STATISTICAL ANALYSIS PLAN

Study Title:	Post Marketing Study of an *in vitro* diagnostic test for direct oral anticoagulants (Apixaban, Edoxaban, Rivaroxaban, Dabigatran) in urine
Sponsor Identification:	DOASENSE GmbH Waldhofer Strasse 102 69123 Heidelberg, Germany
Sponsor Study Number:	DOA-CS-002
HLZ Medizinische Statistik Study Number:	1-2019
Responsible Biostatistician:	Professor Dr. Christel Weiß Medizinische Fakultät Mannheim der Universität Heidelberg Heinrich-Lanz-Zentrum Abteilung für Medizinische Statistik und Biomathematik Theodor-Kutzer-Ufer 1-3 68167 Mannheim Tel.: +49(0)621/383-9901/9903 Fax: +49(0)621/383-9909 E-Mail: christel.weiss@medma.uni-heidelberg.de
Date of SAP:	15 May 2019
Version:	3.0
Scope:	Final analysis

SIGNATURE PAGE

By our signature below, we declare that we have reviewed this statistical analysis plan and that we agree with the planned statistical analysis.

Author:

Dr. Svetlana Hetjens
Statistician
HLZ Medizinische Statisik

27.05.2019 — Date
S Hetjens — Signature

Responsible Biostatistician:

Prof. Dr. Christel Weiß
Director Biometrics
HLZ Medizinische Statisik

22.5.2019 — Date
Ch. Weiß — Signature

Sponsor's Representative:

Prof. Dr. Job Harenberg
Managing Director
DOASENSE GmbH

15.05.2019 — Date
J. Harenberg — Signature

Co-ordinating Investigator:

Simone Mangold
MB/R&D Support Consultant
DOASENSE GmbH

22.05.2019 — Date
Simone Mangold — Signature

TABLE OF CONTENTS

1. DOCUMENT HISTORY

Version	Date	Change to previous version
1.0	2019-03-19	No previous version
2.0	2019-05-08	Final version

2. LIST OF ABBREVIATIONS

ACT	activated clotting time
aPTT	Activated partial thromboplastin time
CE	Certification
CI	Confidence interval
CRF	Case report form
DOAC	Direct oral anticoagulants
FPFV	first patient signing informed consent
ICH	International conference on harmonisation
ITT	Intent-to-treat
IVD	In vitro diagnostic test
LC-MS/MS	Liquid chromatography coupled with tandem mass spectrometry
POC	point of care
PPS	Per protocol set
PT	Prothrombin time
SAP	Statistical analysis plan
SAW-CT	Surface Acoustic Waves
SD	Standard deviation

3. RESPONSIBILITIES

The following persons will perform the statistical analysis (including SAS®-programming), the biometrical interpretation, the biometrical reporting and the internal quality control:

Responsible Biostatistician / project Biostatistician

Name: Prof. Dr. Christel Weiß
Title: Director Biometrics

Additional Biostatistician

Name: Dr. Svetlana Hetjens

Title: Statistician

Responsible SAS®-programmer

Name: Prof. Dr. Christel Weiß

Title: Director Biometrics

Additional SAS®-programmer

Name: Dr. Svetlana Hetjens

Title: Statistician

Internal QC of statistical analysis / biometrical reporting

Name: Michael Hagmann

Title: Statistician

4. STEERING COMMITTEE

Name, City	**Function**
Douxfils, Jonathan, Namur, Belgium	Laboratory expert
Crowther, Marc, Hamilton, Canada	Clinical expert
Beyer-Westendorf, Jan, Dresden	Clinical expert
Verhamme, Peter, Leuven, Belgium	Clinical expert
Elalamy, Ismail, Paris	Laboratory expert
Bauersachs, Rupert, Darmstadt, Germany	Clinical expert
Harenberg, Job, Heidelberg	Principal investigator

5. INTRODUCTION

Direct oral anticoagulants (DOACs) such as dabigatran, rivaroxaban, apixaban, and edoxaban are approved in many countries for prevention of ischemic stroke in patients with atrial fibrillation, prophylaxis of postoperative venous thromboembolism following elective knee or hip replacement surgery, treatment of acute venous thromboembolism, prolonged prophylaxis to prevent recurrent thromboembolic events and even management of acute coronary syndrome (i).

For all indications, DOACs can be administered in fixed doses, without the need for routine laboratory-guided dose adjustment. Consequently, routine plasma samples were not collected to assess anticoagulation status in the pivotal studies, but were only drawn in specific patient subgroups. However, it is important to measure anticoagulation in some patient populations, such as prior to surgery, when renal function deteriorates, during bleeding or thrombotic episodes, and to assess adherence to therapy (2). DOACs prolong coagulation or clotting times to different degrees in several clotting assays ([3]).

DOACs inhibit factor Xa or thrombin, which can be detected with chromogenic substrate assays. The oral direct factor Xa inhibitors rivaroxaban, apixaban and edoxaban also prolong the prothrombin time (PT) and activated partial thromboplastin time (aPTT) to different degrees depending on the DOAC and the sensitivity of reagents (3). PT and aPTT are also affected by pathological conditions in various diseases, which can reduce their specificity to anticoagulants such as DOACs. The main advantage of PT and aPTT is that they can be routinely performed in all laboratories around the clock (4).

Heptest, heptest STAT (5) are more useful for measurement of direct oral factor Xa than PT/aPTT and hemoclot (6), thrombin clotting time, and ecarin clotting time tests (7) for determination of oral direct thrombin inhibitors. These more specific tests also have their limitations. For example, Hemoclot test results were highly variable in patients undergoing

mechanical artificial heart valve replacement and receiving 150 mg to 300 mg bid dabigatran, despite exact timing of blood sampling for coagulation analyses, (8) indicating its limited analytical sensitivity and specificity in this clinical setting. Furthermore, these are specialized tests and can only be performed in specialist laboratories at defined times (usually normal working hours).

Chromogenic assays are based on the specific inhibition of factor Xa or thrombin in the presence of enzyme-specific chromogenic substrates. These assays determine the effect of oral direct factor Xa inhibitors rivaroxaban, apixaban, edoxaban (9), and of the oral direct thrombin inhibitor dabigatran (10). DOAC measurement needs to be carefully standardized for each individual chromogenic substrate method using specific calibrators for every DOAC, and these calibrations need to be performed with every coagulation platform. This makes specific chromogenic assays time consuming and restricts their availability and when they can be performed. The availability of the specific coagulation assays for DOACs ranges from less than 2% to 39% in coagulation laboratories, and much less in smaller laboratories and community hospitals (11). Qualitative DOAC detection from coagulation parameters gave results below, within, and above the normal range, but the assay sensitivities and specificities were still low (11).

Liquid chromatography coupled with tandem mass spectrometry (LC-MS/MS) is the gold-standard assay for DOAC quantitative assessment (12, 13). This method is used to compare the sensitivity and specificity of chromogenic substrate assays and of coagulation assays for DOACs (13). These investigations showed a high correlation of DOAC quantification between LC-MS/MS and chromogenic substrate assays and Hemoclot (Pearson correlation $r > 0.95$). Other analyses of plasma samples from patients receiving therapy with these anticoagulants have revealed only a sensitivity and specificity of 50% to 80% (14). However, these methods can only be performed in a small number of specialized laboratories, so their availability remains very limited (15).

