STA® - RIVAROXABAN CALIBRATOR

Calibration Plasmas for Assay of Rivaroxaban Using anti-Xa Method

- Kit containing:
  – 3 x 1-ml Vials of Reagent 1 (STA® - Rivaroxaban Calibrator 0)
  – 3 x 1-ml Vials of Reagent 2 (STA® - Rivaroxaban Calibrator 1)
  – 3 x 1-ml Vials of Reagent 3 (STA® - Rivaroxaban Calibrator 2)
  – 3 x 1-ml Vials of Reagent 4 (STA® - Rivaroxaban Calibrator 3)

(REF 00704US)

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FOR RESEARCH USE ONLY - NOT FOR USE IN DIAGNOSTIC PROCEDURES

1/ SUMMARY AND EXPLANATION

The **STA® - Rivaroxaban Calibrator** kit is a set of calibrator plasmas used with STA-R® and STA Compact® for calibration of the measurement of rivaroxaban anti-Xa activity (2).

Rivaroxaban is a direct anti-Xa anticoagulant molecule whose activity can be determined with the chromogenic assay **STA® - Liquid Anti-Xa** (REF 00311US, 00322US).

2/ TEST PRINCIPLE

Dedicated calibrators are used to determine the mass concentration of rivaroxaban (ng/ml).

The proposed method is a one-step reaction based on a principle of competition; as soon as factor Xa is added to the plasma-substrate mixture, two reactions take place simultaneously, namely:
- hydrolysis of the substrate by factor Xa
- inhibition of factor Xa by rivaroxaban.

After the necessary period of time for the competitive reaction to reach equilibrium, the quantity of paranitroaniline that is released is inversely proportional to the concentration of rivaroxaban present in the test medium.

3/ KIT REAGENTS

An Assay Value insert with 4 barcodes, one for each reagent, is provided in the box. Each barcode contains the following information: lot number, kit code number, reagent code number, expiration date, and rivaroxaban value established with analyzers of the STA® line for the relevant lot.

- **Reagent 1**: STA® - Rivaroxaban Calibrator 0, human plasma containing no rivaroxaban, lyophilized.
- **Reagent 2**: STA® - Rivaroxaban Calibrator 1, human plasma containing a well-defined quantity of rivaroxaban, lyophilized (see the Assay Value insert).
- **Reagent 3**: STA® - Rivaroxaban Calibrator 2, human plasma containing a well-defined quantity of rivaroxaban greater than that of Reagent 2, lyophilized (see the Assay Value insert).
- **Reagent 4**: STA® - Rivaroxaban Calibrator 3, human plasma containing a well-defined quantity of rivaroxaban greater than that of Reagent 3, lyophilized (see the Assay Value insert).

WARNING - POTENTIAL BIOHAZARDOUS MATERIAL
The reagents provided in this kit contain materials of human and/or animal origin. Whenever human plasma is required for the preparation of these reagents, approved methods are used to test the plasma for the antibodies to HIV 1, HIV 2 and HCV, and for hepatitis B surface antigen, and results are found to be negative. However, no test method can offer complete assurance that infectious agents are absent.Therefore, users of reagents of these types must exercise extreme care in full compliance with safety precautions in the manipulation of these biological materials as if they were infectious.

4/ CAUTION

Store at 2-8 °C. For *in vitro* use only. Take care to use only the reagents from the same kit or the same lot. The disposal of waste materials must be carried out according to current local regulations.
The STA® - Rivaroxaban Calibrator kit is designed for use with analyzers of the STA® line suitable to these reagents. Read the Reference Manual of the analyzer model carefully before starting. Exercise great care in the handling of these reagents and of samples.

5/ REAGENT PREPARATION AND STORAGE

- **Preparation**
  Reconstitute each vial (Reagent 1, 2, 3 or 4) with 1 ml of distilled water. Allow the reconstituted material to stand at room temperature (18-25 °C) for 30 minutes followed by swirling of the vial before use.
- **Storage**
  The reagents in unopened vials are stable until the expiration date indicated on the box label, when stored at 2-8 °C.
  Once reconstituted, Reagents 1, 2, 3 and 4 remain stable for 8 hours on STA-R® and STA Compact®.
  Do not to freeze.

6/ REAGENTS AND EQUIPMENT REQUIRED BUT NOT PROVIDED

- **STA® - Liquid Anti-Xa** (REF 00311US, 00322US).
- **STA® - Owren-Koller** (REF 00360).
- **STA® - Rivaroxaban Control** (REF 00706US).
- STA-R® or STA Compact®.
- Common clinical laboratory equipment and materials.

