# Instructions for use – RVV

REF 1071EU – RVV  UDI 59998629921071EU_1VH

## Intended purpose

CE 2265 IVD **The RVV is a ready to use reagent for in-vitro diagnostic professional use, intended for detection of direct FXa antagonists in citrated blood during viscoelastometry analysis.**

CAUTION: A use of the device outside of its intended purpose, may lead to the test results being incorrectly interpreted by the user.

## Indications for use

Indicated to be used when the presence of FXa antagonists in the patient's blood is suspected.

## Contra-indications for use

The assay shall not be used in patients treated with unfractionated heparin, as this can lead to false-positive results in the RVV assay.

## Intended users

- trained healthcare professionals,
- trained laboratory professionals.

## Environment of use

Indoors in a typical setting of a laboratory, equipped and designed to ensure standard electrical connections, adequate lighting as well as standard environment settings regarding temperature, humidity and pressure to ensure the functionality of typical electrical devices like electrical medical devices and personal computers.

## Intended patient population

Adult patients suspected to be exposed to direct FXa antagonists.

## Principle of the assay


The RVV assay is a functional whole-blood based assay to be used on viscoelastometry analyzers [1] which uses Russell's viper venom to directly activate coagulation factor X as the trigger for thrombin generation in whole blood.

Viscoelastometry allows for the detection of whole blood formation in whole blood, and thus detects coagulation initiation (by the clotting time, CT), blood clot firmness (by the maximum clot firmness, MCF, or related parameters, such as the A20, amplitude 20 minutes after CT) and clot stability or fibrinolysis (by the maximum lysis, ML).

Direct FXa inhibitors (apixaban, rivaroxaban and edoxaban) are anticoagulants that selectively and reversibly inhibit activated factor Xa, a key enzyme in the common pathway of the coagulation cascade [2].

In the RVV assay thrombin generation is mediated via the coagulation factors X and V. In addition to Russell's viper venom the reagent also contains calcium chloride for the recalcification of the citrated blood sample. The use of a combination of Russell's viper venom and calcium chloride to test for direct FXa antagonists was proposed by Exner et al in 2013 [3] and further validated by Douxfils et al [4]. It was introduced as an IVD assay as the RVV-test [5]. Its sensitivity and specificity for the detection of direct FXa antagonists was demonstrated in a large independent clinical study [5].

When no anticoagulants are present in the sample, the initiation of thrombin generation is fast and therefore the clotting time (CT) of the RVV assay is short. Direct FXa antagonists inhibit the factor Xa (FXa) formed by the Russell's viper venom. Therefore, in the presence of direct FXa antagonists, the CT is prolonged, which allows to detect the FXa antagonists.

Due to its mode of action Russell's viper venom triggered viscoelastometry is sensitive for FXa antagonists, but it is also prolonged by unfractionated heparin, dabigatran and other anticoagulants [6]. As a rapid whole-blood based assay it is therefore not specific for the effects of direct FXa antagonists.

## Materials provided

10 sealed single-use pouches containing one pipet tip with reagent each, providing a dry chemistry reagent composed of Russell's viper venom and calcium chloride. Each pouch contains one desiccant bag.

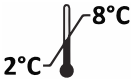
## Additional materials and devices required

- Viscoelastometry analyzer and receptacles (Cups & Pins),
- Electronic pipette for 340 µL with 3 sec aspiration / dispensing cycles,
- Blood collection tube (3.2% sodium citrate) for coagulation testing.

## Reagent preparation

The reagent is ready to use.

## Storage and stability




Store at +2 to +8 °C. The unopened reagent tips are stable until the expiration date stated on the pouch label. Unopened pouches may be stored at room temperature for up to 1 month. Opened pouches are for immediate use within 1 minute after opening the pouch.

CAUTION: Incorrect storage conditions may affect reagent stability and lead to wrong test results.

## Warnings and precautions

For professional use by trained personnel.

CAUTION: Do not use tips from defective pouches or from pouches missing the desiccant pack.

CAUTION: Intended for single use - do not reuse.

CAUTION: Any serious incident that has occurred as a result of the use of the device has to be reported to the manufacturer and the competent authority of the Member State in which the user and/or patient is established.

CAUTION: Failure to comply with these instructions for use may result in device handling errors leading to wrong test results.

CAUTION: Human blood samples should be handled with care, following general precautions recommended for bio-hazardous materials [7].

CAUTION: General precautions (e.g., wear gloves and minimize skin exposure to specimens and reagents) should be followed when handling all materials.

NOTE: Dispose of waste according to local regulations.

NOTE: A material safety data sheet is available upon request.