To detect DOACs in blood samples in emergency situations, point of care (POC) coagulometer instruments may offer advantages over conventional clotting assays. However, the results from plasma samples of patients receiving dabigatran and rivaroxaban are variable, which limits their usefulness in acute clinical conditions (16). A whole-blood clotting time assay based on Surface Acoustic Waves (SAW-CT) has been evaluated in stroke patients treated with phenprocoumon, dabigatran, rivaroxaban, and apixaban. This assay can determine the anticoagulant level in patients receiving dabigatran, rivaroxaban, and phenprocoumon, but not apixaban (17). The *Hemochron Signature* system uses testing cards that measure the activated clotting time (ACT) and PT, and determines blood coagulation by photo-detection. The ACT and PT are prolonged in the presence of vitamin K antagonists, DOACs, and heparins if reagents do not contain heparin neutralizers. They can be used to qualitatively rule out the presence of clinically relevant concentrations of rivaroxaban and dabigatran, with sensitivities of 96% and 98% but with low specificities of 67% and 66%, respectively (18). The *Cascade Abrazo* POC test system includes specific testing cards and is currently under development. This blood test will detect inhibitors to thrombin and factor Xa and is based on photo-detection of coagulation (19). Perosphere Technologies offers a broad array of anticoagulant testing, including DOACs, unfractionated heparin, low molecular weight heparin, fondaparinux, and warfarin using a broad-spectrum POC coagulometer (20).

The certified in vitro diagnostic medical device (IVD) *DOAC Dipstick* was developed based on the rationale that DOACs are excreted into and may be detectable in the urine (21). When detecting DOACs in urine, it is important to consider that plasma coagulation proteins will not be present, unlike in blood or plasma samples. This may be an advantage because blood cells, plasma proteins, and naturally-occurring coagulation inhibitors will not be able to interact with DOACs and may not interfere with the results of the *DOAC Dipstick* (22). This test gives qualitative results regarding the absence or presence of DOACs in a patient's urine sample for both direct oral factor Xa inhibitor and oral thrombin inhibitors. This single-use assay has a

turnaround time of 10 min and results can be determined by visual identification of specific colors with the naked eye.

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.

6. STUDY OVERVIEW

6.1 Design Overview

This prospective, open-label, controlled, not randomized, multicenter, national Post Marketing Study in Germany.

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). Each patient will participate for approximately 1 to 2 hrs in the Post Marketing Study.

6.2. Determination of Sample Size

Two groups of medications (Thrombin inhibitor, Factor Xa inhibitors) will be tested with the IVD and test results compared to LC-MS/MS 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%.

To improve the strength of the study result, the following adjustments were made in the version 3.0 of the SAP compared to the version 2.0: α=0.01 and a 2-sided test with a null proportion of 0.01.

The re-calculation of the required sample size remained unchanged compared to version 2.0 of the document to show that the assumed rate of 2.5% false-negative/false-positive tests is statistically significantly lower than 5% would require 440 patients per each test group including an assumed drop-out rate of 12%,, with α=0.01 and β=0.20 (80% power).

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 2-sided test with a null proportion of 0.01.

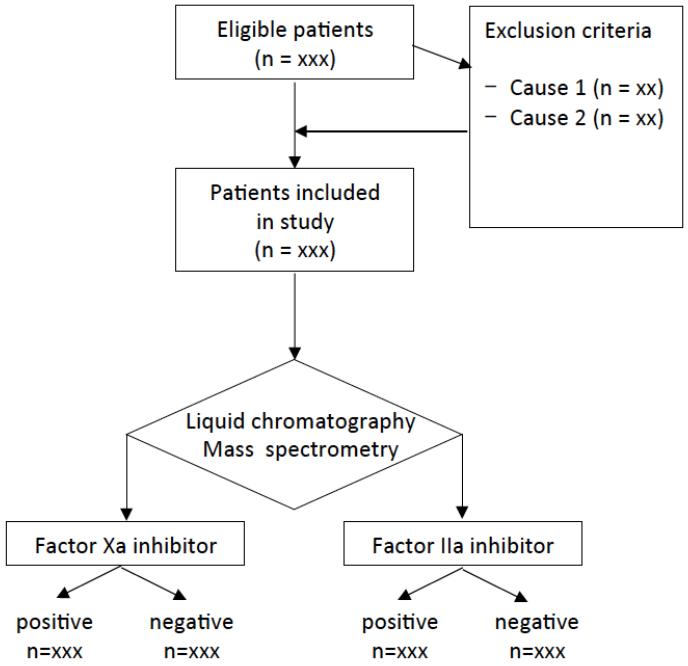
Remark to the calculation of the anticipated dropout rate: **As the present post marketing study is a non-invasive investigation, the dropout rate may be lower than expected.** Only patients fulfilling all of the inclusion criteria and none of the exclusion criteria will be included. Patient`s written informed consent is part of the inclusion criteria.

6.2 Study Objectives

6.2.1 Primary Objective

- Assess the diagnostic performance of the IVD DOAC Dipstick test for oral direct Factor Xa and Thrombin inhibitors from urine samples of patients in comparison to the results of the concentration of the DOACs determined by liquid chromatography mass spectrometry (LC-MS/MS)

The flow chart for adjudication of eligible patients to group A (factor Xa inhibitors) and thrombin inhibitors in shown in the following figure:



6.2.2 Secondary Objectives

- Comparison of the investigator-assessed POCT results with the results obtained by reflectance photometric reading (DOASENSE Reader)
- Determination of the inter-centre reliability of DOAC Dipstick test at centres including a minimum of 30 patients.
- 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.

6.2.3 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.

6.2.4 Patient identification

The medical records at the investigational site will identify patients. No screening log of patients is included in the study.

With signing of informed consent, the patients will be assigned a specific identification number for the Post Marketing Study (2-digits for the study center followed by a 3-digit code for patients starting by 001 with consecutive number for every new patient having signed the informed consent.

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.

Patients who withdraw consent at any time after signing informed consent are counted as drop out and data will be or not transferred to the data manager or will be eliminated by

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

Table: DOAC urine test strip

Name:	DOAC Dipstick
Manufacturer:	DOASENSE GmbH, Heidelberg
Model/Type:	DOASENSE REF Number: 0001

Lot number:	DS 18021901
Expiration date:	2019 05 as of December 2018
Intended use:	Analysis of the presence or absence of DOACs in urine and of normal or low concentration of creatinine
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.

6.2.6 Emergency procedures

Such procedures will not be required because no invasive measures will be taken during the study. Patients provide only spontaneously collected urine which means, that no invasive procedure will be used to collect patient`s urine such as a catheterization of the urinary bladder.