7/ SPECIMEN COLLECTION AND TREATMENT

Sample collection must be in conformity with the recommendations for haemostasis tests.

- Blood (9 vol.) is collected in 0.109 M (i.e., 3.2 %) trisodium citrate anticoagulant (1 vol.).
- Centrifugation: 15 minutes at 2000-2500 g.
- Plasma storage: 6 hours at 20 ± 5 °C.

8/ PROCEDURE

8.1. Assay reagents
Prepare the STA® - Liquid Anti-Xa reagents as indicated on the package insert of this kit.

8.2. Calibration
Assay calibration is performed with Reagents 1, 2, 3 and 4 of the STA® - Rivaroxaban Calibrator kit.
When they are ready for use, load them into the instrument according to the recommendations of the Reference Manual of the analyzer model. The vial position in the instrument is the following:
– on STA-R® model, place the calibrator vials in the R0 area of the product drawer
– on STA Compact® model, place the calibrator vials in one of the positions 1 to 18 or 35 to 38 of the product drawer.
Then scan the information contained in the barcodes of the Assay Value insert into the instrument.
When a rivaroxaban assay is selected, the instrument, at the operator's command, carries out the assay calibration according to the parameters entered in the analyzer for the relevant assay. The calibration curve can be examined on the screen of the instrument in the "Calibration" menu (see the Reference Manual).

8.3. Plasmas to be tested
Plasmas are tested undiluted. They are loaded in the instrument (see the Reference Manual of the analyzer model).
Then select the test(s) to be performed.

8.4. Quality control
It is necessary to run controls in order to ensure accuracy and reproducibility of the results. Use STA® - Rivaroxaban Control. Prepare these controls and scan the information contained in the barcodes printed in the Assay Value insert into the instrument. These controls are used undiluted.

8.5. Assay
Refer to the "Standardized Operating Procedures" of the instrument for full details on how to proceed from this point.
The rivaroxaban assay of the plasmas to be tested is automatically carried out by the analyzer at 405 nm as soon as the samples have been loaded.

9/ RESULTS

The rivaroxaban level (in ng/ml) of the plasmas being tested is displayed in the "Test Status/Test Panel" screen of the analyzer (see the Reference Manual).

Ensure that the values obtained for the controls are within the ranges stated in the Assay Value insert provided in the control box. If the control values are outside the stated ranges, check all components of the test system to ensure that all are functioning correctly, i.e., assay conditions, reagents, calibration, integrity of the plasmas being tested, etc. If necessary, repeat the assays.

10/ LIMITATIONS

- Other anticoagulants (UFH, LMWH, fondaparinux...) can interfere with the assay.
- The method is insensitive to the following substances:
  Hemoglobin (up to 2.0 g/l), unconjugated bilirubin (up to 200 mg/l - 342 µmol/l) and triglycerides (up to 10 g/l).

11/ PERFORMANCE CHARACTERISTICS

11.1. Characteristic of the calibrators
The level of rivaroxaban of Reagents 2, 3 and 4 may vary from one lot to another, but is clearly indicated in ng/ml for each lot (see the Assay Value insert provided in the box).
This level is determined by high-performance liquid chromatography-mass spectrometry (HPLC-MS).

11.2. Characteristics of the method

- **Detection Treshold - Working Range**
  The detection threshold is 25 ng/ml and the linearity range extends to 500 ng/ml.
- **Precision**
  Precision studies were performed using samples containing rivaroxaban (22 days, 2 runs per day) on STA-R® with STA® - Liquid Anti-Xa. The following results have been obtained:

	Repeatability		Within-laboratory precision	
Sample	Sample 1	Sample 2	Sample 1	Sample 2
n	22	22	22	22
$\bar{X}$ (ng/ml)	86	308	86	308
SD (ng/ml)	2.2	5.8	2.9	8.5
CV (%)	2.5	1.9	3.3	2.8

REFERENCES

1. ROHDE G.:
"Determination of rivaroxaban - a novel, oral, direct Factor Xa inhibitor - in human plasma by high-performance liquid chromatography - tandem mass spectrometry". J. Chromatography B Elsevier, 43-50, 2008.
2. SAMAMA M.M., CONTANT G., SPIRO T.E., PERZBORN E., GUINET C., GOURMELIN Y., LE FLEM L., MARTINOLI J.L.:
"Evaluation of the anti-factor Xa chromogenic assay for measuring rivaroxaban plasma concentrations using calibrators and controls", ASH, 5071 b JN04 Abstract, 2010.

Significant changes are indicated by dotted lines in the margin.

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