Post Marketing Study Plan

Post Marketing Study of an *in vitro* diagnostic test for direct oral anticoagulants (Apixaban, Edoxaban, Rivaroxaban, Dabigatran) in urine

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Sponsor:	DOASENSE GmbH Waldhofer Strasse 102 69123 Heidelberg, Germany Phone: +49 (0) 6221-825 9785 Fax: +49 (0) 6221-825 9786
The Post Marketing Study will be carried out and essential documents will be kept and archived in accordance with Good Clinical Practice Guideline.	

1 Approval of the Post Marketing Study

By signing below, the signatories agree to conduct the Post Marketing Study according to this Study Plan.

1.1 Sponsor

J. Harenberg 4th May 2018

Prof. Dr. Job Harenberg Signature Date

Managing Director
DOASENSE GmbH

1.2 Lead Investigator

J. Harenberg 4th May 2018

Prof. Dr. Job Harenberg Signature Date

1.3 Investigator at participating site

Prof. Dr. J. Harenberg J. Harenberg 4th May 2018

Name Signature Date

Site

Privatpraxis
Handschuhsheimer Landstr. 62
69121 Heidelberg

2 Synopsis

Title of the clinical investigation:	Post Marketing Study of an *in vitro* diagnostic test for direct oral anticoagulants (Apixaban, Edoxaban, Rivaroxaban, Dabigatran) in urine
Objectives:	Assess the accuracy and specificity of the *in vitro* diagnostic (IVD) test for oral direct Factor Xa and Thrombin inhibitors from urine samples of patients
Diagnosis and main criteria for inclusion:	Adult patients (>18 years) either under therapy with Rivaroxaban, Apixaban, and Edoxaban, or with Dabigatran for at least 1 week
Design:	Prospective, open-label, controlled, not-randomized, multicenter
Methodology:	Comparison of the test strip results after visual reading and reflectance photometric reading to the results of bioanalytical quantification of Rivaroxaban, Apixaban, and Edoxaban or Dabigatran
Blinding:	Not applicable.
Control:	Patients taking oral direct Factor Xa inhibitors (Test group A) are negative for an oral Thrombin inhibitor and can serve as negative control for Test group B (test for oral Thrombin inhibitor), and vice versa
Number of patients:	880 (n=440 under therapy with oral direct Factor Xa inhibitor, n=440 under therapy with oral Thrombin inhibitor)
Test products:	Direct oral anticoagulant (DOAC) urine dipstick test
Primary endpoints:	True positive and true negative rate of the point of care test (POCT) by comparison with the results obtained by bioanalytical quantification
Secondary endpoints:	Investigator readings of POCT test results as compared to LC-MS/MS Results obtained by the reflectance photometric reading (DOASENSE Reader) as compared to naked eye reading Results of the questionnaire
Plan for statistical analyses:	For each diagnostic test the proportions of false negative and false positive results will be assessed together with confidence intervals.

Table 2-1: Assessments at the visit

Assessment	Day 1
Informed consent	X
Demographic data	X
Medication history (DOACs, other)	X
Collection of urine sample	X
Visual reading presence/absence of DOACs (IVD)	X
Reflectance photometric reading (DOASENSE Reader) of the dipstick	X
Urine aliquot for bioanalysis	X
Completion of questionnaire	X

DOACs: direct oral anticoagulants, IVD: *in vitro* diagnostic test

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4 Abbreviations

BfArM	Federal Institute for Drugs and Medical Devices (*Bundesinstitut für Arzneimittel und Medizinprodukte*) competent authority
BMI	Body mass index, calculated as body weight [kg] divided by the square of the body height [m]
CA	Competent Authority
CE	Certification (*Conformité Européene* [European Conformity])
CRF	Case Report Form
DIN EN	German Industrial Standard European Standard *(Deutsche Industrienorm Euronorm)*
DOAC	Direct oral anticoagulant
EC	Ethics Committee
FPFV	First Patient First Visit
IVD	*In vitro* diagnostic
LC-MS/MS	Liquid chromatography-tandem mass spectrometry
LPLV	Last Patient Last Visit
MPG	German Act on Medical Devices *(Medizinproduktegesetz)*
PID	Patient identification number
POCT	Point-of-care test
SOP	Standard operating procedure

5 Ethics and legal aspects

5.1 Ethics Committee and Competent Authority

This Post Marketing Study will be submitted to

- the competent authority (CA, Bundesinstitut für Arzneimittel und Medizinprodukte, BfArM [Federal Institute for Drugs and Medical Devices]) for notification, and
- to the responsible Ethics Committee (EC) for medical professional consultation

according to the applicable regulatory and legal requirements.

No approval is required as per §23 b, German Act on Medical Devices (*Medizinproduktegesetz,* MPG), as no invasive sampling or treatment is planned.

Both bodies will be notified if there are substantial changes in the planned assessment.

5.2 Ethical conduct of the Post Marketing Study

The Post Marketing Study will be performed in accordance with the ethical principles that have their origin in the Declaration of Helsinki.

5.3 Patient Information and procedure for obtaining consent

The investigator or sub-investigator of the investigational site (i.e., the patient's family doctor or medical practice/outpatient care unit) will inform patients about all aspects of the Post Marketing Study, in particular about the characteristics and objectives, duration and extent of participation, about test methods used.
The information will be non-technical language, and patients will be able to ask questions.

Informed consent has to be given voluntarily and in writing.

Patients will also be informed about the anticipated risks and benefits, data protection and access to their personal data in the context of the Post Marketing Study.

Patients and investigators (or sub-investigator) have to sign the informed consent twice, one original will be given to the patient, and one will remain with the investigator.

5.4 Data protection and data access

The investigator and the patient will agree in writing to the direct access to patient data to examine, analyze, verify, and reproduce any records that are important to the assessment of the Post Marketing Study.

All parties granted access to the data agree to take any precaution necessary to ensure the confidentiality of the patient's data and the sponsor's proprietary information.

All clinical investigation related records and data of the patients will be pseudonymized.

Each patient will receive a unique patient identification number (PID) identifying the patient in the investigation site's patient database and identified in the clinical investigation with a specific identifier (e.g., random number or participant number).

The investigator ensures that any documents or data disclosed to the sponsor do not contain any information disclosing the identity of the patient.

5.5 Compliance statement

This Post Marketing Study will be conducted and reported in accordance to the current versions of

- Ethical principles that have their origin in the Declaration of Helsinki, 1964,
- *In vitro* diagnostic medical device directive 98/79/European Community
- German Act on Medical Devices, and the German medical device ordinance (*Medizinprodukteverordnung*)
- DIN EN 13612 Performance Assessment of *in vitro* diagnostic medical devices

as applicable.

6 Key personnel involved in the Post Marketing Study

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Mariannhillstraße 2c
97074 Würzburg, Germany
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e-mail: u.spannagel@doasense.de

The sponsor and the lead investigator will approve and sign the Post Marketing Study including its amendments.

Each investigator will sign the Post Marketing Study Plan including its amendments.

The investigator at the study site may delegate tasks to adequately trained personnel. A list of personnel and tasks will be maintained at the respective site.

7 Introduction

7.1 Background and test principle

The *in vitro* diagnostic (IVD) test assessed in this study is a dipstick to test for absence or presence of direct oral anticoagulants (DOACs) in urine.

The test can be of value in clinical situations, where establishing the absence or presence of DOACs in the patient prior to procedures is important for clinical decision making, such as emergency procedures, stroke (before starting thrombolytic therapy), trauma, general surgery or other invasive procedures. Trained personnel can perform the test and results are rapidly available.

The point-of-care test (POCT) is a color-indicator diagnostic medical urine dipstick test for assessing the presence of oral direct Factor Xa inhibitors (Rivaroxaban, Apixaban, and Edoxaban) and Thrombin inhibitor (Dabigatran). These DOACs show a high renal clearance by immediate glomerular filtration – which allows testing for these substances in urine when they are present in blood. Chromogenic assays in urine showed a high sensitivity and specificity, and a low inter-observer variability [1][2].