6.2.7 Blinding

All urine samples will be labelled with a QR code that contains a 9-digit unique number (will not contain any patient identifying information), provided on a laboratory order sheet from Laboratory Limbach, Heidelberg (annex 1) and will be frozen at -20°C immediately at

the study center. The codes are attached to the urine tubes before freezing and to the CRF on the prespecified position. Before analysis or upon earlier request by the study center, samples will be collected by Laboratory Limbach and transported within the same day at -20°C to Laboratory Limbach, Im Breitspiel 17, Heidelberg. Personal performing the LC-MS/MS and evaluating the data are unaware of the patient's treatment.

6.3 Study Time points

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.

Upon patient`s written informed consent the investigator will document the patient's demographic characteristics and record the time of last intake of DOAC in the CRF.

Then, the patients will provide a sample of urine and give it to the respective site personnel. The patient requires no further action.

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

Then, the site personnel will perform the visual and reflectance photometric reading of the DOAC Dipstick.

Thereafter, the site personnel will complete the questionnaire.

Assessment	Day 1
Informed consent	X
Demographic data, medical history	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 DOAC Dipstick	X
Urine aliquot for bioanalysis (LC_MS/MS)	X
Completion of questionnaire	X

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

7. STUDY POPULATION

Patients will be included into the trial if they are on anticoagulant therapy for one week or longer for the indications: non-valvular atrial fibrillation (NVAF) for prevention of cerebral and non-cerebral embolic events and venous thromboembolism (VTE) for prevention of thrombotic events. The inclusion and exclusion criteria define the study population more specifically.

7.1 Inclusion Criteria

Remark: The first inclusion criterion of the Study Plan Version 7.0 eliminated and included into the exclusion criteria due to an overlap of the content.

- 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

7.2 Exclusion criteria

- 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.

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).

7.3 Population characteristics

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

- Age (years)
- Gender (male, female)
- Body weight (kg)
- Body height (cm)
- Medical and surgical history relevant to DOAC treatment
- Medication history – DOAC (type, dose, 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.

8. DEFINITIONS AND DERIVED VARIABLES

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

Patients will collect a sample of urine into the container provided by DOASENSE GmbH and hand it over to the site personnel.

The site personnel will first perform

1) Visual testing using DOAC Dipstick according to the instructions for use (annex 2)

2) immediately thereafter analyze the patient's DOAC Dipstick with the DOASENSE Reader (annex 3). The printout of the DOASENSE Reader is attached to the respective field in the CRF with the numbers to be written with a pencil beside the printout because the thermopaper of the printout may lose colour over time.
3) put two aliquots of 5 mL urine into the tubes provided for measurements of DOACs and creatinine.
4) to discard the remaining urine.
5) Analysis of DOASENSE Control Urines that comprise a negative and positive control for DOACs and creatinine before and after inclusion of every 12th patient
6) Compilation of a questionnaire on the handling of DOAC Dipstick and its analysis by the study personnel that perform the test (visual and with DOASENSE Reader) after having performed the analyses of the 5th and 15th patient

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

8.1 Demographic variables

The variables are described in paragraph 2.3. Medical personnel at the site will record the patient's demographic data in a prepared Case Report Form (annex 4).

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.

8.2 Visual assessment of colours of pads of DOAC Dipstick

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

The site personnel will perform the visual 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.

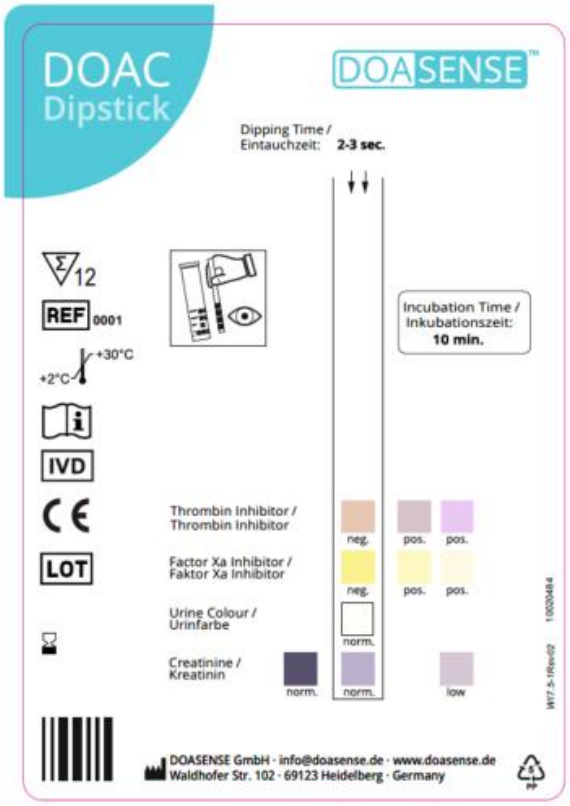
The study personnel will document the results in the CRF and the printout of the reflectance photometric color reader (DOASENSE Reader) will be copied and fixed with an adhesive into the CRF.

After 10 minutes, the reactions on the pads are completed. The colours of the pads are identified by naked eye of the study personnel and judged as follows by comparing the colour label attached to the test tube.

The text for the identification of the colour by the observer is taken form the instructions for use (annex 2) and as follows:

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.



Pad					
4	Thrombin Inhibitor		☐ neg	☐ pos	☐ pos
3	Factor Xa Inhibitor		☐ neg	☐ pos	☐ pos
2	Urine color		☐ norm		☐ abnormal
1	Creatinine	☐ norm	☐ norm		☐ low

Legend left figure: Thrombin inhibitor pad, Factor Xa inhibitor and Creatinine pad are depicted with 3 colours: the left colour is described always first, the middle colour always second and the right colour always third in sequence in the text.

Legend right table: Scheme for documentation of colour identified by the observers at centres by comparing the results of the colour of DOAC Dipstick 10 min after incubation with patient's urine with the colours printed on the label of the tube (left, figure) containing the Dipsticks

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.

8.3 Assessment of DOAC Dipstick by DOASENSE Reader

Measurement

After the urine strip has been immersed in the urine sample for approx. 2-3 seconds according to the instructions for use, the strip is placed on the strip holder of the DOASENSE Reader after 10 minutes and the START button is pressed. The device pulls the strip holder and carries out the measurement. The result of the four pads is displayed on the screen and printed on the thermal printer. After measuring the strip, the device ejects the strip holder. The strip is removed and disposed of.

The output of DOASENSE Reader are 3-digit values reprinting the %REM values (reflectance units) of the 4 pads and printed by the Reader of the printer for each measurement that is attached with an adhesive by the study personal into the CRF. These values are then written with a ball point pen on the right part of the printed values because the printout may lose color over time.

8.4 Analysis by LC-MS/MS

Urine samples will be analyzed at Laboratory Limbach by LC-MS/MS. The method is described elsewhere ([23]). The results are given as ng/ml. Normal range of values is 0.1 to 4.0 ng/ml (23). Normal values are interpreted as “negative”. Higher values represent “positive” results. Optionally, the upper limit of normal range may be changed to 8 ng/ml if values between 4 ng/ml and 8 ng/ml occur in more than 0.5% of patients.

