Evaluation of DOAC Dipstick Test for Detecting Direct Oral Anticoagulants in Urine Compared with a Clinically Relevant Plasma Threshold Concentration

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Abstract
Measuring direct oral anticoagulant (DOAC) concentrations might be necessary in certain clinical situations but is not routinely performed. The DOAC Dipstick is a new rapid test for detecting DOACs in urine. The aim of this study was to evaluate the possible uses and limitations of the DOAC Dipstick and to compare visual analysis and DOASENSE Reader analysis of DOAC Dipstick pads. Plasma and urine samples were collected from 23 patients taking DOACs. DOAC concentrations in plasma and urine were measured by chromogenic substrate assays and in urine also by the DOAC Dipstick. Plasma concentrations were dichotomized at a threshold of ≥30 ng/mL. Patient samples were compared with samples from control individuals not using anticoagulants (n = 10) and with DOASENSE control urines. The Combur-10 test was used to measure parameters that may affect urine color and hence the interpretation of the DOAC Dipstick result. DOAC Dipstick test results were positive in 21/23 patient urine samples at a plasma DOAC concentration of ≥30 ng/mL and in 2/23 patient urine samples at a plasma DOAC concentration of <30 ng/mL. Inter-observer agreement was above 90% for visual analysis of patient urine samples and was 100% for DOASENSE Reader analysis of patient urines and for analysis of control group urines and DOASENSE control urines. Abnormalities in urine color detected by the Combur-10 test did not affect the DOAC Dipstick results. DOAC Dipstick detects DOACs in urine at a plasma threshold of ≥30 ng/mL. Positive DOAC Dipstick results should be confirmed by measuring DOAC plasma concentration.

Keywords
apixaban, dabigatran, direct oral anticoagulants, edoxaban, rivaroxaban

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Introduction

Direct oral anticoagulants (DOACs) comprise direct thrombin inhibitors (DTIs) (dabigatran) and direct factor Xa inhibitors (DXIs) (rivaroxaban, apixaban, edoxaban).1 DOAC use has increased in Estonia in the past years, mainly to prevent ischemic stroke in patients with non-valvular atrial fibrillation and to prevent and treat deep venous thrombosis and pulmonary embolism.1

DOAC concentrations are not routinely monitored but it is sometimes necessary to know the DOAC concentration of a patient in certain clinical situations such as acute hemorrhage or before thrombolysis, emergency surgery, or other invasive procedures.2–4 However, measuring DOAC concentrations in plasma is invasive, time consuming, and can only be performed in selected medical centers in Estonia. Furthermore, DOAC concentrations measured in plasma may be influenced by plasma proteins.2–4 Measuring DOAC concentrations in urine samples may overcome these challenges.

The DOAC Dipstick (DOASENSE GmbH, Germany) is a new rapid test for detecting DXIs and DTIs in urine. The test strip contains pads that specifically detect DXIs and DTIs as well as urine color and creatinine. The pad colors are visually compared with a color scale to determine the result. Measuring DOAC concentrations is much quicker in urine samples than in plasma samples and it takes approximately 10 minutes to obtain a result with the DOAC Dipstick. However, because the DOAC Dipstick result is interpreted visually, it is observer-dependent and may be invalid if the urine color is abnormal. These potential errors can be eliminated by using the Reader (DOASENSE GmbH, Germany).

The DOAC Dipstick has other advantages over conventional blood assays. For example, the test pads do not react with heparin, nadroparin, fondaparinux, and coumadin. The thrombin inhibitor pads can also detect r-hirudin and argatroban at high concentrations, so these may be confused with dabigatran when interpreting the test result but this is highly unlikely to occur.5–9 A recent multicenter study showed that the DOAC Dipstick can detect DXIs and DTIs with an accuracy of 97.3% and 99.3%, respectively and that sensitivity, specificity, and negative and positive predictive values for the DOAC Dipstick were all 95% or higher.9