The principle of the diagnostic test is based on the development of different colors on the indicator part of the dipstick in the presence or absence of oral direct Factor Xa (Rivaroxaban, Apixaban, and Edoxaban) and Thrombin inhibitors (Dabigatran). The colors for the test were chosen so that they could easily be read by the naked eye, with little possibility of incorrect identification of colors [1].

7.2 Description of the *in vitro* diagnostic test

The dipstick test consists of a plastic strip with 4 indicator test pads (Figure 7-1):

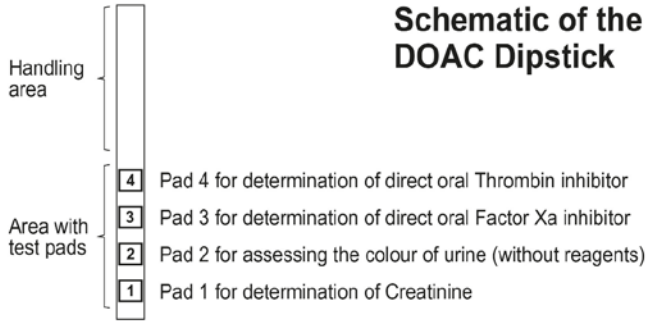


Figure 7-1: Schematic representation of DOAC Dipstick

The container contains a reference color scale for the visual comparison of the test results.

7.3 Rationale of the Post Marketing Study

This trial is conducted to assess the performance and handling of the IVD for oral direct Factor Xa and Thrombin inhibitors from urine samples of patients on treatment with DOACs in an actual point-of-care setting in comparison to results obtained by liquid chromatography-tandem mass spectrometry (LC-MS/MS) from urine samples.

7.4 Risks and benefits of the investigational device and Post Marketing Study procedures

No interventions or invasive procedures in the patients are planned.

The patients will not have additional risks due to participation in the investigation, and will not benefit directly by participating. Only patients who already take oral direct Factor Xa or Thrombin inhibitors will be included into the trial. They will provide a spontaneous urine sample for testing and will not come into contact with the test itself.

8 Clinical investigation objectives and endpoints

8.1 Primary objective

Primary objective of the investigation is to

- Assess the accuracy and specificity of the IVD for oral direct Factor Xa and Thrombin inhibitors from urine samples of patients

Primary endpoint:

- True positive and true negative rate of the POCT by comparison with the results obtained by bioanalytical quantification

8.2 Secondary objectives

Secondary objectives of the investigation are:

- Comparison of the investigator-assessed POCT results with the results obtained by reflectance photometric reading (DOASENSE Reader)
- Determination of the sensitivity and specificity of the results of the reflectance photometric reading (DOASENSE Reader) by means of concentrations of Rivaroxaban, Apixaban, Edoxaban and Dabigatran determined by LC-MS/MS
- Investigate the handling and usability of the IVD for oral direct Factor Xa and Thrombin inhibitors in clinical setting using a questionnaire for the investigator / sub-investigator

Secondary endpoints:

- Investigator readings of POCT test results
- Results obtained by the reflectance photometric reading (DOASENSE Reader)
- Concentrations of Rivaroxaban, Apixaban, Edoxaban and Dabigatran determined by LC-MS/MS
- Results of the questionnaire

9 Design of the Post Marketing Study

9.1 Overall design and plan-description of the Post Marketing Study

This prospective, open-label, controlled, not randomized Post Marketing Study will be conducted as a multicenter Post Marketing Study in Germany.

Two groups of patients will be included:

- Test group A: Patients under therapy with oral direct Factor Xa inhibitor (Rivaroxaban, Apixaban, and Edoxaban) (n=440)
- Test group B: Patients under therapy with an oral Thrombin inhibitor (Dabigatran) (n=440)

The Post Marketing Study will be conducted at the patient's family doctor or medical practice/outpatient care unit (referred to as "investigational site" in the following).

The Post Marketing Study will consist of a single visit, which is performed during a routine visit at the investigational site.

The Post Marketing Study starts with first patient signing informed consent (FPFV) and ends with the last patient providing the last sample (last patient last visit, LPLV).

Patients fulfilling the criteria described in Sections 9.3.1 and 9.3.2 will be included in the Post Marketing Study after having given informed consent.

Each patient will participate for approximately 1 h in the Post Marketing Study.

Medical personnel at the site will record the patient's demographic data (see Section 9.5.2).

Patients will provide a sample of spontaneous urine.

The urine samples will be tested in the outpatient care unit with the IVD and the DOASENSE Reader. In addition, aliquots of urine will be analyzed by LC-MS/MS.

The personnel performing the test will complete a questionnaire on handling of the IVD.

Primary endpoints are described in Section 8.1.

9.2 Discussion of Post Marketing Study design, including the choice of control groups

This clinical Post Marketing Study investigates the sensitivity and specificity of a POCT for DOACs, i.e., the rate of correct positive, false positive, correct negative and false negative results in the point-of-care setting.

The IVD is a test to determine absence or presence of DOACs in urine – Test A (pad 3) tests for oral direct Factor Xa inhibitors (Rivaroxaban, Apixaban, and Edoxaban), Test B (pad 4) for an oral Thrombin inhibitor (Dabigatran).

No control group of patients not treated with a DOAC is required, as patients take either oral direct Factor Xa inhibitors (Test group A) or an oral Thrombin inhibitor (Test group B), never both. Thus, patients in Test group A are negative for an oral Thrombin inhibitor and can serve as negative control for Test group B, and vice versa.

The presence or absence of oral direct Factor Xa inhibitors or an oral Thrombin inhibitor will be determined with 3 methods:

- Naked-eye reading by the investigator or delete by comparing the colors after end of the reactions to the reference color scale
- Reflectance photometric reading using the DOASENSE Reader
- Analysis of the concentrations of Rivaroxaban, Apixaban, Edoxaban and Dabigatran LC-MS/MS in urine. The sensitivity and specificity of this method is at least 98% [1][2].

9.3 Selection of population

The patients participating in this Post Marketing Study will be recruited directly at the point of care, i.e., the respective outpatient care unit. Only patients fulfilling all of the inclusion criteria and none of the exclusion criteria will be included.

9.3.1 Inclusion criteria

- Patient is able to understand and follow instructions of the study
- Fully signed and dated written informed consent
- Age >18 years
- Patient is either under therapy with Rivaroxaban, Apixaban, and Edoxaban or Dabigatran for at least 1 week

9.3.2 Exclusion criteria

Only patients fulfilling none of the criteria below will be included:

- Patient not able to provide urine samples.
- Patient not able to understand the informed consent or severe mentally disabled.
- Patients in the end-stage of a severe disease.

9.3.3 Discontinuation of patients from treatment or assessment

Patients are free to withdraw their participation any time following written informed consent. The data of these patients will be discarded and eliminated from the assessment (drop-out patients).

9.3.4 Patient replacement

Not applicable.

9.3.5 Premature discontinuation of the Post Marketing Study

The sponsor has the right to terminate the entire Post Marketing Study or parts thereof at any time.

The investigator has the right to terminate participation in the Post Marketing Study at any time.

A premature termination must be discussed between the involved parties before becoming effective.

There are no statistical criteria for clinical investigation termination.

9.3.6 Patient identification

Patients will be identified by their medical records of the investigational site.

With signing of informed consent, the patients will be assigned a specific identification number for the Post Marketing Study.

The investigator will maintain a patient identification log, which lists all patients enrolled in the Post Marketing Study, with an identification code linked to their names, alternative identification or contact information. This list will not be provided to the sponsor.