Methodological reasons may lead to a repeated measurement of the urine sample diluted 1:15. Analyses will be repeated with this dilution if concentration of DOAC in urine is > 50.000 ng/ml or is expected to be above this value. The repetition is required to ensure that the concentration is in the linear range of detection range of LC-MS/MS. However, the dilution affects the normal range, which is 15-fold higher compared to samples that are

analysed without this dilution. For the statistical evaluation these mean of the normal range without dilution will be used.

Remark: The personal at Limbach laboratory is blinded regarding the information which DOAC the patient is taken. The personal does not have access to any information in the CRF which DAOC the patient was taking.

8.5 Analysis of Creatinine in urine

Creatinine is determined in urine by the method of Jaffe et al at Laboratory Limbach (24). Normal values are below 0.25 g/l. Values below 0.25 g/l are interpreted as "low" and higher values are interpreted as "normal". The normal range of creatinine may change upon the results of the measurement because reduced renal function with low creatinine is a contraindication for the therapy with a DOAC. If a diagnosis of renal insufficiency is documented in a patient, low creatinine value has to be interpreted as correct low. However, changes may be required upon the results of the determination of creatinine by laboratory Limbach. These decisions are made by the Steering Committee.

Data are transferred unchanged by software to an Excel file at Laboratory Limbach. This file is stored on a password secured USB flash drive and brought to the Institute for Biometry and Statistics by the Co-ordinating Investigator. Prof. Weiss imports the data from Excel to SAS. Treatment of patients and data of LC-MS/MS remain blinded during this procedure. Six months after termination of the analysis of DOACs by LC-MS/MS and creatinine by Jaffe Method urine samples are discarded. This period may be changed upon request of the Sponsor of the study.

8.6 Assessment of DOASENSE Control Urines

At start of study, and after every 12th examination of patient urine samples, on a DOAC Dipsticks are analysed by the visual reading and by DOASENSE Reader using DOASENSE Control Urines that are a negative control (0 ng/mL Rivaroxaban and Dabigatran, 1.5 g/L

creatinine and normal urine colour) and a positive control (800 ng/mL Rivaroxaban and Dabigatran and no creatinine) as described above. The results are documented on a separate page of the CRF (Quality Control to be performed at start of Study, and after every 12th Examination of Patient Urine Samples (9.5.3. Page 21, Study Protocol)) (annex5) provided by DOASENSE.

8.7 Questionnaire on user handling of DOAC Dipstick

The aim of the content of the questionnaire is to gather information on the handling and the performance of DOAC Dipstick by the user. A questionnaire that contains statements on the handling of DOAC Dipstick and the colour scale has to be compiled by the study personnel who perform the testing with DOAC Dipstick after the analysis of the 5th and 15th patient (annex 6).

8.8 Clinical Efficacy and Safety Endpoints

This is a non-interventional study to determine the accuracy of the IVD DOAC Dipstick in comparison to the concentration of the analytes in spontaneously obtained urine sample of patients on chromic treatment with DOACs. Clinical endpoints are not part of the determination of the accuracy of DOAC Dipstick in the study.

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.

Therefore, clinical efficacy and safety endpoints are not defined for this study.

9. EFFICACY PARAMETERS

9.1 Primary Efficacy Endpoint

The primary efficacy endpoint is to assess the performance of the results for oral direct Factor Xa and Thrombin inhibitors of the **qualitative** visual analysis of the IVD **DOAC Dipstic**k from urine samples of patients on treatment with DOACs in comparison with the **quantitative** results of DOACs obtained by **LC-MS/MS** quantification.

In case the cut-off values are different for apixaban, edoxaban and rivaroxaban, as determined by LC-MS/MS this has to be taken into account for the analysis. In such case LC-MS/MS may be transformed to qualitative data for the analysis of the primary endpoint.

9.2 Secondary Efficacy Endpoints

9.2.1 DOACs

I. Comparison of the **qualitative DOAC Dipstick** results with the **qualitative** results of the **DOASENSE Reader** for DOACs
II. Comparison of the **qualitative** results of the **DOAC Dipstick** with the **quantitative** results of **DOASENSE Reader**.
III. Comparison of the **qualitative** results of the **DOASENSE Reader** with the **quantitative** results of **DOASENSE Reader**. Differences between readers have to be respected and if so, included into this analysis. Each center has an individual reader. Readers are standardized before delivery from the producer by adequate tastings, which, however do not include samples of patients under treatment. This has to be analyzed statistically in an adequate way. Number of samples may not be sufficient for all readers.
IV. Determination of the inter-center reliability for centers including more than 30 patients
V. Comparison of the **qualitative** results of the **DOASENSE Reader** with the **quantitative** results of **LC-MS/MS**. The results of Rivaroxaban, Apixaban and

Edoxaban have to be analyzed separately because of different concentration ranges during therapy by LC-MS/MS.

VI. Comparison of the **quantitative** results of the **DOASENSE Reader** with the **quantitative** results of **LC-MS/MS** is performed. The results of Rivaroxaban, Apixaban and Edoxaban have to be analyzed separately because of different concentration ranges during therapy by LC-MS/MS.

VII. Analysis of an **interaction** of **demographic data** on the results of **DOAC Dipstick**, **DOASENSE Reader** and **LC-MS/MS** (demographic data, creatinine)

VIII. Analysis **dose, last intake and duration of treatment** with each DOAC on **qualitative** results of **DOAC Dipstick** and **DOASENSE Reader** and **quantitative** results of **LC-MS/MS**

IX. Analysis of an **interaction** of **demographic data** on the results of **DOAC Dipstick**, **DOASENSE Reader** and **LC-MS/MS** (demographic data, creatinine)

X. Analysis **dose, last intake and duration of treatment** with each DOAC on **qualitative** results of **DOAC Dipstick** and **DOASENSE Reader** and **quantitative** results of **LC-MS/MS**

XI. Results of DOAC Dipstick and Doasense Reader are compared between Rivaroxaban, Apixaban and Edoxaban using of LC-MSMS because DOAC Dipstick and Doasense Reader do not differentiate between the 3 direct oral factor Xa inhibitors.

9.2.2. Creatinine

XII. Comparison of **quantitative creatinine** of Limbach (Jaffe Method) with **qualitative** results of **DOASENSE Reader**. A cut-off value of creatinine (g/L) needs to be determined for the colours low to normal by DOASENSE Reader. Differences between Readers needs to be analyzed.

XIII. Comparison of results of **qualitative of creatinine by DOAC Dipstick** with **qualitative** results of **DOASENSE Reader**. Analysis of normal ranges of the **quantitative** results of **Urine Colour** Pad of **DOASENSE Reader**.

9.2.3. Urine Color

XIV. Analysis of normal ranges of the **quantitative** results of **Urine Colour** Pad of **DOASENSE Reader**.

XV. Analysis of an interaction of the **qualitative results** of the pad **Urine Colour** on the results of the qualitative results of the pads of Factor Xa inhibitor, thrombin inhibitor and creatinine of **DOAC Dipstick** with the qualitative results **DOASENSE Reader.**

9.2.4. Other

XVI. Analysis of the results of **DOASENSE control urines** over time and influence on results of DOAC Dipstick and DOASENSE Reader.