A threshold plasma DOAC concentration of ≥30 ng/mL has been regarded as important for supporting clinical decision making in patients admitted to hospital for stroke, major hemorrhage or major joint injury.4 The performance of the DOAC Dipstick has been evaluated in urine samples by comparing DOAC concentrations measured with the DOAC Dipstick with those measured by liquid chromatography mass-spectrometry, dichotomized according to the 30 ng/mL plasma cut-off value.9 Here, we aimed to analyze the advantages and disadvantages of using the DOAC Dipstick in a hospital setting. We recruited patients with venous thromboembolism and non-valvular atrial fibrillation from internal medicine and cardiology departments because patients with previously mentioned diseases are mostly treated in these departments. Patients were recruited from the in- and out-patient departments and were treated with apixaban, rivaroxaban, and dabigatran. We compared DOAC results in urine measured using the DOAC Dipstick with DOAC concentrations in plasma and urine measured using chromogenic assays. A DOAC plasma concentration of ≥30 ng/mL was chosen as clinically significant according to previously published data.2,4 We also analyzed the agreement between visual analysis and DOASENSE Reader analysis of DOAC Dipstick pads and well as agreement in visual analysis between observers. We used the Combir-10 test to detect abnormal urine color and its effects on DOAC Dipstick results.

Participants and Methods

This was a clinical pilot study conducted at the North Estonia Medical Centre and was approved by the Tallinn Medical Research Ethics Committee (nr 227). The study design is summarized in Figure 1.

Study participants were recruited from the in- and out-patient wards of the internal medicine and cardiology departments at the North Estonia Medical Centre. Inclusion criteria were ongoing DOAC treatment and age ≥18 years. Exclusion criteria were age <18 years and DOAC treatment for fewer than 7 days. All participants gave written informed consent prior to participation in the study. We analyzed plasma and urine samples from 23 patients (age range 38-94 years) receiving antithrombotic therapy with apixaban (n = 10), rivaroxaban (n = 10), or dabigatran (n = 3) and from 10 control volunteers (age range 30-67 years) who did not receive any antithrombotic therapy. Edoxaban was not used in the study population.

Information was collected from participants using a prespecified questionnaire and medical records. We collected information on the participant’s gender, age, weight, height, body mass index (BMI) (kg/m²), DOAC type, DOAC dose, time of last DOAC intake and time of last urination before urine samples were collected for analysis.

Collection of Plasma and Urine Samples

Blood samples were collected in BD Vacutainer® Citrate Tubes (BD Diagnostics, Plymouth, UK) with 3.2% buffered sodium citrate solution for DOAC measurement and in BD Vacutainer® PST™ Tubes (BD Diagnostics, Plymouth, UK) containing spray-coated lithium heparin for estimated glomerular filtration rate (eGFR) evaluation. The time between medicine intake and analysis was documented. Urine specimens were collected in 10-mL BD Vacutainer tubes (BD Diagnostics, Plymouth, UK). All samples were transported to the laboratory by a pneumatic tube transportation system, handled according to local laboratory guidelines, and tested at the time of arrival to the laboratory.

Within 2 hours of collection, blood samples were centrifuged at room temperature for 15 minutes at 1500 g and analyzed. Urine samples were collected within 15 minutes of
blood collection and analyzed immediately using the DOAC Dipstick as described.8

Laboratory Methods

All blood and urine samples were analyzed in the North Estonia Medical Centre’s laboratory. Laboratory staff were chosen randomly to visually analyze the DOAC Dipstick pads and were trained by one of the leading researchers (MP) in the DOASENSE instructions for use. The reference scale for visual investigation is shown in Figure 2.

DOAC concentrations were measured in plasma and urine using chromogenic assays. The STA-ECA II assay was used to measure dabigatran and the STA-Liquid Anti-Xa assay with DOAC-specific calibrators was used to measure rivaroxaban and apixaban (Diagnostica Stago, France) on a STA-R Evolution coagulation analyzer (Diagnostica Stago, France). The chromogenic assay results were expressed as absolute values and these values were dichotomized according to the plasma cut-off of 30 ng/mL for comparison with the DOAC Dipstick results.