9.4 Identification and description of the *In vitro* Diagnostic Test

9.4.1 Identification, traceability and labelling of the *In vitro* Diagnostic Test

Table 9-1: DOAC urine test strip

Name:	DOAC Dipstick
Manufacturer:	DOASENSE GmbH, Heidelberg
Model/Type:	DOASENSE REF Number: 0001
Lot number:	DS 18021901
Expiration date:	2018 08
Intended use:	Urine dipstick to test for presence or absence of DOACs
Contraindications:	known intolerance to DOACs
CE certification:	CE marked DIMDI registration number: DE/CA 38/00141004
Packaging:	Container (12 test strips) including reference color chart

CE: Certification (Conformité Européene [European Conformity])
DOAC: Direct oral anticoagulant

The packaging and the instructions for use will indicate that the device is only intended for the Post Marketing Study according to this Study Plan.

The sponsor will supply the investigators and clinical investigation sites with the test, including all relevant documentation.

Used test strips will be discarded into the standard containers of the site for collection of tubes containing blood or urine samples. Unused test strips will be returned to the sponsor.

9.4.2 Emergency procedures

Not required.

9.4.3 Method of assigning patients to test arms

Two groups of patients will participate in the test, based on their DOAC therapy. The same test will be performed in each patient's urine sample.

9.4.4 Blinding

Not applicable.

9.4.5 Restrictions and precautions

The IVD has to be handled according to handling instructions. No special requirements or precautions (e.g., gloves) have to be taken.

Urine sample handling by the site personnel has to be performed according to standard procedures of the site.

9.5 Procedures and assessments related to the Post Marketing Study

All measurements are carried out according to standard medical methods of the investigational site. The personnel involved is adequately trained in required measurements.

All sites will receive the same model/type of reflectance photometric dipstick color reader (DOASENSE Reader).

9.5.1 Visit description

During a routine visit in the outpatient care unit of the site, patients who match the entry criteria (based on their medical records at the site) will be informed about the Post Marketing Study, and informed consent will be obtained.

The investigator will document the patient's demographic characteristics (Section 9.5.2) and record the time of last intake of DOAC.

Then, the patients will provide a sample of urine and give it to the respective site personnel. No further action is required by the patient.

The site personnel will put two aliquots of urine (5 mL) into provided test tubes for bioanalysis (Section 9.5.3.2) and prepare the sample for shipment according to the shipment instructions.

Then, they will perform the visual and reflectance photometric reading of the dipstick (Section 9.5.3.1).

Thereafter, they will complete the questionnaire.

Case report forms (CRFs) and completed questionnaires will be sent to:

Prof. Dr. Christel Weiß, Dipl Math
Medical Statistics, Biomathematics, and Information Processing
Medical Faculty Mannheim of the University of Heidelberg
Ludolf-Krehl-Straße 13-17
68167 Mannheim, Germany

9.5.2 Population characteristics

The following data will be recorded for each patient in a CRF:

- Age (years)
- Sex
- Body weight (kg)
- Body height (cm)[1]
- Medical and surgical history relevant to DOAC treatment
- Medication history – DOAC (type, start of treatment, last intake before providing urine sample)
- Other medication history relevant to DOAC therapy

Data will be taken from the patient's medical records.

9.5.3 Performance endpoints

Patients will collect a sample of urine into the container usually used at the site and hand it over to the site personnel.

The site personnel will put two aliquots of 5 mL into the test tubes provided for bioanalytical measurements (see Section 9.5.3.2), and perform direct urine tests as described in Section 9.5.3.1. After completion of the test, the remaining urine is discarded.

At start of study, and after every 12th examination (one per test tube) of patient urine samples, the visual reading and reflectance photometric reading procedures described below will be performed on one negative control (0 ng/mL) and one positive control (800 ng/mL) for each group of inhibitors: Dabigatran (Thrombin inhibitor) and Rivaroxaban (Factor Xa inhibitor).

[1] Body mass index deleted, as not required.

9.5.3.1 Urine dipstick test – visual and reflectance photometric color reading

Please note: Site personnel with known red-green blindness/ weakness cannot perform the test.

The site personnel will perform the urine test as follows:

The test strip is dipped into urine or into control samples for 2 to 3 seconds, so all test pads are wet with urine. Then, the test strip is placed on a flat surface (room temperature, at light) with the pads facing upwards (so color changes can be seen). After 10 Minutes the site personnel will compare the results visually with the reference scale on the dipstick container. Then, the dipstick will be placed into the reflectance photometric reader according to the handling instructions.

The results will be documented in the CRF by the study personnel and the printout of the reflectance photometric color reader (DOASENSE Reader) will be copied and fixed with an adhesive into the CRF.

After 10 minutes, the reactions on the pads are completed:

Test Pad 1: (Creatinine)

- The color of pad 1 corresponds to colors "**norm**." on the tube label → creatinine in urine is normal. Pad 3 and pad 4 can be evaluated.
- The color of pad 1 is darker than the colors "**norm.**" on the tube label → high creatinine does not affect DOAC excretion into urine.
  Pad 3 and pad 4 can be evaluated.
- The color of pad 1 is **"low"** or lighter than the respective color of the tube label → creatinine in urine is low, indicating renal insufficiency.
  Colors of pad 3 and pad 4 may be false negative.

Test Pad 2: (Urine color)

- The color of the pad is white as the respective color marked "**norm"** on the tube label → the results of pad 1, 3 and 4 are valid.
- The color of the pad is darker than the color printed on the tube label → colors of pad 1, pad 3 and pad 4 may be distorted. The test is invalid.

Test Pad 3 (Medication Apixaban, Edoxaban, Rivaroxaban):

- The color of pad 3 clearly is yellow as the respective color marked "**neg**." on the tube label → direct oral Factor Xa inhibitor is absent in the urine sample.
- The color of pad 3 is less yellow than the color marked "**neg**." on the tube label, thus the results is **"pos."** → direct oral Factor Xa inhibitor is present in urine.
- The color of pad 3 is white as the respective color marked "**pos**." on the tube label → direct oral Factor Xa inhibitor is present in urine.

Test Pad 4 (Medication Dabigatran):

- The color of pad 4 is ochre as the respective color marked "**neg**." on the tube label → direct oral Thrombin inhibitor is absent in the urine sample.
- The color of pad 4 is between the ochre color marked "**neg.**" and the rose color marked "**pos**." on the tube label → direct oral Thrombin inhibitor is present in urine.
- The color of pad 4 is rose as the respective rose color marked "**pos.**" on the tube label → direct oral Thrombin inhibitor is present in urine.

If pad 3 and pad 4 are both "pos.", the test is invalid,
because it is unlikely that a person is treated with both types of DOACs.

The study personnel will document the results in the CRF and the printout of the reflectance photometric color reader (DOASENSE Reader) will be copied and fixed with an adhesive into the CRF.

After all readings are completed and documented, the used test strip will be discarded.

9.5.3.2 Bioanalysis of the urine samples

The urine aliquots will be stored in a deep freezer at about -20°C[2] at the study center. The analytical laboratory will collect one aliquot, and one sample will be sent to CRS for storage.

