL Advance/Futura | 4 | 1.06 (4.2 %) | 1.15[5] (0.8 %) | 1.10[6] (2.7 %) | 1.26[7] (7.6 %) |

[1]p=0.01, [2]p=0.01, [3]p=0.02, [4]p=0.0002, [5]p=0.006, [6]p=0.03, [7]p=0.04.

## Discussion

Several DOAC such as dabigatran etexilate and rivaroxaban, have been developed for prophylaxis and treatment of thromboembolic disorders. Due to their predictable pharmacokinetic and pharmacodynamic profile and wide therapeutic window, these agents do not require therapeutic monitoring. However, their presence significantly influences coagulation assays, especially in high-dose use or after blood sampling at the peak level. Hence, it is recommended that laboratories are aware of the sensitivity of their assays to each DOAC (4, 5). Therefore, the Belgian EQAS for blood coagulation organised a nationwide survey to inform the Belgian clinical laboratories on how dabigatran and rivaroxaban affect their routine tests of haemostasis. For practical and ethical reasons, an *in vitro* approach with lyophilised plasma samples spiked with dabigatran or rivaroxaban was chosen.

We selected the plasma concentrations for our study, taking into account pharmacological and clinical data published so far. In the paragraph on pharmacodynamic properties in the 'Summary of Product Characteristics' of dabigatran ([8], version at the end of 2011), Boehringer Ingelheim stated that plasma concentrations above 200 µg/l measured at through after 150 mg dabigatran twice daily dosing were associated with an increased risk of bleeding. We wanted to show the laboratories the impact of dabigatran at a concentration possibly associated with this safety issue. As cut-off values for bleeding risk were not available for rivaroxaban, we used a similar approach as for dabigatran by considering a plasma concentration above the 90$^{th}$ percentile of rivaroxaban trough levels as being associated with an increased risk of bleeding. In the 'Summary of Product Characteristics' of rivaroxaban (9), it is stated that the 90$^{th}$ percentile of rivaroxaban plasma concentrations measured at trough was about 239 µg/l in patients treated with rivaroxaban

**Table 9: Influence of instrument on antithrombin results.** Results are expressed as ratio, i. e. the ratio of the antithrombin activity of the sample spiked with dabigatran or rivaroxaban compared with the antithrombin activity of the plasma pool. Data are presented as in Table 6.

| Reagent | N | Instrument | N | 100D | 250D | 120R | 290R |
|---|---|---|---|---|---|---|---|
| **Activity IIa** | **39** | | | | | | |
| Stachrom AT III | 27 | STA-R Evolution | 18 | 1.11 (3.5 %) | 1.231 (3.4 %) | 1.00 (4.3 %) | 1.00 (0.9 %) |
| | | STA Compact | 8 | 1.07 (2.7 %) | 1.191 (1.9 %) | 1.01 (1.0 %) | 1.00 (1.3 %) |

[1]p=0.02.

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20 mg once daily for the treatment of acute deep venous thrombosis.

After processing all the data, the participants were provided with a full report including the results of the survey as well as comments and recommendations edited by the advisory board (interpretation of test results with reference to the dose and time interval between drug intake and blood sampling, situations in which measuring the plasma concentration of the drug may be necessary, …), in order to draw attention to the importance of careful interpretation of coagulation test results in patients taking dabigatran or rivaroxaban.

All but three Belgian clinical laboratories performing coagulation testing participated in the survey. As expected, many different reagent/instrument combinations were reported. In total, six PT, 10 aPTT, 11 fibrinogen and seven antithrombin reagents were used in various combinations with 23 different instruments. Most participants used manufacturer-specific reagent/instrument combinations (►Tables 6-9).

Both, dabigatran and rivaroxaban significantly prolonged the PT and aPTT in a concentration- and reagent-dependent manner. PT reagents were more influenced by rivaroxaban than by dabigatran and aPTT reagents more influenced by dabigatran than by rivaroxaban. However, as the molar concentration is higher for rivaroxaban than for dabigatran (30% for the higher concentration and 25% for the lower concentration), the difference in the effect of dabigatran and rivaroxaban on aPTT and PT results could also have been partly due to the difference in molar concentration and not only to the drug target.