Plasma creatinine was measured on a Cobas 6000 analyzer (Roche Diagnostics, Germany) to calculate the eGFR according to the CKD-EPI equation (ml/min/1.73m²). Urine was analyzed using the Combir-10 test (Cobas u pack), which contained an iodated mesh to reduce interference with ascorbic acid and was analyzed on a Cobas u 6500 analyzer (Roche Diagnostics, Germany). The following values were considered normal for the Combir-10 test parameters: urine pH: 4.5–8.0, specific gravity: 1.015–1.025, and “negative” for glucose, protein, nitrite, ketones, bilirubin, urobilinogen, blood, and leukocytes.

The DOAC Dipstick (LOT DS18110802, DOASENSE GmbH, Germany) was used to qualitatively detect rivaroxaban, apixaban, or dabigatran in urine samples. Three independent observers compared the pad colors with those of a reference scale as described previously.8 The same Dipstick was then analyzed by a Reader (DOASENSE GmbH, Germany).

Control assays were performed with urine samples from control participants (n = 10) and with negative and positive Doasense control urines on nine separate days by three independent observers and the DOASENSE Reader.

Statistical Analysis

Statistical analysis was performed with IBM SPSS statistics (version 23, IBM, USA). Descriptive statistics were used to analyze age and gender. DOAC, eGFR, and BMI results were expressed as median with minimal and maximal values.

Results

Patient data and DOAC concentrations in plasma and urine are presented in Table 1. Concentrations of apixaban, rivaroxaban, and dabigatran were all within the expected range of 26–465 ng/mL, except for one patient on rivaroxaban who had a plasma concentration of 3 ng/mL (Table 2, case 4).

The median DOAC concentrations in urine were 819 ng/mL for apixaban, 2359 ng/mL for rivaroxaban, and 1940 ng/mL for dabigatran (Table 1).

The DXI pad of the DOAC Dipstick was analyzed as positive by visual analysis and the Reader in 20/20 patients on apixaban and rivaroxaban. Plasma concentrations of apixaban and rivaroxaban were ≥30 ng/mL in 18/20 patients and 3.0 and 26.0 ng/mL in the remaining two patients (Table 2, cases 4 and 5).

The DTI pad of the DOAC Dipstick was positive in 3/3 patients treated with dabigatran and plasma concentrations were ≥30 ng/mL. There were no differences in color assessment between observers and the Reader.

The DXI and DTI pads were both positive in one patient (case 1) treated with apixaban. The plasma concentration of apixaban was ≥30 ng/mL and dabigatran concentration was not
measured in the plasma or urine samples because there was no record of the participant taking dabigatran. The concentration of apixaban was high in the urine sample of this patient (Table 2).

In one patient (case 2), the plasma concentration of apixaban was $\geq 30$ ng/mL but the urine concentration of apixaban was 142 ng/mL. This participant had renal insufficiency (eGFR $< 30$ mL/min/1.73m$^2$), so the DOAC Dipstick result was likely false negative because of a reduced excretion of DOACs. This participant was not taking dabigatran, so we did not measure dabigatran concentration in the plasma and urine samples.

In one patient (case 3), the observer evaluated the DXI pad as negative whereas the DOASENSE Reader identified it as positive, in agreement with the plasma concentration of $\geq 30$ ng/mL.

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**Table 1.** Characteristics of the Study Participants. Values are Given as Number (n) or as Median and Minimal (min) and Maximal (max).