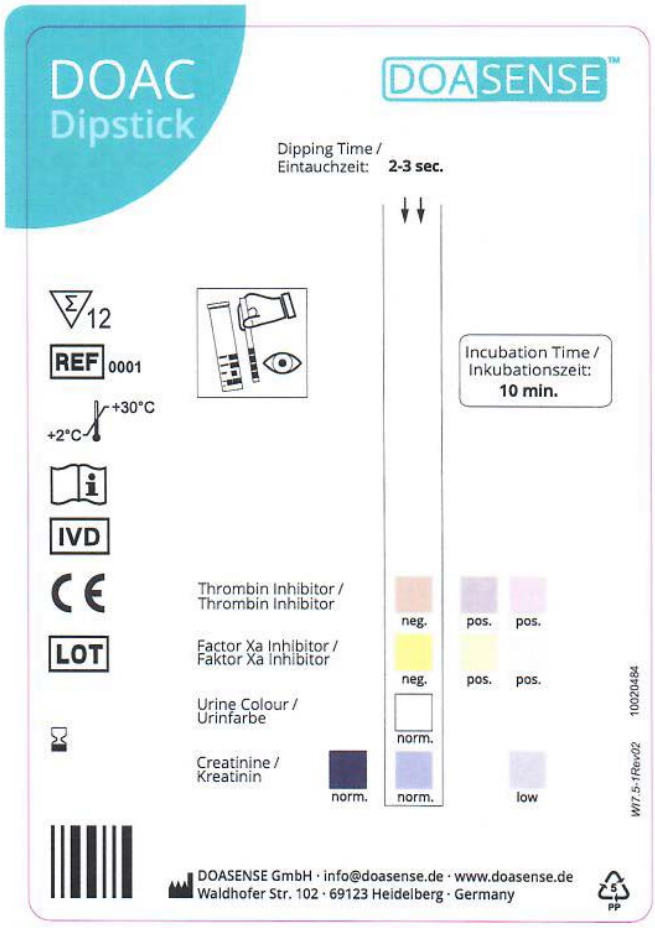


Figure 9-1: Color chart label of pads 1, 2, 3, and 4 of the DOAC Dipstick

9.5.4 Safety endpoints

There will be no intervention; therefore, no safety endpoints will be measured.

[2] Storage of urine aliquots was changed to deep freezer at -20 C.

9.5.5 Anticipated adverse device effects

The patients who participate in this investigation will come into contact only with their own urine. They will not come in contact with any of the study materials. No additional study-related investigation will be performed for the study. Therefore, no study-related adverse events will occur.

9.6 Statistical methods and determination of sample size

9.6.1 Statistical and analytical plans

For each diagnostic test the proportions of false negative and false positive results will be assessed together with confidence intervals. The urine concentration serves as a gold standard. Furthermore, McNemar tests will be conducted in order to compare the sensitivity, the specificity, accuracy, negative predictive value, positive predictive value and likelihood probability of the two different medications. Kappa coefficients will be calculated in order to quantify the strength of agreement between two diagnostic test methods.

As the study design is not randomized the two groups will be compared according to biographic data (i.e. age, gender, concentration in urine) by common statistical tests (Chi^2 test, t-test) in order to investigate their equality. In the case of differences between groups statistical adjustment will be done (i.e. propensity score) in order to avoid the influence of a bias.

9.6.2 Sample size determination

Two groups of medications (Thrombin inhibitor, Factor Xa inhibitors) will be tested with the IVD and test results compared to bioanalytical results in urine.

The objective of the investigation is to show that the proportion of false negative and false positive tests with the IVD is below 5%.

The required sample size to show that the assumed rate of 2.5% false-negative/false-positive tests is statistically significant lower than 5% would require 384 patients per each test group, with α=0.05 and β=0.20 (80% power).

Accounting for a potential drop-out rate of 12%, a sample size of n=440 patients per test group was considered adequate to demonstrate adequate performance of the IVD. This sample size has been assessed with the SAS procedure PROC POWER (SAS Institute Inc., Cary, NC, USA, release 9.3) using the ONESAMPLEFREQ statement under the assumption that the test will be conducted as a 1-sided test with a null proportion of 0.05.

10 Data collection

10.1 Case Report Form and Questionnaire

The sponsor will provide individual CRFs to the investigator, together with instructions for completion.

The investigator is responsible to complete the CRF.

The investigator will maintain a list of personnel authorized to make entries into the CRF. These have to be adequately trained in the procedures of the investigation.

10.2 Document and data control

All documents related to the Post Marketing Study will be identifiable, traceable, and appropriately stored.

The investigator ensures that all data entries on the CRFs and other documentation are accurate, legible, complete, and entries are made in a timely manner. Copies of source documents or printouts of electronic source documents will be signed and date by a member of the investigation team.

Any corrections in the CRF have to be clearly signed and dated by authorized site personnel. In case of non-obvious corrections, the reason for correction has to be explained. The original entry has to remain legible and may not be obscured by the correction.

10.3 Source data

CRFs, informed consent forms and patient's medical records are source data.

- CRF
- Signed informed consent
- DOASENSE Reader printouts (signed and dated copies, as the originals are thermographic paper)
- Questionnaire on IVD handling

10.4 Data management

Data management procedures will be described in a separate document.

10.5 Document retention

The sponsor will archive the sponsor's file, and all relevant documentation for at least 10 years after end of the study, or longer if regulatory or legally required.

The investigator will archive informed consent form and original CRFs for at least 10 years after end of the study.

11 Quality assurance and quality control, audits, and inspections

11.1 Quality control and quality assurance

The sponsor is responsible for implementing and maintaining quality assurance and quality control systems with written standard operating procedures (SOPs) to ensure that the Post Marketing Study is conducted, data are generated, documented (recorded), and reported in compliance with this Study Plan and the applicable regulatory and legal requirements.

The sponsor is responsible for securing agreement from all involved parties to ensure direct access to all Post Marketing Study-related sites, source data/documents, and reports for the purpose of monitoring and auditing by the sponsor, and inspection by domestic and foreign regulatory authorities.

Quality control should be applied to each stage of data handling to ensure that all data are reliable and have been processed correctly.

Agreements, made by the sponsor with the investigator/institution and any other parties involved with the Post Marketing Study, should be in writing, as part of the protocol or in a separate agreement.

11.2 Monitoring

The sponsor will appoint a monitor, who will verify that the conduct of the Post Marketing Study complies with this Study Plan and the applicable regulatory and legal requirements.

The planned monitoring activities will be outlined in a monitoring plan, and reported in monitoring reports.

11.3 Audit

The investigator) shall allow representatives from Quality Assurance of DOASENSE to have access to its study relevant documentation and raw data.

An audit is defined as an inspection and assessment on the general organization of the Study Monitor system, as well as study related information.

Activities related to the Post Marketing Study and documents may be audited to determine if performance and assessment of the Post Marketing Study were compliant with the Post Marketing Study Plan and applicable regulatory and legal requirements. Auditors who are independent from the Post Marketing Study and its conduct will perform audits.

The investigator will permit the auditors access to the facilities and documents at agreed times.

11.4 Inspection

Regulatory authorities may conduct an official review of all Post Marketing Study documents, facilities, records and other material related to the clinical investigation / Post Marketing Study.

The investigator must cooperate with any inspection.

The patients will agree with signing the informed consent to the access of their data by authorities.

12 Confidentiality of patient data

All information collected during the course of this investigation is kept strictly confidential.

The patients participating in the study are identified by their PID. The monitor has limited access to the patient's medical records for source data verification.

Any information, which could identify a patient, remains with the investigator where it is archived with investigation documents.

Patients will remain anonymous for the purposes of data analysis.

Should the investigation require future review, relevant regulatory authorities and ECs will be allowed access to all relevant information for audit and inspection purposes.

13 Post Marketing Study Report

Upon completion of the Post Marketing Study, the sponsor will write a clinical report, which includes a critical assessment of all data obtained in the investigation.

The Post Marketing Study report will be approved and signed by the sponsor and the lead investigator.

14 Publication policy

In case the sponsor intends a publication of the study results, the investigator and the sponsor's representative(s) prior to publication thereon shall discuss manuscripts. Regard shall be given to the sponsor's legitimate interests, e.g., containing optimal patent protection, coordination of submissions to health authorities or with other ongoing studies in the same therapeutic field, protection of confidential data, and information, etc.

The sponsor's comments shall be given within 30 days after receipt of the publication draft. If there is no consensus, the senior author of the manuscript and the sponsor's representative(s) shall further discuss and mutually agree on the final wording or disposition of the publication.

The above-described procedure also applies to information on prematurely discontinued and other non-completed studies.

Results from investigations shall not be made available to any third parties by the investigating team outside the publication procedure as set out above.