In this study, all PT reagents were Quick type methods, which are, in general, more sensitive to dabigatran and rivaroxaban than Owren type PT assays (10–12). Quick (plain) reagents differ from Owren (combined) reagents by the fact that Owren type assays are independent of the contents of factor V and fibrinogen in the patient's plasma since they contain adsorbed bovine plasma providing these factors and that they use a smaller sample volume in the reaction mixture (6, 7, 11, 13). Therefore, since Owren methods use a higher final dilution of the plasma sample, they are probably less sensitive to interfering substances (5, 11, 12).

Expression of the PT results as INR, which is common practice in monitoring VKA therapy, did not reduce but even increased the variability between reagents, indicating that the INR cannot be applied to rivaroxaban nor to dabigatran (4, 5). Indeed, the conventional INR system was developed specifically for monitoring the VKAs and corrects for varying sensitivities of thromboplastin reagents to reductions in the levels of vitamin K–dependent clotting factors and not for different sensitivities to DOAC (14, 15).

The agreement between laboratories using the same reagent was good despite differences in reagent lots. However, as mentioned in earlier publications (10–12, 16–25), there was a wide variation in responsiveness between reagents. Among PT reagents, Neoplastin R® was the most sensitive to rivaroxaban while Innovin® and Thromborel S® were the least sensitive. The difference in sensitivity was not related to the source of tissue factor (e.g. rabbit brain, human placenta and relipidated recombinant human tissue factor) since recombinant human Innovin® was the least sensitive to rivaroxaban and Neoplastin R®, another recombinant human thromboplastin, the most sensitive. The difference in sensitivity was neither related to the mean normal prothrombin time (MNPT) nor to the ISI values used by the participants. Indeed, median MNPT were 10.9 seconds (sec) (range: 8.6–11.6 sec), 11.6 sec (range: 10.5–12.7), 10.8 sec (range: 10.0–12.3 sec), 13.1 sec (12.3–14.0 sec) and 13.8 sec (12.5–14.4 sec) for Innovin®, Thromborel S®, RecombiPlasTin 2G®, Neoplastin CI Plus® and Neoplastin R®, respectively, and median ISI values 1.04 (range: 0.93–1.13), 1.03 (range: 0.99–1.12), 0.97 (range: 0.95–1.05), 1.32 (range: 1.29–1.33) and 0.98 (range: 0.95–1.07). The variation in responsiveness could be due to the composition of the reagents. Indeed, it has been demonstrated that the PT sensitivity toward rivaroxaban increases by decreasing the concentration of tissue factor or by increasing the concentration of phospholipids or NaCl and that increasing the percentage phosphatidylserine generally decreases rivaroxaban sensitivity, while including phosphatidylethanolamine generally increases rivaroxaban sensitivity (26). The TriniCLOT PT Excel S® reagent, only used by three participants, proved to be very sensitive to rivaroxaban (data not shown). The lipid component in this reagent has been shown to be responsible for a reduced activation of factor V resulting in limited FXa formation and a strong sensitivity to inhibition by rivaroxaban (27).

The aPTT reagents differed also in their sensitivity to DOAC but this variability was substantially less compared to the variability in responsiveness of the PT reagents to rivaroxaban. Actin FSL® was the least sensitive to dabigatran while the other aPTT reagents showed slightly higher sensitivities. aPTT reagents vary in type of contact activator (e.g. ellagic acid, kaolin or micronised silica), phospholipid source (e.g. rabbit brain cephalin, soy bean phospholipid extract or synthetic phospholipid mixtures), total concentration of phospholipids and relative percentage of individual phospholipid components (e.g. % of phosphatidylserine, phosphatidylinositol, phosphatidylethanolamine, phosphatidylcholine,…). These factors could influence the responsiveness of aPTT reagents to DOAC as has been demonstrated for the sensitivity to heparin, lupus anticoagulant antibodies and clotting factor deficiencies (28–32).