XVII. Descriptive analysis of **Drop-out** patients (case reports)

XVIII. Analysis of the results of the **questionnaire** and interaction on results of **DOAC Dipstick**. Center effects need to be analysed.

Additional statistical evaluations may be required during analysis due to the complexity of data.

10. STATISTICAL METHODOLOGY

10.1 Statistical and Analytical Issues

10.1.1 Data management

All study practices and statistical methods are based on the International Conference on Harmonization (IHC) document “Statistical Principles for Clinical Trials.”

A database containing patient IDs and codified numbers will be restricted to the office of Prof. Christel Weiss, Department of Biometry and Statistics, Medical Faculty Mannheim. This is a locked office, and the data will only be accessible to the investigators named on this application. This database for will contain patient IDs, and info on disease status, treatment/control, follow up visits, and sample analysis – only info pertinent to the outcome measures. All sample data will be assessed using codified descriptors and will contain no patient ID info. Once the LC-MS/MS data has been analyzed, unmasking treatment and control groups will be conducted at the office of Prof. Weiss, by the named investigators. Any publications of study results will be completed devoid of any information that could be used to identify patients included in the study. Treatment groups will summarize data. Baseline characteristics, and safety outputs total overall columns will be included to summarize all subjects.

10.1.2 Subject disposition – Descriptive Statistics

The subject disposition table will summarize the following and will be presented for all subjects by treatment group and overall.

- The number (%) of subjects entered into the study after Baseline visit
- The number (%) of subjects withdrawn before treatment completion
- The number (%) of subject after treatment completion
- The number (%) of subjects who complete the study according study protocol

The number (%) of subjects who complete and withdraw from the study and the primary reason for withdrawal will be summarized by treatment group and overall for all subjects.

10.1.3 Demographic Characteristics - Descriptive Statistics

Demographic data presented will be group of DOACs (group A and group B). Demographic data will be summarized using summary statistic for continuous variables (number of subjects, mean, standard deviation, median, minimum, and maximum).

10.1.4 Prior and Concomitant Medications – Descriptive Statistics

Prior and concomitant medications taken by or administered to a subject will be recorded. Prior medications are defined as the medication that started and stopped before Baseline. Concomitant medications are defined as the medications that started before Baseline and continued into the study.

The main medication (i.e. the specific DOAC medication) and the concomitant medication relevant to DOAC therapy will be categorized in groups with ascending numbers (Annex 7). The grouping of concomitant medication may change after descriptive statistics. It is aimed to have a minimum number of n=30 per group.

10.1.5 Medical History – Descriptive Statistics

Investigators should document all medical conditions relevant to DOAC therapy. Any medical condition present at the time informed consent is obtained is to be regarded as concomitant and will result in the subject being ineligible for the study.

Main diagnosis as indication for anticoagulant therapy and concomitant diagnoses will be categorized in groups with ascending numbers (Annex 7). The grouping of concomitant diagnoses may change after descriptive statistics. It is aimed to have a minimum number of n=30 per group.

All data will be summarized by treatment group. Baseline characteristics, and safety outputs total overall columns will be included to summarize all subjects. For all baseline, demographic, safety and efficacy outputs data will be summarized by treatment group.

In detail, each DOAC Dipstick contains 4 pads that are analyzed and documented from every patient by the observers by visual analysis and by DOASENSE Reader. The pads are described in 3.2. Therefore, all 4 pads are evaluated from every patient`s urine.

In all statistical tables, p-values will be reported as specified by the statistical program used, at least up to three decimal places. P-values less than 0.001 will be reported as provided by SAS (e.g. '<0.001'). All tests will be two-sided at the $\alpha = 0.01$ level of significance, if not stated otherwise.

For each diagnostic test the proportions of false negative and false positive results will be assessed together with confidence intervals. The urine concentration, determined by LC-MS/MS, serves as a gold standard. Furthermore, McNemar tests will be conducted in order to compare the sensitivity, the specificity, accuracy of the two different medications. Chi2 tests will be used in order to compare negative predictive value and positive predictive values. If the preconditions of a Chi2 test are not fulfilled Fisher's exact test will be used instead. 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 (Chi2 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.

Where that any of the statistical methods described herein prove unsuitable during analysis, more appropriate methods will be used. All changes in methodology will be documented in the clinical study report.

Additional ad-hoc analyses may be conducted as deemed suitable.

Table: The treatment label for listings, tables and figures will be the following:

Consecutive number	**Treatment**	**Parameter**	**Label**
1	Group A, Group B	Demographic data dose, duration, and last intake per DOAC	Descriptive statistics, comparison between groups
2	Parameters: FXa DOACs together FXA DOACs individual Creatinine	DOAC Dipstick Reader qualitative and quantitative LC-MS/MS, Jaffe Method	Descriptive statistics
3	Factor Xa Inhibitors together	DOAC Dipstick, LC-MS/MS	Accuracy parameters
4	Thrombin inhibitor	DOAC Dipstick, LC-MS/MS	Accuracy parameters
5 a	Factor Xa Inhibitors Separately Apixaban Edoxaban Rivaroxaban	DOAC Dipstick, LC-MS/MS	Accuracy parameters
5b	Factor Xa Inhibitors separately Apixaban Edoxaban Rivaroxaban Creatinine	DOAC Dipstick, DOASENSE Reader qualitative	Accuracy parameters

5c	Factor Xa Inhibitors separately Apixaban Edoxaban Rivaroxaban	DOAC Dipstick, DOASENSE Reader quantitative	Accuracy parameters
5d	Factor Xa Inhibitors separately Apixaban Edoxaban Rivaroxaban	DOASENSE Reader qualitative, DOASENSE Reader quantitative	Accuracy parameters
6	Urine colour, normal and abnormal	DOAC Dipstick, DOASENSE Reader qualitative and quantitative	Descriptive, analysis of difference between groups
7	Factor Xa Inhibitors together Thrombin inhibitor Factor Xa inhibitors separately Apixaban Edoxaban Rivaroxaban	DOAC Dipstick DOASENSE Reader qualitative and quantitative LC-MS/MS	Interaction with demographic data
8	DOASENSE Control urines	DOAC Dipstick, DOASENSE Reader qualitative and quantitative	Descriptive Statistics Analysis of influence on parameters of DOAC Dipstick

9	Drop out	Demographic data, withdrawal of consent lack of dose, duration of therapy <7 days, Lack of results of DOAC Dipstick and of DOASENSE Reader No urine samples available at Limbach	Descriptive Statistics
10	Time dependence of results on test parameters	All test parameters of DOAC Dipsick	Descriptive statistics
11	Unexpected results	Examples: Non-compliance (LC_MSMS and DOAC Dipstick both negative) Factor Xa and thrombin inhibitor positive both with DOAC Dipstick and or LC-MSMS	Descriptive statistics
12	Questionnaire	Items	Descriptive Statistics parameters
13	Questionnaire Items	DOAC Dipstick	Interaction analysis

10.1.6 Interim Analysis

No interim analysis will be performed.