The sponsor will not quote from publications by investigators in its scientific information and/or promotional material without full acknowledgement of the source (i.e., author and reference).

15 Insurance

No special insurance will be taken out. The patients will provide a urine sample during a routine visit at the study center.

16 Responsibilities and finances

Responsibilities and finances will be specified in separate contracts.

17 References

[1] Harenberg J, Krämer S, Du S, Weiss C, Krämer R.: Concept of a point of care test to detect new oral anticoagulants in urine samples. Thrombosis Journal. 2013 Aug 1; 11: 15 PMC [article] PMCID: PMC3766639, PMID: 23915217, DOI: 10.1186/1477-9560-11-15.

[2] Harenberg J, Du S, Wehling M, Zolfaghari S, Weiss C, Krämer R, et al.: Measurement of dabigatran, rivaroxaban and apixaban in samples of plasma, serum and urine, under real life conditions. An international study. Clin Chem Lab Med. 2016 Feb;54(2):275-83. doi: 10.1515/cclm-2015-0389. PubMed [citation] PMID: 26167981.

18 Amendments

Not applicable.

19 Appendix

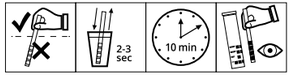
Handling instructions for the DOAC dipstick test/Package insert.

DOAC Dipstick

CE IVD REF 0001 Σ 12

DIAGNOSTIC DOAC IN URINE DIPSTICK TEST

EN



Intended Use:
The diagnostic test strip DOAC Dipstick is intended for qualitative detection of the absence or presence of direct oral anticoagulants (DOAC in human urine by visual identification of colours. The DOAC Dipstick is an in vitro diagnostics test intended for professional use only.

Summary and Explanation
The clinical importance of positive results of a DOAC in human urine relates to the presence of DOAC in blood. DOACs are excreted rapidly into urine starting 1 to 2 hours after intake of the medication. A specific and rapid detection indication by a point of care test may support diagnosis of anticoagulant therapy with DOACs especially in emergency medicine. Medical decision-making may be accelerated. Typical indications are patients with ischemic or haemorrhagic stroke with indication for fibrinolytic therapy or administration of a specific antidote, major trauma, emergency procedures, spontaneous thrombotic and bleeding events during oral anticoagulant therapy, and situations without available medication history.

Material Provided with the Test

Test strip:	12
Test strip tube container with printed colour scale and cap:	1
Instructions for Use:	1

Materials Required but Not Provided
Clean container made of Polypropylene for sample collection.
Timer.

Principle of the DOAC Test Strip
The test consists of a change of colour upon reaction of Factor Xa or Thrombin with a Factor Xa or Thrombin chromogenic substrate in relation to the amount of DOAC present in the urine sample. Colours for oral direct Factor Xa and Thrombin inhibitors are different. The colour of the pads on the test strips changes within 10 min and can be identified by naked eye. The colours allow the detection of DOAC in a urine sample, with interpretation as "negative" in the absence of a DOAC and as "positive" in the presence of a DOAC. Respective colours for comparison are printed on the test tube containing the test strips.

The DOAC Dipstick has four different test pads used for analysis, as follows:

Schematic of the DOAC Dipstick:

Pad 1 for determination of Creatinine
Pad 2 for assessing the colour of urine (without reagents)
Pad 3 for determination of oral direct Factor Xa inhibitor
Pad 4 for determination of oral direct Thrombin inhibitor

Principle of Tests
Creatinine – The test is based on reaction of creatinine with 3.5-dinitrobenzoic acid in alkaline medium (Benedict-Behre reaction).
Factor Xa Inhibitors – The test is based on the release of a chromophore from a Factor Xa specific peptide by Factor Xa and the inhibition by an oral direct Factor Xa inhibitor.
Thrombin Inhibitors – The test is based on the release of a chromophore from a Thrombin specific peptide by Thrombin and the inhibition by an oral direct Thrombin inhibitor.
Urine Colour – This pad does not contain any reagents and is used for assessing the impact of the colour of the patient urine used.

Warning and Precautions
Do not use expired devices.
Do not reuse the test components.
Follow good laboratory practice and safety guidelines. Wear lab coats, disposable latex gloves and protective glasses where necessary. Prepared or used test strips have to be treated as hazardous waste according to national biohazard and safety guidelines or regulations. All reagents of this kit have been found to be uninfectious. However, a presence of used materials with human urine cannot exclude infectious agents. For this reason used test strips should be treated as potential biohazards in use and for disposal.

10020484

If Contamination of clothing occurs: Rinse skin with water or shower.
Avoid contact with skin and eye. If skin irritation occurs: Consult a physician in all serious cases of health damage.
In case of an accidental ingestion **wash up the mouth and drink approximately 0.5**
l of water. In case of eye contact rinse the eye quickly and thoroughly using a stream of clean water. **Procedure Notes Before Performing the Test**
Do not use test strips with an expiry date that has already passed. Carefully read the instructions for use before starting the test.
The instructions must be followed exactly to obtain accurate results.
This test is for professional in vitro diagnostic use only.
Do not touch test pads of the strip. Handle the test strips only at the handling area opposite to the area with the test pads.
Do not open the tube containing the test strips unless you are ready to conduct the test.
Remove only as many test strips as required and reseal the tube immediately with the cap. The cap contains a desiccant.

Collection of Urine Sample:
1. Each urine sample must be collected in a clean container made of Polypropylene. Only freshly collected urine may be used.
2. Shake the container smoothly before dipping the test strip.
3. Use urine immediately after collection.

Assay Procedure - Use of test strips:
1. Immerse the test strip for 2 to 3 seconds into the urine sample so that all test pads are completely covered by the urine.
2. After removing the test strip from the urine, some liquid may be attached at the borders or edges of the test strips. Whip off the excess urine on a filter paper or on a cellulose or cotton wool surface to absorb runoff. The test pads should not be touched.
3. Place the test strip on a flat surface, so that you can see the test pads, and wait for 10 minutes (incubation time of tests). Use a timer to control the time.
4. After 10 minutes incubation time immediately compare the test pads by naked eye to the corresponding colour scales on the label of the tube container. Refer to the next section regarding the visual determination of the colours.

Visual Determination of Colours:
Colours are compared to colours of a colour scale, which is printed on the label of the container tube containing the test strips by naked eye.
Start by comparing the colour of test pad 2 ("Urine Colour") to the respective colour area on the label.

Test Pad 2 Interpretation:
If the colour of pad 2 is different to the colour printed on the tube, the tests of pad 1, pad 3 and pad 4 are invalid, as their respective colours may be falsified by the impact of excess urine colour. Thereafter, identify the colour of test pad 1 and compare to the colour of pad 1 on the label of the tube or the package insert.

Test Pad 1 Interpretation:
If the colour of pad 1 indicates a "high" creatinine value, the test results of pad 3 and pad 4 are invalid.

Then identify the colour of test pad 3 or of test pad 4 depending on the type of DOAC the patient is treated with. If therapy with a specific DOAC is unknown, both pad 3 and pad 4 should be identified by naked eye. Compare the colour of pad 3 and or pad 4 with the colour scales printed on the tube.

All colours different from the colour "negative" ("neg.") as shown on the tube label are "positive" ("pos.").

Test Pad 3 Interpretation (medication apixaban, rivaroxaban):

- If the colour of pad 3 clearly is as yellow as the respective colour marked "neg." on the label colour scale, the result is negative, indicating that no Factor Xa Inhibitor anticoagulant is present in the urine sample.
- If the colour of pad 3 is not clearly as yellow as the respective colour marked "neg." on the label and more similar to the yellow colour marked "pos." on the label colour scale, the result is positive, indicating that the Factor Xa Inhibitor anticoagulant is present in urine.
- If the colour of pad 3 clearly is white or tends to be similar to the respective white colour marked "pos." on the label colour scale, the result is positive, indicating that the Factor Xa Inhibitor anticoagulant is present in urine.