It is important that laboratories and clinicians are aware of the influence of dabigatran and rivaroxaban on the PT and aPTT reagents used in order to be able to correctly interpret the results. Indeed, the same PT or aPTT result in a patient taking dabigatran or rivaroxaban will point to a much lower drug concentration when determined with a sensitive than a less sensitive reagent.

As previously reported (12, 17, 33), the presence of dabigatran led to falsely reduced fibrinogen concentrations when measured with the Clauss fibrinogen assay Fibrinogen C®. A concentration of 100 µg/l dabigatran caused a negative median bias of 16% and 250 µg/l a negative median bias of almost 40%. This fibrinogen method uses a low thrombin concentration (~ 35 NIH units/ml) and is, therefore, more sensitive to increasing concentrations of dabigatran (17). An alternative Clauss fibrinogen assay (QFA thrombin) from the same manufacturer with a high thrombin concentration (~100 NIH units/ml) was not affected. The other Clauss type clotting assays were unaffected by dabigatran at the drug con-

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centrations studied although interferences have been described with the reagents Multifibren U® (12) and STA-Fibrinogen® (17), containing ~50 and 80 NIH units/ml of thrombin, respectively.

The presence of both dabigatran and rivaroxaban led to falsely elevated PT derived fibrinogen concentrations when measured with RecombiPlasTin 2G® on the Elite Pro®, ACL 9000®, ACL advance® or ACL Futura®. A concentration of 120 µg/l rivaroxaban caused a positive median bias of 10% and 290 µg/l a positive median bias of almost 30%. A concentration of 250 µg/l dabigatran caused a positive median bias of 15%. Both drugs had no effect when the fibrinogen concentration was determined on instruments of the ACL Top® series. In surveys of the Belgian EQAS for blood coagulation, the same phenomenon is observed on samples from patients taking VKA: PT derived fibrinogen results obtained on such samples with RecombiPlasTin 2G® are also systematically and markedly higher when determined on an Elite Pro®, ACL 9000®, ACL advance® or ACL Futura® than when determined on an instrument of the ACL Top® series (data not shown).

As our results show, it is important that clinicians are aware of the effect of dabigatran and rivaroxaban on the reagent/instrument combination used for fibrinogen testing to avoid inappropriate result interpretation, misdiagnosis and treatment errors.

As mentioned in earlier publications (11, 12, 18, 23, 34), the presence of dabigatran caused an overestimation of the antithrombin level when measured with a thrombin-based antithrombin activity assay and the presence of rivaroxaban an overestimation of the antithrombin level when measured with an antithrombin activity assay based on FXa inhibition. The factitious elevation of antithrombin activity was approximately 20% at 250 µg/l dabigatran and approximately 30% at 290 µg/l rivaroxaban. Hence, these assays have the potential to falsely indicate a normal result in a patient with antithrombin deficiency. Therefore, it is important that clinicians are aware of the effect of dabigatran or rivaroxaban on the method used in the laboratory, otherwise the diagnosis of antithrombin deficiency may be missed. Overestimation can be avoided by using a FXa-based antithrombin method to measure antithrombin activity in patients on dabigatran and an thrombin based method to measure antithrombin activity in patients on rivaroxaban (35).