10.1.7 Definitions

In the following table, the definitions and calculation of derived variables are summarised.

Variable / Term	Definition / Way of calculation
BMI	Weight / Height
Interval therapy duration	Start of therapy - date of last medication intake
Interval urine collection	Date of last medication - Date of urine delivery

10.1.8 Analysis Sets

Analysis set	Definition
Per protocol (PP) population	All patients of the Full analysis population without any relevant protocol violations (major protocol deviations) and complete data for the primary efficacy variable. The relevant protocol violations have to be defined prior to database closure and unblinding by a systematic data review. For this purpose, protocol violations that occurred during the trial such as violations of inclusion/exclusion criteria or forbidden concomitant medications or violation of visit time windows will be assessed as 'major' or 'minor' depending on their potential to interfere with the objectives of the trial.

Major protocol deviations and the assessment of analysis sets will be defined during last data review before data base closure. All definitions given in the Minutes of the Final Data Review will be taken into account in the analysis.

10.1.9 Multicentre Studies

The results will not be stratified by centre in general. The inter-center variability will be analysed.

10.1.10 Subgroup Analyses

No subgroup analysis is planned in this trial.

10.1.11 Dropouts and Missing Data

Subject inclusion/exclusion criteria will be determined at baseline visit, and subjects who do not meet all criteria will not be entered into the study. Those subjects with signed informed consent deemed eligible to participate will be allocated a 2-digit number of the center at which patients are recruited followed by a 3-digit number that is consecutive according to the inclusion.

If patients do not fulfil posthoc all inclusion or exclusion criteria – except missing or late signature of written informed consent – e.g. reduced renal function, less than 7 days on therapy, intake of two different DOACs at the same time, change of dose of DOAC within the past 7 days and other findings, they will be evaluated individually and may be described as case reports.

If there is no "informed consent" or if the date of the "informed consent" is after the "start date" it is a dropout.

If a subject withdraws consent at any time after entering the study, will result in patient removal of all data from the study.

If data (dd.mm.yyyyy) is specified with 99.99.yyyy in the table, it will be replaced as follows:

1. 99.99.yyyyy (day and month missing) is replaced by 01.07.yyyy. Example: 99.99.2017 = 01.07.2017
2. 99.mm.yyyyy (day missing) is replaced by 15.mm.yyyy. Example: 99.08.2018 = 15.08.2018

For twice daily intake of medication: If time (hour/min) is not specified, the mean of the time (i.e. 02:00 p.m. or 02:00 a.m., between 08:00 a.m. and 08:00 p.m. or 08:00 a.m. and 08:00 p.m.,

whatever applies according the documentation in the CRF, i.e. is taken for the analysis, respectively.

For once daily intake of medication: If time (hour/min) is not specified, the mean of the time (i.e.08:00 p.m.) between 08:00 a.m. at the day before and the day of urine sampling is taken for analysis.

10.2 Efficacy Analyses

10.2.1 Primary Efficacy Variable

The primary efficacy endpoint is to assess true negative rates of the results for oral direct Factor Xa and Thrombin inhibitors of the **qualitative** visual analysis of the IVD **DOAC Dipstic**k from urine samples of patients on treatment with DOACs in comparison with the **quantitative** results of DOACs obtained by **LC-MS/MS** quantification.

Statistical method: determination of a cut-off value with Logistic regression analysis, Mc Namar test, furthermore kappa index, accuracy, sensitivity, specificity, positive and negative predictive values with 95% confidence intervals.

10.2.2 Secondary Efficacy Variables

DOACs

I. Comparison of the **qualitative DOAC Dipstick** results with the **qualitative** results of the **DOASENSE Reader** for DOACs
   Statistical methods: determination of a cut-off value with Logistic regression analysis, McNemar test, kappa index, accuracy, sensitivity, specificity, positive and negative predictive values (with confidence intervals).
II. Comparison of the **qualitative** results of the **DOAC Dipstick** with the **quantitative** results of **DOASENSE Reader**.
   Statistical methods: determination of a cut-off value with Logistic regression analysis,

McNemar test, kappa index, accuracy, sensitivity, specificity, positive and negative predictive values (with confidence intervals).

III. Comparison of the **qualitative** results of the **DOASENSE Reader** with the **quantitative** results of **DOASENSE Reader**. Differences between readers have to be respected and if so, included into this analysis. Each center has an individual reader. Readers are standardized before delivery from the producer by adequate tastings, which, however do not include samples of patients under treatment. This has to be analyzed statistically in an adequate way. Number of samples may not be sufficient for all readers.
Statistical methods: determination of a cut-off value with Logistic regression analysis, McNemar test, kappa index, accuracy, sensitivity, specificity, positive and negative predictive values (with confidence intervals).

IV. Determination of the (kappa index, accuracy, sensitivity, specificity, positive and negative predictive values, cut-off value) **qualitative** results of the **DOAC Dipstick** compared to the **quantitative** results of **LC-MS/MS**.

   a. The results of rivaroxaban, apixaban and edoxaban have to be analysed separately because of different concentration ranges during therapy by LC-MS/MS. The results of the pad for factor Xa inhibitor of group B will be used to determine the statistical values. The results of the factor Xa inhibitors of group B by LC-MS/MS in urine will also be used for the statistical values.
   b. The results of dabigatran are analysed from group B as well as from group A to determine the statistical values.

Statistical methods: determination of a cut-off value with Logistic regression analysis, McNemar test, kappa index, accuracy, sensitivity, specificity, positive and negative predictive values.

V. Comparison of the **qualitative** results of the **DOASENSE Reader** with the **quantitative** results of **LC-MS/MS**. The results of Rivaroxaban, Apixaban and Edoxaban have to be analyzed separately because of different concentration ranges

during therapy by LC-MS/MS.

Statistical methods: determination of a cut-off value with Logistic regression analysis, McNemar test, kappa index, accuracy, sensitivity, specificity, positive and negative predictive values.

VI. Comparison of the **quantitative** results of the **DOASENSE Reader** with the **quantitative** results of **LC-MS/MS** is performed. The results of Rivaroxaban, Apixaban and Edoxaban have to be analyzed separately because of different concentration ranges during therapy by LC-MS/MS.

Statistical method: t-test.