Test Pad 4 Interpretation (medication dabigatran):

- If the colour of pad 4 is ochre as the respective colour marked "neg." on the colour scale on the tube, no Thrombin Inhibitor anticoagulant is present in urine.
- If the colour of pad 4 is not ochre as the respective colour marked "neg." on the colour scale of the tube, the result is "pos." as shown on the label colour scale, indicating that the Thrombin Inhibitor anticoagulant is present in urine.
- If the colour of pad 4 is purple similar to the respective purple colour marked "pos." on the label colour scale, the Thrombin Inhibitor anticoagulant is present in urine.

If both pad 3 and pad 4 are "pos.", then the test is invalid.

Limitations
The test results are qualitative. No quantitative interpretation should be made based on the test results.
The results always have to be interpreted and evaluated in connection with other clinical information by a physician before diagnosing. No treatment decisions should be made solely on the basis of the outcomes of a DOAC Dipstick analysis. Additional laboratory analysis (e.g. determination of blood coagulation parameters) may be required.
Persons with colour vision deficiency or colour blindness may not perform the DOAC Dipstick test.
Expected Values and Reference Ranges
Creatinine – Reference Range: 0.25 – 3.0 g/l, (2.2 – 26.5 mmol/l) (Ref.: Needleman).
Factor Xa Inhibitors – Normal values in urine are below 5ng/ml (LC-MS/MS method). Patients under DOAC treatment typically display values above 150ng/ml (see paragraph performance characteristics).
Thrombin Inhibitors – Normal values are below 5ng/ml (LC-MS/MS method). Patients under DOAC treatment typically display values above 150ng/ml (see paragraph performance characteristics) (Ref.: Schreiner).

Performance Characteristics
The cut off values for rivaroxaban and dabigatran by visual analysis using identification of colours as "negative" and "positive" were 100 ng/ml in normal human urine spiked with concentrations ranging from 0 to 1500 ng/ml. Sensitivity and specificity of detection was 0.92 and 0.93 (rivaroxaban) and 0.90 and 0.93 (dabigatran). Using urine samples of patients treated with dabigatran, rivaroxaban and apixaban, and of control patients not treated with DOACs, sensitivities and specificities were all 1.0 (Ref.: Harenberg 2017).
Concentrations of DOACs in urine are higher due to the lower volume of urine compared to the volume of distribution of DOACs in blood and were below 5 ng/ml (LC-MS/MS method) in patients not treated with DOACs and above 150 ng/ml for all DOACs during steady state of treatment and 12 or 24 hours after intake of medication (mean values 5.600 ng/ml Dabigatran, 2.700 ng/ml Rivaroxaban, 1.800 ng/ml Apixaban, n=29 each) (Ref.: Schreiner).

Interferences
No information is reported in the literature on drug-drug and drug-other compounds interactions – except the coloured compounds in urine described herein. The number of interactions tends to be low to very low due to the high specificity of the enzymes with the respective substrates. No interaction occurs between the components of pad 3 with pad 4 and vice versa.
Heparins do not react on pads 3 and 4 (Harenberg 2017).
Coloured compounds in urine such as bilirubin, urobilinogen and blood (macrohaematuria) may modify the results of pad 1, pad 3 and pad 4. The impact of the colour of the urine sample can be assessed by pad 2 (see above for interpretation).
Creatinine – In urines with high buffering capacity false negative reading may be obtained. With high concentrations of ketone bodies (>50 mmol/l) false positive reading may occur. Blood >2000 Ery/µl may cause false positive results.

Storage Conditions
The recommended storage temperature of the closed test strip tube container is between +2 °C to +30 °C. Each device may be used until the expiration date printed on the label. After opening, the tube has to be closed tightly immediately after removing of the strips used for evaluation, and the closed tube has to be stored away from direct sunlight at tempe¬ratures not exceeding +30 °C. Storage above +30 °C will adversely affect the stability and test performance of the product.

References
Schreiner R et al. Determination of rivaroxaban, apixaban, edoxaban and dabigatran by ultra-performance liquid chromatography-tandem mass-spectrometry and chromogenic assays from urine samples of patients. J Thromb Haemostas 2017, Abstract, in press.
Harenberg J, et al. Reliability and validity of a point of care test from urine samples of patients on therapy with apixaban, rivaroxaban and dabigatran. J Thromb Haemostas 2017, Abstract, in press.
Harenberg J, et al Measurement of dabigatran, rivaroxaban and apixaban in samples of plasma, serum and urine, under real life conditions. An international study. Clin Chem Lab Med. 2016 Feb;54(2):275-283.
Du S, et al. Determination of dabigatran in plasma, serum, and urine samples: comparison of six methods. Clin Chem Lab Med. 2015 Jul;53(8):1237-1247.
Harenberg J, et al. Patients' serum and urine as easily accessible samples for the measurement of non-vitamin K antagonist oral anticoagulants. Semin Thromb Hemost. 2015 Mar;41(2):228-236.
Favaloro EJ, et al. Laboratory testing in the era of direct or non-vitamin K antagonist oral anticoagulants: a practical guide to measuring their activity and avoiding diagnostic errors. Semin Thromb Hemost. 2015 Mar;41(2):208-227.
Harenberg J, et al. Concept of a point of care test to detect new oral anticoagulants in urine samples. Thromb J. 2013 Aug 1;11(1):15.
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USED SYMBOLS

Date of Revision: 30.6.2017

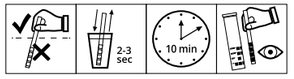
CE CE Mark - Device comply with the Directive 98/79/EC
IVD In vitro Diagnostics
Expiry date
LOT Lot number
Σ Content is sufficient for <n> tests
Manufacturer
REF Catalogue number
Storage temperature
Follow carefully instructions for use

DOASENSE™ GmbH, Waldhofer Str. 102, 69123 Heidelberg, Germany
Email: info@doasense.de, www.doasense.de

DOAC Dipstick

CE
IVD REF 0001 Σ 12

DIAGNOSTISCHER TESTSTREIFEN ZUR ANALYSE VON DOACS IN URIN



DE

Verwendungszweck
Der diagnostische Teststreifen DOAC Dipstick ist für die qualitative Bestimmung der Abwesenheit oder Anwesenheit direkter oraler Antikoagulantien (DOAK bzw. englisch DOAC) in menschlichem Urin durch visuelle Identifikation von Farben vorgesehen. Der DOAC Dipstick ist ein in-vitro diagnostischer Test nur für Fachgebrauch durch medizinisches Personal.

Zusammenfassung und Erläuterung
Die klinische Bedeutung eines positiven Ergebnisses eines DOAK in menschlichem Urin geht einher mit der Anwesenheit des DOAK in Blut. DOAK werden schnell in den Urin ausgeschieden, beginnend 1 bis 2 Stunden nach der Einnahme der Medikation. Eine spezifische und schnelle Erfassung durch einen patientennahen Point-of-Care-Test (POCT) könnte die Diagnostik der gerinnungshemmenden Therapie mit DOAK unterstützen, insbesondere in der Akutmedizin. Die medizinische Entscheidungsfindung könnte beschleunigt werden. Typische Indikationen zum Einsatz eines solchen Tests sind Patienten mit einem ischämischen oder hämorrhagischen Schlaganfall, bei einer Indikation zu einer fibrinolytischen Therapie eines thrombotischen Ereignisses unter DOAK, die Überprüfung der Notwendigkeit der Verabreichung eines Antidot für DOAK, bei schwerem Trauma, vor Notfalleingriffen, bei spontanen thrombotischen Ereignissen oder schweren Blutungen unter einer blutverdünnenden Therapie, oder in Situationen in denen eine Medikamentenanamnese nicht verfügbar ist.