The sensitivity to dabigatran and rivaroxaban did not only depend on the reagent but also on the instrument used, and instrument-related differences were found for all tested parameters. The principle of clot detection (mechanical measurement for the Stago instruments and photo-optical for the other instruments) could play a role in the sensitivity to DOAC. Furthermore, for instruments with optical detection, there are numerous variables potentially affecting the sensitivity to DOAC as wavelength and data analysis used for the determination of the clotting time (endpoint, delta optical density, first or second derivative). The largest instrument-related differences in this study were observed for the PT-derived fibrinogen results obtained with RecombiPlasTin 2G®. At 290 µg/l rivaroxaban, the PT derived fibrinogen concentration was overestimated by almost 30% when determined on an ACL Elite Pro®, ACL 9000®, ACL Futura® or ACL Advance® but not when determined on an instrument of the ACL Top® series. This instrument-related difference accounts for the high coefficient of variation (19.4%) of the sample containing 290 µg/l rivaroxaban in ►Table 4 and is likely to be due to the difference in measuring principle between the instruments: ACL 9000® and Elite Pro® using nephelometry to detect clot formation and based on centrifugal analysis technology, ACL Advance® and ACL Futura® using turbidimetric clot detection and the ACL Top® series using turbidimetric clot detection and reading the PT clotting assay at 671 nm. Smaller instrument-related differences were observed for Thromborel S® and Actin FS® when used in combination with Siemens instruments with different algorithms for determination of the clotting time and/or different measuring principles: BCS® and BCS XP® using fixed absorbance for determination of the clotting time (i.e. calculating the time for a predefined change of absorbance over the baseline optical density [36]), Sysmex CA-1500® and CA-7000® using nephelometric clot detection and point of inflexion for determination of the clotting time (i.e. recording the endpoint when the total change of absorbance/scatter of 50% has been reached causing the inflexion of the curve [36]) and Sysmex CS2100i® using turbidimetric clot detection and point of inflexion. Hence, it is important that laboratories define the sensitivity of their own

## What is known about this topic?

- The direct oral anticoagulants dabigatran and rivaroxaban have variable effects on routine coagulation tests depending on the type and concentration of the drug and the type of reagent used.
- The Subcommittee on Control of Anticoagulation of the Scientific and Standardization Committee of the International Society on Thrombosis and Haemostasis and the British Committee for Standards in Haematology recommend that laboratories are aware of the sensitivity of their assays to these drugs.

## What does this paper add?

- This paper provides detailed information on the effect of dabigatran and rivaroxaban on the coagulation assays PT, aPTT, fibrinogen and antithrombin as performed with a large number of reagent/instrument combinations in a routine setting based on the results of a nationwide survey, in which virtually all Belgian clinical laboratories participated.
- The results of this study indicate that the sensitivity to dabigatran and rivaroxaban does not only depend on the reagent but also on the instrument used. Some of the instrument-related differences were of clinical importance.
- Our data demonstrate that there is a wide variation in responsiveness between reagent/instrument combinations but that the agreement between laboratories using the same reagent/instrument combination is good. Hence, our data provide clinical laboratories with useful information on the impact of dabigatran and rivaroxaban on the coagulation assays PT, aPTT, fibrinogen and antithrombin as performed with their reagent/instrument combinations and may help them to interpret these tests properly in patients taking dabigatran or rivaroxaban.

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reagent/instrument combination to each DOAC in order to interpret coagulation testing properly in patients taking these drugs.

The use of plasma samples spiked with dabigatran or rivaroxaban and not from patients taking dabigatran or rivaroxaban is a limitation of our study. Indeed, our results do not reflect the inter-individual variability from patients taking these drugs (14, 23, 37–42). However, our *in vitro* approach with use of the same normal plasma pool for both drugs allowed us to study the impact of therapeutic concentrations of both dabigatran and rivaroxaban on various reagent/instrument combinations in a large number of laboratories. Strong points of our study include the blinded study design, the inter-laboratory setting with almost 200 participating laboratories and the variety of reagent/instrument combinations employed which made it possible to study also the impact of instrumentation on the obtained results.

The Belgian EQAS plans to send on a regular basis samples spiked with different concentrations of DOAC in order to keep laboratories aware of the influence of these drugs on their routine coagulation tests.

In conclusion, this paper provides detailed information on the effect of dabigatran and rivaroxaban on routine coagulation assays as performed with a large number of reagent/instrument combinations. The current study also illustrates the value of a national EQAS as a tool to provide laboratories insight into the response of their reagent/instrument combination to dabigatran and rivaroxaban and underlines its educational role.

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**Conflicts of interest**

None declared.

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