| Characteristics          | Apixaban | Rivaroxaban | Dabigatran | Total | Control |
|--------------------------|----------|-------------|------------|-------|---------|
| n                        | 10       | 10          | 3          | 23    | 10      |
| Male/female              | 5/5      | 5/5         | 0/3        | 10/13 | 5/5     |
| Age (years)              | 75.5 (38-94) | 75.0 (43-88) | 79.0 (41-83) | 77.0 (38-94) | 43 (30-67) |
| BMI (kg/m$^2$)           | 31 (26-48) | 29.6 (24-43) | 28.7 (28-31) | 29.6 (24-48) | N/A     |
| eGFR (mL/min/1.73m$^2$)  | 42.5 (17-116) | 73.5 (36-103) | 57.0 (47-98) | 62.0 (17-116) | N/A     |
| DOAC dose daily          | 2.5 mg bid | 15 mg od    | 150 mg bid |       |         |
|                          | 5 mg bid  | 20 mg od    |            |       |         |
| DOAC plasma (ng/mL)      | 126.0 (75.0-275.0) | 159.5 (3.0-465.0) | 48.2 (46.0-52.0) |       | N/A     |
| DOAC urine (ng/mL)       | 819 (142-4016) | 2359 (124-9085) | 1940 (1290-3560) |       | <7 (0-7) |
| DOAC Dipstick positive/negative | 10/0        | 10/0        | 3/0        | 23/0  | 0/10    |

N/A, not applicable.
and the high apixaban concentration measured in the urine sample (Table 2).

The DOAC Dipstick results for the DOASENSE control urines were interpreted correctly after visual assessment of DXI and DTI pad colors by three independent observers on nine different days (kappa value between observers: 1.0; kappa value between observer and Reader: 1.0) (Table 3).

The DOAC Dipstick results for control individuals were interpreted correctly as negative by visual and DOASENSE Reader analysis. The concentrations of all DOACs were <7 ng/mL in urine (Table 1).

Some results from the Combur-10 test indicated abnormal urine color (Tables 2 and 4), but these abnormalities did not affect the DOAC Dipstick results. For example, one patient (case 1) had cloudy urine, but this did not affect the color of the DOAC Dipstick pad (Tables 2 and 4).

**Discussion**

Laboratory evaluation of DOAC plasma concentration is currently not widely available in Estonian hospitals. Because DOAC use is rapidly increasing in Estonia, we need tests that
| pH (norm: 4.5-8.0) | Specific Gravity (norm: 1.015-1.025) | Leukocytes (norm: neg.) | Blood (norm: neg.) | Protein (norm: neg.) | Nitrate (norm: neg.) | Bilirubin (norm: neg.) | Glucose (norm: neg.) | Ketone (norm: neg.) | Urobilinogen (norm: neg.) | Urine Color in Test Tube (norm: norm.) | Color Pad Visual (norm: norm.) |
|-----------------|-------------------------------------|------------------------|------------------|---------------------|---------------------|----------------------|---------------------|----------------|-------------------------|----------------------------------|-----------------------------|
| 5.0             | 1.021                               | neg                    | neg              | neg                 | neg                 | neg                   | neg                 | neg            | neg                     | norm                             | norm                         |
| 5.0             | 1.028                               | neg                    | neg              | neg                 | neg                 | neg                   | neg                 | neg            | neg                     | norm                             | norm                         |
| 5.0             | 1.007                               | neg                    | neg              | neg                 | neg                 | neg                   | neg                 | neg            | neg                     | norm                             | norm                         |
| 5.0             | 1.006                               | 2+                     | 1+               | 2+                  | pos                 | 1+                   | neg                 | 1+             | 1+                      | norm                             | norm                         |
| 5.0             | 1.009                               | 2+                     | neg              | neg                 | neg                 | neg                   | neg                 | neg            | neg                     | norm                             | norm                         |
| 5.0             | 1.017                               | 2+                     | 5+               | neg                 | neg                 | 1+                   | neg                 | 1+             | 2+                      | norm                             | cloudy                       |
| 5.0             | 1.026                               | 1+                     | neg              | 1+                  | neg                 | 1+                   | neg                 | 1+             | 1+                      | norm                             | norm                         |
| 5.0             | 1.008                               | neg                    | 1+               | neg                 | neg                 | neg                   | neg                 | neg            | neg                     | norm                             | norm                         |
| 5.0             | 1.012                               | neg                    | neg              | neg                 | neg                 | neg                   | neg                 | neg            | neg                     | norm                             | norm                         |
| 5.0             | 1.013                               | neg                    | 2+               | 1+                  | neg                 | neg                   | neg                 | neg            | 1+                      | norm                             | norm                         |
| 5.0             | 1.013                               | 2+                     | neg              | neg                 | neg                 | neg                   | neg                 | neg            | neg                     | norm                             | norm                         |
| 5.0             | 1.017                               | 1+                     | neg              | neg                 | neg                 | neg                   | neg                 | neg            | neg                     | norm                             | norm                         |
| 5.0             | 1.022                               | neg                    | 1+               | neg                 | neg                 | neg                   | neg                 | neg            | neg                     | norm                             | norm                         |
| 5.0             | 1.023                               | neg                    | neg              | neg                 | neg                 | neg                   | neg                 | neg            | neg                     | norm                             | norm                         |
| 5.0             | 1.024                               | 2+                     | neg              | neg                 | neg                 | neg                   | neg                 | neg            | 1+                      | norm                             | norm                         |
| 5.0             | 1.045                               | neg                    | 1+               | neg                 | neg                 | neg                   | neg                 | neg            | neg                     | norm                             | norm                         |
| 5.5             | 1.009                               | 1+                     | 5+               | neg                 | neg                 | neg                   | neg                 | neg            | neg                     | norm                             | norm                         |
| 5.5             | 1.011                               | 1+                     | 5+               | neg                 | neg                 | neg                   | neg                 | neg            | 1+                      | norm                             | norm                         |
| 5.5             | 1.014                               | 2+                     | neg              | neg                 | neg                 | neg                   | neg                 | neg            | neg                     | norm                             | norm                         |
| 5.5             | 1.022                               | 3+                     | 1+               | neg                 | neg                 | neg                   | neg                 | neg            | neg                     | norm                             | norm                         |
| 7.0             | 1.009                               | neg                    | 1+               | neg                 | neg                 | neg                   | neg                 | neg            | neg                     | norm                             | norm                         |
| 7.0             | 1.023                               | 2+                     | 5+               | 1+                  | neg                 | 1+                   | neg                 | 3+             | 3+                      | norm                             | norm                         |
| 8.0             | 1.020                               | 3+                     | 5+               | 1+                  | neg                 | neg                   | neg                 | neg            | neg                     | norm                             | norm                         |