Materialien eines Testkits

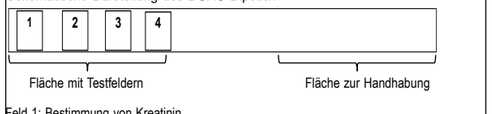
Teststreifen:	12
Behälter für die Teststäbchen, bedruckt mit der Farbskala und Verschluss mit Trockenmittel:	1
Gebrauchsanweisung:	1

Andere Materialien, die benötigt aber nicht enthalten sind:
Sauberer Behälter aus Polypropylen für die Sammlung von Urin.
Zeitmesser.

Prinzip des Teststreifen für DOAK
Der Test beruht auf einem Farbumschlag durch die Einwirkung von den Gerinnungsenzymen Faktor Xa und Thrombin auf die Freisetzung eines Farbstoffs, der an ein Substrat gebunden ist. Die Freisetzung des Substrats ist negativ proportional abhängig von der Menge an DOAK in Urin. Die Farben sind unterschiedlich für Faktor Xa Hemmer und Thrombin Hemmer der DOAK. Sie entwickeln sich über 10 Minuten auf den Testfeldern und können mit dem bloßen Auge identifiziert werden. Sie erlauben eine Feststellung der Ab- oder Anwesenheit eines DOAK in Urin und werden als “negativ” oder “positiv” bewertet. Entsprechende Farben sind auf dem Behälter der Teststreifen zum Vergleich für die Zuordnung zu “negativ” und “positiv” abgebildet.

Der Teststreifen “DOAC Dipstick” besteht aus vier verschiedenen Feldern:

Schematische Darstellung des DOAC Dipstick



Feld 1: Bestimmung von Kreatinin
Feld 2: Identifikation der Urinfarbe (keine Reagenzien)
Feld 3. Bestimmung direkter Faktor Xa Hemmer der DOAK
Feld 4: Bestimmung direkter Thrombin Hemmer der DOAK

Prinzip der Tests
Kreatinin – Der Test beruht auf einer Reaktion von Kreatinin mit 3.5-Dinitrobenzol Säure in alkalischem Medium (Benedict-Behre-Reaktion).
Faktor Xa Hemmer – Der Test beruht auf der Freisetzung eines Farbstoffs von einem Faktor Xa spezifischen Substrat durch das Enzym und einer Hemmung von Faktor Xa durch einen oralen Faktor Xa Hemmer.
Thrombin Hemmer – Der Test beruht auf einer Freisetzung eines Farbstoffs von einem Thrombin spezifischen Substrat durch das Enzym und einer Hemmung von Thrombin durch einen oralen Thrombin Hemmer.
Urinfarbe – Dieses Feld enthält keine Reagenzien und wird zur Feststellung der Farbe des Urins der Patienten benutzt.
Warnung und Vorsichtsmaßnahmen
Nicht benutzen nach Ablauf des Verfallsdatums auf dem Behälter.
Einen Teststreifen nicht mehrfach benutzen.
Die Richtlinien für Gute Laborpraxis beachten: Laborkittel, Einmalhandschuhe und Schutzbrillen tragen, wenn nötig.
Gebrauchsfertige oder benutzte Teststäbchen sollen als gefährlicher Abfall behandelt werden, entsprechend den nationalen Richtlinien für gefährliche Biostoffe und Sicherheitsrichtlinien.
Alle Reagenzien des Test sind nicht infektiös. In Gegenwart von Urin können infektiöse Agenzien nicht ausgeschlossen werden. Aus diesem Grund sollten die Teststäbchen wie gefährliche Biostoffe zur Nutzung und Entsorgung behandelt werden.

Bei einer Verunreinigung der Kleidung durch die Teststreifen bzw. Urin: Behandlung der Haut mit Wasser oder Dusche.
Kontakt mit Haut oder Auge vermeiden. Falls eine Hautirritation durch Berührung der Testfelder erfolgt: bei gesundheitlichen Schäden Hausarzt oder Facharzt aufsuchen. Bei Kontakt mit dem Auge dieses umgehend und ausgiebig mit sauberem Wasser spülen. Bei einem unbeabsichtigten Verschlucken den Mund ausgiebig mit Wasser spülen und etwa 0,5 l Wasser trinken.
Zur Beachtung vor Nutzung des Test
Teststreifen nicht nach Verfallsdatum nutzen.
Sorgfältig die Gebrauchsanweisung lesen.
Die Gebrauchsanweisung muss genau befolgt werden, um ein zuverlässiges Ergebnis zu erhalten.
Der Test ist nur für den Fachgebrauch durch medizinisches Personal als in-vitro Diagnostikum zu nutzen.
Die Testfelder des Teststreifens nicht berühren. Den Testreifen nur auf der zur Handhabung vorgesehenen Seite anfassen, die sich am an dem Ende des Teststreifens befindet, auf welchem keine Testfelder aufgebracht sind.
Den Behälter erst unmittelbar vor der Durchführung des Tests öffnen.
Nur so viele Teststreifen aus dem Behälter nehmen wie erforderlich. Den Behälter unmittelbar nach dem Entnehmen von Teststreifen wieder mit dem Deckel verschließen. Der Deckel enthält ein Trockenmittel.

Gewinnung der Urinprobe:
1. Jede Urinprobe muss in einem sauberen Behälter aus Polypropylen gesammelt werden. Nur frisch gesammelter Urin darf verwendet werden.
2. Den Behälter vor Eintauchen des Teststäbchens leicht schütteln.
3. Den Urin unmittelbar nach dem Sammeln für den Test verwenden.

Ablauf des Tests – Benutzung der Teststreifen:
1. Den Teststreifen 2 bis 3 Sekunden in den Urin eintauchen, so dass alle Testfelder mit Urin bedeckt sind.
2. Nach Herausnehmen des Testreifens aus dem Urin Reste des Urin an der Unterseite und den Rändern des Teststre ifens vorsichtig über ein aufsaugendes Papier streifen. Die Fläche mit den Testfeldern darf nicht berührt werden.
3. Den Teststreifen für 10 Minuten mit der Rückseite auf eine glatte Oberfläche legen. Die Zeitdauer mit einem Zeitmesser prüfen.
4. Nach 10 Minuten umgehend die Farben der Testfelder mit dem Auge beurteilen und mit den korrespondierenden Farben auf dem Etikett des Behälters vergleichen. Zur Interpretation der Farben siehe den nächsten Abschnitt.

Visuelle Bestimmung der Farben:
Die Farben der Testfelder werden mit der Farbskala auf dem Etikett des Behälters verglichen.
Beginnen Sie indem Sie die Farbe von Feld 2 des Teststreifens mit der Farbe „Urinfarbe“ auf dem Etikett des Behälters vergleichen.

Feld 2 Interpretation:
Falls die Farbe von Feld 2 eindeutig anders ist als die Farbe des entsprechenden Felds auf dem Etikett des Behälters, so ist der Test ungültig, da die Farben der übrigen Testfelder durch die Urinfarbe verfälscht werden könnten. Der Test ist nicht verwertbar.
Anschließend wird die Farbe von Feld 1 des Testreifens abgelesen und mit den Farben von Feld 1 auf dem Etikett des Behälters verglichen.

Feld 1 Interpretation:
Wenn die Farbe von Feld 1 der Farbe „high“ auf dem Etikett des Behälters entspricht, dürfen Feld 3 und Feld 4 nicht interpretiert werden. Der Test ist dann ungültig, weil ein erhöhter Wert von Kreatinin falsche Werte für Feld 3 und Feld 4 ergeben kann. Der Test ist nicht verwertbar.

Danach werden die Farben von Feld 3 bzw. Feld 4 in der Farbe identifiziert, welche abhängig von dem DOAK das der Patient einnimmt, reagieren. Ist die Einnahme des spezifischen DOAK nicht bekannt, sollen beide Testfelder ausgewertet werden. Die Farben von Feld 3 und Feld 4 werden mit den Farben der Farbskala auf dem Etikett des Behälters verglichen.