VII. Analysis of an **interaction** of **demographic data** on the results of **DOAC Dipstick**, **DOASENSE Reader** and **LC-MS/MS** (demographic data, creatinine)

Statistical method: multivariable regression analysis using SELECTION=FORWARD method

VIII. Analysis **dose, last intake and duration of treatment** with each DOAC on **qualitative** results of **DOAC Dipstick** and **DOASENSE Reader** and **quantitative** results of **LC-MS/MS**

Statistical method: multivariable regression analysis using SELECTION=FORWARD method

IX. Analysis of an **interaction** of **demographic data** on the results of **DOAC Dipstick**, **DOASENSE Reader** and **LC-MS/MS** (demographic data, creatinine)

Statistical method: multivariable regression analysis using SELECTION=FORWARD method

X. Analysis **dose, last intake and duration of treatment** with each DOAC on **qualitative** results of **DOAC Dipstick** and **DOASENSE Reader** and **quantitative** results of **LC-MS/MS**

Statistical method: multivariable regression analysis using SELECTION=FORWARD method

Creatinine

XI. Comparison of **quantitative creatinine** of Limbach (Jaffe Method) with **qualitative** results of **DOASENSE Reader**. A cut-off value of creatinine (g/L) needs to be determined for the colours low to normal by DOASENSE Reader. Differences between Readers needs to be analyzed.
Statistical methods: determination of a cut-off value, McNemar test, kappa index, accuracy, sensitivity, specificity, positive and negative predictive values.

XII. Comparison of results of **qualitative of creatinine by DOAC Dipstick** with **qualitative** results of **DOASENSE Reader**. Analysis of normal ranges of the **quantitative** results of **Urine Colour** Pad of **DOASENSE Reader**.
Statistical methods: McNemar test, kappa index, accuracy, sensitivity, specificity, positive and negative predictive values.

Urine Colour

XIII. Analysis of normal ranges of the **quantitative** results of **Urine Colour** Pad of **DOASENSE Reader**.
Statistical method: Descriptive statistic

XIV. Analysis of an interaction of the **qualitative results** of the pad **Urine Colour** on the results of the qualitative results of the pads of Factor Xa inhibitor, thrombin inhibitor and creatinine of **DOAC Dipstick** with the qualitative results **DOASENSE Reader.**

Other

Statistical method: multivariable regression analysis using SELECTION=FORWARD method

XV. Analysis of the results of **DOASENSE control urines** over time and influence on results of DOAC Dipstick and DOASENSE Reader.
Statistical method: McNemar test, kappa index, accuracy, sensitivity, specificity, positive and negative predictive values.

XVI. Descriptive analysis of **Drop-out** patients (case reports)
Statistical method: Descriptive statistic

XVII. Analysis of the results of the **questionnaire** and interaction on results of **DOAC Dipstick**. Center effects need to be analysed.
Statistical method: multivariable regression analysis using SELECTION=FORWARD method.
Additional statistical evaluations may be required during analysis due to the complexity of data.

10.3 Safety Analyses

This is a non-invasive study and patients do not get in contact with the IVD DOAC Dipstick. Adverse events directly related to the participation of the study do not occur. Any unexpected event, that occurs during the study visit will be recorded on a Site Contact Report according to the specification.

11. PLANNED DEVIATION FROM THE STUDY PROTOCOL

The planned analysis will be performed according to the study protocol, its amendments and this statistical analysis plan. If there are contradictions between the study protocol or its amendments and this statistical analysis plan, the analysis will be performed according to this analysis plan. Any deviance from the planned analysis according to the study protocol has to be described in the integrated report.

12. SOPS FOR ANALYSIS AND REPORTING

Sponsor/ HLZ Medical Statistic's standard operating procedures as well as the ICH guideline E9 will be applied to this analysis.

13. FORMAT OF THE REPORT

All tables, graphs and listings will be in portrait orientation. They will fit on DIN A4 paper with margins of 1.5 cm on the top, 1 cm at the right and left and 1.25 cm at the bottom including header and footer.

The statistical methods used for the analysis will be summarised and the results will be discussed from a biometrical point of view. A separate biometrical report will not be written, but the results and the methodology section will be included into the integrated report.

14. DATA BASE LOCK AND UNBLINDING

After the data cleaning process is finalised and signed by the sponsor, the database will be locked. The Biostatistician and the SAS®-programmer will have only read access to the locked database.

After the data base closure will be validated by an independent statistician person. The data entry is done twice. Then the database will be archived on USB flash drive and given a write protection on the server.

15. SOFTWARE

All statistical analyses will be performed with SAS®, Version 9.3 or later on a MS-Windows platform.

16. PROGRAM VALIDATION

Program validation with a subset of data:

The following key-tables of the analysis will be generated for a subset of the data (for approximately 15 patients). These tables will be checked against the patient data listings for descriptive statistics. P-values and confidence intervals will be calculated independently with second programs. All discrepancies, which are not based on rounding (i.e. the figures have to

coincide at the reported precision in the report) will be investigated. The SAS® programs will be used for the final analysis, if no discrepancies will occur:
List of selected analysis for this type of validation:

I. Comparison of the **qualitative DOAC Dipstick** results with the **qualitative** results of the **DOASENSE Reader** for DOACs
II. Comparison of the **qualitative** results of the **DOAC Dipstick** with the **quantitative** results of **DOASENSE Reader**.

This validation process will be performed with preliminary data prior to the data base lock. All log- and output-files as well as data files will be stored. The person, responsible for the validation will sign a printout of these files. If the check of a table will be performed by a pocket calculator, the table has to be annotated with the results of the second calculation and signed.

17. PRELIMINARY TERMIANTION OF THE STUDY

The study can be terminated earlier by the decision of the sponsor. The sponsor's aim is to obtain the anticipated aim of the study.
Safety reasons are not an issue and the study cannot be terminated due to safety issues of the investigation.
Efficacy aspects may be a reason to terminate the study earlier. This argument may be relevant because decision-making process of medical personal can be speed up by the use of the DOAC Dipstick since no other accurate POC test are not available. The need of such a testing in emergency medicine is continuously growing due to the continuous increase of prescription of DOACs.

18. LIST OF POST-TEXT-TABLES

Per protocol analysis

Tables

1. Demographic data of patients of group A and Group B, p values
2. Descriptive data of tested parameters per group
3. Results of regression analysis for Rivaroxaban with LC-MC/MC
4. Results of Regression analysis for Dabigatran with LC-MC/MC
5. Results of Regression analysis for Creatinine Jaffe Method
6. Results of Regression analysis of Urine Colour (REM% values)
7. Group A: correct and false negative and positive data of DOAC Dipstick versus LC-MS/MS, p-value
8. Group B: correct and false negative and positive data of DOAC Dipstick versus LC-MS/MS, p-values
9. Apixaban: correct and false negative and positive data of DOAC Dipstick versus LC-MS/MS, p-value
10. Edoxaban: correct and false negative and positive data of DOAC Dipstick versus LC-MS/MS, p-value
11. Rivaroxaban: correct and false negative and positive data of DOAC Dipstick versus LC-MS/MS, p-values
12. Group A: Creatinine: correct and false of low and normal data of DOAC Dipstick versus Jaffe-Method, p-value
13. Group B: Creatinine: correct and false of low and normal data of DOAC Dipstick versus Jaffe-Method, p-value
14. All analyses of no 3 to 13 with qualitative results DOAC Dipstick versus quantitative Results of DOAC Dipstick
15. Comparison of negative and positive or low and normal results of all pads of DOAC