Neg, negative; pos, positive; norm, normal.
are more precise than prothrombin time/international normalized ratio and activated partial thromboplastin time to measure DOAC concentrations in plasma. Although DOACs affect the prothrombin time/international normalized ratio and activated partial thromboplastin time, these effects are non-consistent, making these assays unreliable in emergency situations.\(^\text{11}\) The DOAC Dipstick has solved this problem by detecting DOACs in urine. Similar test strips are used in many patients admitted to an emergency care unit for differential diagnosis. Consequently, urine sampling for DOAC test strip may follow the same collection technique, which would not require an additional urine sampling method. The DOAC Dipstick gives rapid results, is easy to interpret, and can be used in the laboratory as well as at the bedside to save time. However, accuracy of the DOAC Dipstick results can be hampered by urine color or blood cells in the urine and can depend on how long urine was present in the bladder before the sample was collected. Therefore, a positive DOAC Dipstick result may not always reflect a clinically significant DOAC concentration in the plasma and actual plasma concentrations should be verified by further tests. In contrast, a negative result on the DOAC Dipstick can be considered reliable.\(^\text{9}\) Because DOAC plasma concentrations cannot be routinely measured in most Estonian hospitals, the DOAC Dipstick may be useful in emergency situations.

In this study, we qualitatively measured DOAC concentrations in urine samples from 23 patients using the DOAC Dipstick and compared these concentrations with quantitative DOAC concentrations in plasma and urine samples from the same patients and controls. Based on previous reports, we selected \(\geq 30\) ng/mL as a clinically significant DOAC plasma concentration,\(^\text{2,4}\) but DOAC urine concentrations have not been studied widely, so there is currently no reference concentration for urine samples.