## Residual risks, undesirable side-effects, and information for the patient

The following residual risks were identified during the risk management activities for the device:

- In case of an off-label use of the product, test results may be incorrectly interpreted by the user.
- In case of device handling errors, patient's coagulation may be incorrectly reflected.
- In case of the use of the expired product, patient's coagulation may be incorrectly reflected.
- In case of unacceptable transport and storage conditions, patient's coagulation may be incorrectly reflected.

Warnings related to the residual risks are provided throughout the document.

No undesirable side-effects were identified during the post-market activities for the device.

No information for the patient is required to be provided for the device.

## Sample collection

CAUTION: Collect a venous blood sample according to the recommended procedures [8-9] using a blood collection tube with 3.2% sodium citrate. Samples should be analyzed within 3 hours from blood collection. Store the blood at room temperature. Always ensure blood collection tubes are filled to the indicated fill volume to avoid excessive citrate levels.

## Test procedure

1. Check the expiry date of the device. The expiry date format is yyyy-mm-dd.

   CAUTION: Do not use the expired product. The use of the expired product may lead to wrong test results.

2. Allow the reagent tip pouch to reach room temperature.
3. If the sample is cold (< 22°C) it is advised to allow the sample to warm up for 5 min on the heated position of the viscoelastometry analyzer. In evaluations on the effect of pre-warming blood tubes which had room temperature little to no effect was observed vs. tubes which were not pre-warmed.
4. Create the test in the software of the viscoelastometry analyzer according to the analyzer manual.
5. Place the Cup and Pin into the analyzer according to the analyzer manual.
6. Tear open the reagent tip pouch, attach the reagent tip to the electronic pipette and aspirate 340 µL sample from the blood tube.
7. Dispense the blood sample into the Cup.
8. Aspirate and dispense the sample once again to facilitate thorough mixing of the reagents with the blood sample. Ensure sample pipetting is performed without interruption of the process.
9. Start the test as described in the analyzer manual.
10. The test will stop, or you can stop the test as described in the analyzer manual.
11. Remove the Cup & Pin and dispose according to local regulations.

## Quality control

Plasma-based quality control materials can be used to confirm the stability of test results determined with the RVV assay over time.

## Result interpretation and expected values

The effect of direct FXa antagonists on the RVV assay is detected by the clotting time (CT).

The reference range for the clotting time (CT) is 47-89 sec. This was determined in a clinical study including 122 healthy individuals, aged 18.9 - 79.2 years, 51.6% female and 48.4% male, by the calculation of the 95% central interval (2.5° percentile – 97.5° percentile).

The ability of the RVV assay to detect direct FXa antagonists at a concentration of >= 50 ng/mL was evaluated in a clinical study which included 102 samples from patients taking FXa antagonists. The apixaban, rivaroxaban or edoxaban levels were quantified with using mass spectrometry on the Shimadzu LCMS-8060 analyzer (Shimadzu, Kyoto, Japan).

The determined FXa antagonist levels ng/ml) were as follows:

| Parameter | Apixaban | Rivaroxaban | Edoxaban |
|---|---|---|---|
| N | 58 | 29 | 15 |
| Mean | 205.5 | 154.1 | 220.5 |
| SD | 131.2 | 124.8 | 134.3 |
| Min | 0 | 0 | 36.7 |
| Max | 488 | 442 | 453 |

The RVV assay CT results for samples with a direct FXa antagonist concentration of > 50 ng/mL were as follows:

| | RVV-CT |
|---|---|
| N | 87 |
| 2.5° – 97.5° percentile | 116.8 – 386.7 sec |
| Mean ± SD | 238 ± 73.6 sec |
| Range (min – max) | 110 – 430 sec |

Based on the data of the examined clinical collectives - patients treated with direct FXa antagonists (n=102) and reference collective (n=122) the following sensitivity, specificity, positive predictive value, negative predictive value, positive and negative likelihood ratio were determined, using a predetermined cut-off of 105 sec for the RVV CT value for detecting direct FXa antagonists at a concentration of 50 ng/mL:

| Total N | 224 |
|---|---|
| Sensitivity | 100% |
| Specificity | 94% |
| Positive predictive value (PPV) | 92% |
| Negative predictive value (NPV) | 100% |
| Positive likelihood ratio (LR+) | 17.13 |
| Negative likelihood ratio (LR–) | 0 |

The 50 ng/mL cut-off was defined according to literature, which defines that “A DOAC level of less than 50 ng/mL may be considered a minimal, clinically insignificant anticoagulant effect” (Douketis

JD, Spyropoulos AC. Perioperative Management of Patients Taking Direct Oral Anticoagulants: A Review. JAMA. 2024 Sep 10;332(10):825-834).