Alle Farben, die nicht eindeutig der Farbe „negativ“ („neg.“) der Farbskala auf dem Etikett des Behälters entsprechen, sind als „positiv“ („pos.“) zu interpretieren.

Feld 3 Interpretation (Behandlung mit Apixaban, Rivaroxaban):
• Wenn die Farbe auf Feld 3 eindeutig gelb ist wie die Farbe „neg.“ der entsprechenden Farbskala auf dem Etikett des Behälters, dann ist das Ergebnis der Untersuchung negativ und bedeutet, dass sich kein oraler Faktor Xa Hemmer im Urin befindet.
• Wenn die Farbe auf Feld 3 nicht eindeutig so gelb ist wie die Farbe „neg.“ der entsprechenden Farbskala auf dem Etikett des Behälters, dann ist das Ergebnis der Untersuchung „pos.“ (siehe Etikett des Behälters), d. h. positiv. Dies bedeutet, dass sich ein oraler Faktor Xa Hemmer im Urin befindet.
• Wenn die Farbe auf Feld 3 eindeutig weiß ist wie die Farbe „pos.“ der entsprechenden Farbskala auf dem Etikett des Behälters, dann ist das Ergebnis der Untersuchung positiv und bedeutet, dass sich ein oraler Faktor Xa Hemmer im Urin befindet.

Feld 4 Interpretation (Behandlung mit Dabigatran):
• Wenn die Farbe auf Feld 4 eindeutig ocker ist wie die Farbe „neg.“ der entsprechenden Farbskala auf dem Etikett des Behälters, dann ist das Ergebnis der Untersuchung negativ und bedeutet, dass sich kein oraler Thrombin Hemmer im Urin befindet.
• Wenn die Farbe auf Feld 4 nicht eindeutig so ocker ist wie die Farbe „neg.“ der entsprechenden Farbskala auf dem Etikett des Behälters, dann ist das Ergebnis der Untersuchung „pos.“ (siehe Etikett des Behälters), d. h. positiv. Dies bedeutet, dass sich ein oraler Thrombin Hemmer im Urin befindet.
• Wenn die Farbe auf Feld 4 eindeutig rosa ist wie die Farbe „pos.“ der entsprechenden Farbskala auf dem Etikett des Behälters, dann ist das Ergebnis der Untersuchung positiv und bedeutet, dass sich ein oraler Thrombin Hemmer sich im Urin befindet.

Wenn Feld 3 und Feld 4 beide das Ergebnis “pos.” anzeigen, ist der Test ungültig und nicht verwertbar.

Limitierungen
Es handelt sich um qualitative Testergebnisse. Eine Interpretation zu quantitativen Werten darf aus den Ergebnissen nicht abgeleitet werden.
Die Ergebnisse dürfen nur in Zusammenhang mit den klinischen Informationen zu dem Patienten interpretiert werden. Eine Therapieentscheidung darf nur von einem Arzt und unter Hinzuziehung der klinischen Informationen zu dem Patienten vorgenommen werden. Eine Therapieentscheidung ausschließlich auf Basis des Ergebnisses des DOAC Dipstick Tests ist nicht zulässig. Zusätzliche klinische, laborchemische oder gerinnungsanalytische Untersuchungen können erforderlich sein.
Personen mit einer Farbsehschwäche oder Farbenblindheit dürfen den DOAC Dipstick Test nicht durchführen.

Erwartete Werte und Referenzbereiche
Kreatinin – Referenzbereich: 0,25 – 3,0 g/l, (2,2 – 26,5 mmol/l) (Ref.: Needleman).
Faktor Xa Hemmer – Normale Werte in Urin sind kleiner als 5ng/ml (LC-MS/MS Methode). Patienten unter einer Behandlung mit DOAK zeigen typischerweise Werte größer als 150ng/ml (siehe Paragraph Leistungsnachweis).
Thrombin Hemmer – Normale Werte liegen unter 5ng/ml (LC-MS/MS Methode). Patienten unter einer Behandlung mit DOAK zeigen typischerweise Werte größer als 150ng/ml (siehe Paragraph Leistungsnachweis) (Ref.: Schreiner).

Leistungsnachweis
Die Cut-off Werte von „negativ“ („neg.“) und „positiv“ („pos.“) für Rivaroxaban und Dabigatran liegen bei 100 ng/ml, gemessen anhand von menschlichen Urinproben, die mit 0 ng/ml bis 1500 ng/ml Rivaroxaban und Dabigatran versetzt worden sind. Die Sensitivität und Spezifität an dieser Umschlagsgrenze der Konzentration beträgt 0,92 und 0,93 für Rivaroxaban sowie 0,90 und 0,93 für Dabigatran. Bei Urinproben von Patienten unter Behandlung mit Apixaban, Rivaroxaban oder Dabigatran liegen die Sensitivität und Spezifität des Nachweises für alle DOAK bei 1,0 im Vergleich zu Urin von Personen ohne Behandlung mit DOAK (Ref.: Harenberg 2017).
Die Konzentrationen von DOAK in Urin sind höher als in Blut oder Plasma wegen des geringeren Verteilungsvolumens in Urin. Die Konzentrationen von DOAK in Urin und Blut sind bei nicht mit DOAK behandelten Personen gleich niedrig (kleiner 5 ng/ml (LC-MS/MS Methode). Unter DOAK Therapie betragen die Konzentrationen in Urin 12 bis 24 Stunden nach Einnahme mindestens 150 ng/ml und im Mittel etwa 5.600 ng/ml für Dabigatran, 2.700 ng/ml für Rivaroxaban, 1.800 ng/ml für Apixaban (n=29 oder 30 je Gruppe) (Ref.: Schreiner).

Wechselwirkungen
In der Literatur gibt es keine Informationen zu medikamentösen oder anderen Wechselwirkungen für den Nachweis in Urin – außer den genannten Wechselwirkungen der Farbsubstanzen. Eine Wechselwirkung von oralen Faktor Xa Hemmern mit Feld 4 und von oralen Thrombin Hemmern mit Feld 3 besteht nicht (Harenberg 2017).
Heparine reagieren nicht mit Feld 3 und Feld 4 (Harenberg 2017).
Farbige Substanzen im Urin wie Bilirubin, Urobilinogen und Blut (Makrohaematurie) können die Farben von Feld 1, Feld 3 und Feld 4 beeinflussen. Eine nicht-normale Urinfarbe kann durch Feld 2 beurteilt werden (siehe Interpretation der Ergebnisse).
Kreatinin – Urin mit einer hohen Kapazität an puffernder Lösung wie Ketone (>50 mmol/l) können hohe Konzentrationen vortäuschen. Blut >2000 Ery/µl kann die Farbe des Testfeldes beeinflussen.

Lagerung
Die empfohlene Temperatur für die Lagerung der Teststreifen in den Behältern beträgt +2 °C bis +30 °C. Die Teststreifen müssen innerhalb des Haltbarkeitsdatums genutzt werden, welches auf dem Behälter angegeben ist. Nach dem Öffnen des Behälters muss er mit der Verschlusskappe gleich nach Entnahme der Teststreifen wieder verschlossen werden. Die Aufbewahrung des Behälters erfolgt unter Vermeidung von direkter Sonneneinstrahlung bei Temperaturen 30° Celsius nicht überschreiten. Temperaturen über 30° Celsius können die Stabilität und Funktionsfähigkeit des Teststreifens beeinträchtigen.

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SYMBOLE

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CE CE Markierung – Produkt entspricht den Vorgaben der Richtlinie 98/79/EC
IVD In vitro Diagnostikum
Verfallsdatum
LOT Chargennummer
Σ Inhalt ausreichend für <n> Tests
Hersteller
REF Katalognummer (Bestellnummer)
Lagertemperatur
Gebrauchsanweisung sorgfältig befolgen

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