DOAC concentrations are about 10- to 50-fold higher in urine than in plasma because 30–80% of absorbed DOACs are excreted into urine.\(^\text{8,12}\) In the present study, we used chromogenic substrate assays to quantify DOACs in plasma and urine samples. Previous reports have shown that DOAC concentrations measured in urine using chromogenic substrate assays are comparable to those measured using liquid chromatography mass spectrometry.\(^\text{13}\) We found that concentrations of apixaban and rivaroxaban were within the same range as those reported but were generally lower, which may be explained by the low number of patients and low plasma concentrations in our participants (Table 1).

In agreement with previous findings, we found the DOAC Dipstick fast and easy to use, with a low inter-observer variability.\(^\text{5,8,9}\) We observed some inter-observer variability in visual interpretation of the DXI inhibitor pad, of which two were interpreted as positive in patients taking rivaroxaban. Plasma concentrations of these two patients were <30 ng/mL and urine concentrations were between 75 and 200 ng/mL. These urine concentrations were very low considering that they should be 10- to 50-fold higher than those in plasma.\(^\text{19}\) The positive result in urine may also be explained by the pharmacokinetics of rivaroxaban when the rivaroxaban plasma concentration decreased to 3 ng/mL. Another explanation for the positive result is that urine accumulated in the bladder over a couple of hours before the urine sample was collected for analysis.

The Combur-10 test screens for parameters that may affect urine color (such as blood, leukocytes, nitrates, and pH) and hence the interpretation of the DXI and DTI pad colors. However, all abnormal results detected by the Combur-10 test were interpreted as a normal urine color on the DOAC Dipstick. This may be explained by the specific reagents used to determine the parameters on the Combur-10 test strip. Nevertheless, our results support the assumption\(^\text{7}\) that mild changes to the urine, such as those caused by microhematuria, do not affect the DOAC Dipstick. The results are limited to the observed range of data and it would be interesting to collect more data including the whole range of results of such urine test strip.

There are some limitations to the present study. First, the number of patients was too small to conclusively interpret DOAC Dipstick results at a plasma threshold of \(\geq 30\) ng/mL in patients treated with dabigatran. The number of patients taking dabigatran may have been low because dabigatran is prescribed less frequently than other DOACs in Estonia.\(^\text{1}\) Second, the DTI and DXI pads were both positive in a patient treated with apixaban, but the positive DTI result was not verified by a chromogenic test because there was no record of the participant taking dabigatran. Third, the positive DOAC Dipstick test in two patients taking rivaroxaban with a DOAC plasma concentration of <30 ng/mL was surprising and may not be a false positive because the DOAC Dipstick may be more sensitive for DOACs in urine than assays used to detect DOACs in plasma are. This finding may also be explained by the pharmacokinetics of DOACs, by the longer interval between urine sampling and last drug intake, and by the time interval between urine excretion into the bladder and urine sampling for analysis. Additional studies may give more insight into these observations. Fourth, there were some discrepancies in interpretation of the pad colors between observers. This may be due to differences in color vision between observers or the warm artificial light source. This inter-observer variation is eliminated by the DOASENSE Reader, which may be especially useful in emergency situations when fast, accurate analysis is needed. However, in two patients (cases 1 and 2), the Reader also gave positive results for both DXI and DTI pads, indicating that the lowest threshold of DOACs that can be determined by the DOAC Dipstick needs further investigation.

**Conclusions**

The DOAC Dipstick is an easy and effective way to assess the presence of DTIs or DXIs in urine in urgent medical situations such as acute hemorrhage or before thrombolysis or emergency surgery. Variations in visual evaluation between observers can be avoided by using the DOASENSE Reader. This is particularly useful in critical situations with limited medical staff and time for minor procedures. We conclude that the DOAC Dipstick is a helpful tool for excluding clinically significant DOAC plasma concentrations at a threshold of \(\geq 30\) ng/mL.
This is especially relevant to smaller hospitals in Estonia, where DOAC plasma tests are not readily available. However, we agree that all positive results in urine samples should be confirmed by measuring DOAC plasma concentrations.

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