Dipstick versus DOASENSE Reader

16. Comparison of quantitative values of all pads by DOASENSE Reader versus LC-MS/MS and Jaffe Method
17. Interaction of Apixaban, Edoxaban and Rivaroxaban results of DOAC Dipstick with results of Creatinine of DOAC Dipstick
18. Interaction of Apixaban, Edoxaban and Rivaroxaban and creatinine results of DOAC Dipstick with results of urine colour of DOAC Dipstick
19. Regression analysis of biographic data with pads of DOAC Dipstick to test interactions
20. Results of dependence of time of last intake of or drug on DOAC Dipstick quantitative, DOASENSE Reader qualitative and quantitative, and LC-MS/MS
21. Results of dose dependence of individual DOACs on parameters of No 20
22. Descriptive results of DOASENSE Control Urines and analysis of differences between centres
23. Analysis of changes results of DOASENSE Control Urines over time and influence on results of pads of DOAC Dipstick over time
24. Descriptive results of Questionnaire over time and differences between centres
25. Interaction of results of questionnaire with results of DOAC Dipstick

19. LIST OF POST-TEXT-TABLES AND FIGURES

Figures

1. Results of all data of tables no 3 to 21 whenever possible as column diagrams with respective text (header)
2. ROC Curves when applicable with respective text (Header)

20. LIST OF PATIENT DATA LISTINGS

Listing of data will be structured as follows (if applicable)

1. Study termination
2. Protocol deviations
3. Excluded patients
4. Demographic and baseline data
   - 4.1 Informed consent
   - 4.2 Demographic data
   - 4.3 Inclusion criteria
   - 4.4 Exclusion criteria
   - 4.5 Anamnesis (medical history & physical examination)
5. Compliance
6. Efficacy
7. Adverse events
8. Laboratory data
9. Vital signs
10. Concomitant medication
11. Comments

21. SHELLS FOR TABLES, LISTINGS, AND FIGURES

Listings contain treatment, identification variable and description of the listed variables as title.

22. ANNEXES

The following annexes 1 to 7 are added.

- Annex1_laboratory_order_sheet
- Annex2_Instruction_for_use_DOAC-Dipstick
- Annex3_Quick_reference_guide_DOASENSE

- Annex4_Case_Report_Form
- Annex5_Quality_Control_test_urine
- Annex6_Questionnaire_on_performance_DOAC_Dipstick
- Annex7_Groups_of_medications_and_diseases

23. REFERENCES

1 Weitz JI, Harenberg J. New developments in anticoagulants: Past, present and future. Thromb Haemost 2017;117:1283-1288

2 Ten Cate H, Henskens YM, Lancé MD. Practical guidance on the use of laboratory testing in the management of bleeding in patients receiving direct oral anticoagulants. Vasc Health Risk Manag 2017;13:457-467.

3 Tripodi A, Marongiu F, Moia M, et al. The vexed question of whether or not to measure levels of direct oral anticoagulants before surgery or invasive procedures. Intern Emerg Med 2018 [Epub ahead of print]

4 Gosselin RC, Adcock DM, Bates SM, et al. International Council for Standardization in Haematology (ICSH) Recommendations for Laboratory Measurement of Direct Oral Anticoagulants. Thromb Haemost 2018;118:437-450

5 Du S, Harenberg J, Krämer S, et al. Measurement of Non-Vitamin K Antagonist Oral Anticoagulants in Patient Plasma Using Heptest-STAT Coagulation Method. Ther Drug Monit 2015;37:375-80.

6 Stangier J, Feuring M. Using the HEMOCLOT direct thrombin inhibitor assay to determine plasma concentrations of dabigatran. Blood Coagul Fibrinolysis 2012;23:138-143

7 Fenyvesi T, Jorg I, Weiss C, et al. Effects of lepirudin, argatroban and melagatran and additional influence of phenprocoumon on ecarin clotting time. Thromb Res 2003;111:89-94

8 Eikelboom JW, Connolly SJ, Brueckmann M, et al. Dabigatran versus warfarin in patients with mechanical heart valves. N Engl J Med 2013;369:1206-1214.

9 Di Minno A, Spadarella G, Prisco D, et al. Clinical judgment when using coagulation tests during direct oral anticoagulant treatment: a concise review. Semin Thromb Hemost 2013;39:840-846

10 Poli S, Härtig F, Spencer C, et al. Diagnostic Accuracy of a Novel Chromogenic Direct Thrombin Inhibitor Assay: Clinical Experiences for Dabigatran Monitoring. Thromb Haemost 2017;117:2369-2375

11 Samuelson BT, Cuker A, Siegal DM, et al. Laboratory Assessment of the Anticoagulant Activity of Direct Oral Anticoagulants: A Systematic Review. Chest 2017;151:127-138

12 Gous T, Couchman L, Patel JP, et al. Measurement of the direct oral anticoagulants apixaban, dabigatran, edoxaban, and rivaroxaban in human plasma using turbulent flow liquid chromatography with high-resolution mass spectrometry. Ther Drug Monit 2014;36:597-605

13 Douxfils J, Gosselin RC. Laboratory Assessment of Direct Oral Anticoagulants. Semin Thromb Hemost 2017;43:277-290

14 Douxfils J, Ageno W, Samama CM, et al. Laboratory testing in patients treated with direct oral anticoagulants: a practical guide for clinicians. J Thromb Haemost 2018;16:209-219

15 Gosselin RC, Douxfils J. Measuring Direct Oral Anticoagulants. Methods Mol Biol 2017;1646:217-225

16 Mani H, Herth N, Kasper A, et al. Point-of-care coagulation testing for assessment of the pharmacodynamic anticoagulant effect of direct oral anticoagulant. Ther Drug Monit 2014;36:624-631

17 Bluecher A, Meyer Dos Santos S, Ferreirós N, et al. Microfluidic coagulation assay for monitoring anticoagulant therapy in acute stroke patients. Thromb Haemost 2017;117:519-528

18 Ebner M, Birschmann I, Peter A, et al. Point-of-care testing for emergency assessment of coagulation in patients treated with direct oral anticoagulants. Crit Care 2017;21:32

19 Poli S. Specific PoC Testing of Coagulation in Patients Treated With DOAC 1 (SPOCT-DOAC 1). https://clinicaltrials.gov/ct2/show/NCT02825394. Accessed May 09, 2019

20 http://www.perospheretech.com/content/research/coagulometer.htm. Accessed May 09, 2019

21 Harenberg J, Warttinger U, Giese C, et al. Reliability and validity of a point of care test from urine samples of patients on therapy with apixaban, rivaroxaban and dabigatran**.** Res Pract Thromb Haemost 2017, 1: PB45

22 Harenberg J, Du S, Krämer S, Weiss C, et al. Patients' serum and urine as easily accessible samples for the measurement of non-vitamin K antagonist oral anticoagulants. Semin Thromb Hemost 2015;41:228-236

23 Harenberg J, Schreiner R, Hetjens S, Weiss C. Detecting Anti-IIa and Anti-Xa Direct Oral Anticoagulant (DOAC) Agents in Urine using a DOAC Dipstick. Semin Thromb Hemost. 2019; 45:275-284