

Reliability and validity of point-of-care tests taken from urine samples of patients on therapy with Apixaban, Rivaroxaban and Dabigatran

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To analyze the reliability and validity of a point-of-care-test taken from urine samples of patients on treatment with Apixaban (A), Rivaroxaban (R) and Dabigatran (D).



Objectives

When every minute counts...

in patients' emergency situations.
 DOACs are excreted at a rate of 30 % to 80 % into urine.

Aim

A point-of-care-test (POCT) has been developed in order to determine the absence or presence

The determination of the

direct oral anticoagulants

(DOACs) may be required

absence or presence of

Results

• The assignment of the colors of the urine samples by naked eye with both lots of test strips is shown in Table 1.

• Examples of negative and positive colors taken from two patient samples are shown for R (Fig. 2) and D (Fig. 3).

 Upper pad (Fig. 2 and 3) is specific to D: Pad shows the color ochre indicating absence of D when patients are treated with A and R.
 Both observers assigned always the color for "negative" for this pad (data not shown).

• Central pad (Fig. 2 and 3) is specific for A and R: Pad shows color yellow indicating for absence of A and R when patients are treated with D.

Fig. 1: Examples of colors of DOAC Dipsticks (taken from tube of

Observers assigned always the color "normal" to this pad with few exceptions (dark urine, not influencing the color of pads 3 and 4).

• Lower pad (Fig. 1 and 2): shows color of urine sample. Observers assigned the color "normal" to this pad (data not shown).

• Table 2 shows the results of the statistical analysis of the assessment of the colors of dipsticks from urine samples of patients and controls (lot 1).

• Table 3: The concentrations of A, R, and D and of controls are given from the urine samples of the patients – mean value, standard deviation (sd), coefficient of variation (CV) and number (N).

Tab. 1: Results of the optical assessment of the colors of urine samples after incubation on DOAC Dipsticks as negative and positive of control groups and patients treated with A, R and D.

... fast DOAC testing matters.

of DOACs in urine samples of patients.

Methods

- Definitions for assignment of colors:
- Pad 2 color of urine: normal or abnormal
- Pad 3 R and A: yellow (negative), not clearly yellow
- and white (positive) Pad 4 – D: ochre (negative),
- not clearly ochre and rose (positive) The examples of the colors are given on the test tube (Fig. 1).
- Test strips were photographed in a lightbox.

- This study was approved by the Ethics Committee II of Heidelberg University.
- Patients signed informed consent prior to investigation.
- Test strips with pads containing the reagents (dry chemistry) for determination of A, R, and D were incubated with patients' urine for 10 min.
- Determinations were performed using test strips of 2 pilot productions.
- Test strips were incubated with urine and color of pads were assessed by the observers and photographed after 10 min.

the product)				
Thrombin Inhibitor / Thrombin Inhibitor				
	neg.	pos.	pos.	
Factor Xa Inhibitor / Faktor Xa Inhibitor				
	neg.	pos.	pos.	
Urine Colour / Urinfarbe				

Fig. 2: Photos taken of pads from urine samples of 2 control patients (left) and 2 patients treated with R (right)

norm.

Upper row pad 4: pad for thrombin inhibitor Central row pad 3: pad for FXa inhibitor Lower row pad 2: pad for urine color

Fig. 3: Photos of pads from urine samples of 2 control patients (left) and 2 patients treated with D (right)

	Apixaban	Rivaroxaban	Dabigatran	Control
Patient	1-29	1-30	1-30	1-30
1 st lot of test strips				
Observer 1	all pos.	all pos.	all pos.	all neg.
Observer 2	all pos.	all pos.	all pos.	all neg.
2 nd lot of test strips				
Observer 1	all pos.	all pos.	all pos.	all neg.
Observer 2	all pos.	all pos.	all pos.	all neg.

Tab. 2: Statistical parameters of colors of pads from patients treated with A, R, D and of control group; results of 1st lot of test strips. 2nd lot not analyzed.

Parameter	Apixaban	Rivaroxaban	Dabigatran	Control
Карра	1.0	1.0	1.0	1.0
Sensitivity	1.0	1.0	1.0	1.0
Specificity	1.0	1.0	1.0	1.0
Accuracy	1.0	1.0	1.0	1.0
PPV*	1.0	1.0	1.0	1.0
NPV**	1.0	1.0	1.0	1.0

* positive predictive value, ** negative predicitve value

Tab. 3: LC-MS/MS analysis of urine samples of patients treated with A, R, D and of control group.

Urine samples were obtained from patients (n=29 or 30 per group) treated with A (5 mg bid), R (20 mg od), D (110 mg or 150 mg bid) and patients without anticoagulant therapy (control groups, C). Urine samples were taken 2 or 12 hrs after intake of medication. • Two observers assessed the colors of the pads visually by naked eye in comparing them to the color negative and positive (see Fig. 1).

 The concentration of DOACs was measured by liquid chromatography tandem massspectro-metry (LC-MS/MS). Upper row pad 4: pad for thrombin inhibitor Central row pad 3: pad for FXa inhibitor Lower row pad 2: pad for urine color Ranges of concentration were: 202 to 6.667 ng/ml (A), 169 to 9.579 ng/ml (R) and 1.057 to 15.996 ng/ml (D).

LC-MS/MS				
	Apixaban	Rivaroxaban	Dabigatran	Control
mean ng/ml	1849	2752	5650.6	> 10
sd	1420	1922	3698	nd*
CV	65.5	69.6	70.0	nd*
N	29	30	30	30
'not determined				

Conclusions

 The current POCT taken from urine samples of patients offers a rapid, reliable and valid way of proving the presence or absence of DOACs in medical emergency situations.

- Pads are specific for A, R and D and do not show cross reaction.
- A prospective study is currently prepared to assess

the performance of an in vitro diagnostic test for DOACs in urine submitted to ClincialTrials.gov (ID: NCT03182829).

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