**Precision**

In a precision study citrated blood with and without the addition of 200 ng apixaban /mL was tested in 3 runs, on three analyzers, three operations and including 3 RVV assay lots (54 determinations per sample). The resulting mean, standard deviation (SD) and coefficient of variation (CV) for the CT were as follows:

| | mean | SD | CV |
|---|---|---|---|
| citrated blood (CB) | 59.6 | 8.5 | 14.2% |
| citrated blood (CB) + 200 ng apixaban/ml | 272.2 | 31.2 | 11.5% |

## Limitations and interferences

Every in vitro diagnostic method can deliver wrong results under certain circumstances. It is therefore advisable to use certain precautions to avoid misinterpretations.

Measurements with noisy curves or irregular shapes should be discarded and repeated. The clinical context and other laboratory values (when available) should be considered when interpreting the RVV assay. Generally, consider repeating a measurement that gives a very surprising or otherwise implausible result.

While in the clinical study a 100% sensitivity and a specificity of 94% were found for the presence of direct FXa antagonists, these figures depend on the local situation and the study protocol [10]. The reference method applied in this investigation was mass spectrometry, which is highly sensitive and precise, but time-consuming and not available in many centers. Anti-Xa analysis can be less precise around the cut-off of 50 ng/mL [11]. The more patients with factor deficiencies or other anticoagulants are included in an investigation (i.e. conditions that can lead to false-positive results) the lower the specificity of an investigation can be [10]. The sensitivity and specificity of the assay will be best, when a highly accurate reference method is used, and when less patients with other suspected anticoagulants or severe diseases are tested. If such conditions are suspected, it may make sense to confirm prolonged RVV assay clotting times using more specific test methods if available.

The mechanism of the RVV assay is not specific to direct FXa antagonists.

Other methods such as the determination of the apixaban, rivaroxaban or edoxaban concentration using anti-factor FXa analysis or mass spectrometry are more specific and sensitive compared to the RVV assay, which represents a rapid whole-blood based assay. However, the anti-factor Xa analysis and / or mass spectrometry are not available in various centers or can have long turn-around times.

Due to its method Russell's viper venom triggered viscoelastometry is sensitive in respect to FXa antagonists [5], but is also prolonged by heparins [6],[12], argatroban [12], dabigatran [6] and can be prolonged by factor deficiencies [6].

Interference by the following substances was tested using citrated blood with the in vitro addition of unfractionated heparin (UFH), low molecular weight heparin (LMWH) and Dabigatran (Pradaxa®, direct thrombin antagonist). In addition, hemodilution was tested using 20% or 40% of saline 0.9%, sodium citrate 3.2%.

Measurements were performed in 6-fold determinations. CT results were as follows:

| | mean (sec) | SD | change (sec) |
|---|---|---|---|
| Citrated blood (control) | 73.7 | 5.9 | n/a |
| UFH 0.5 IU/mL | 471.8 | 22.3 | 398.2 |
| UFH 1 IU/mL | 1599.0 | 121.4 | 1525.3 |
| LMWH 0.5 IU/mL | 212.5 | 14.0 | 138.8 |
| LMWH 1 IU/mL | 401.8 | 26.9 | 328.2 |
| Dabigatran 100 ng/mL | 216.2 | 10.2 | 142.5 |
| Dabigatran 300 ng/mL | 354.2 | 12.9 | 280.5 |
| 20% hemodilution | 68.7 | 5.3 | -5.0 |
| 40% hemodilution | 72.7 | 3.3 | -1.0 |

One can see that UFH, LMWH and dabigatran can prolong the RVV assay while hemodilution had little basically no effect in the experiment.

In summary, as a functional parameter the RVV assay clotting time can be prolonged by other anticoagulants factors and clotting factor deficiencies, which can therefore lead to false-positive results.

## Summary of safety and performance


Summary of safety and performance is provided in electronic format and is available for download on www.apiro.eu/eIFU

## Manufacturer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## Authorized representative


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## Symbols

| Symbol | Meaning |
|---|---|
|  | Manufacturer |
|  | Batch code |
|  | Country of manufacture |
|  | Temperature limit |
|  | Consult instructions for use or electronic instructions for use |
|  | Contains biological material of animal origin |
|  | Not intended for near-patient testing |
|  | Unique device identifier |
|  | Swiss authorized representative |

| Symbol | Meaning |
|---|---|
|  | Use-by date |
|  | Catalogue number |
|  | Do not use if package is damaged and consult instructions for use |
|  | Do not re-use |
|  | Contains sufficient for <n> tests |
|  | Caution / Warning |
|  | CE marking of conformity |
|  | In vitro diagnostic medical device |
|  | Biological risks |

## References


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[3] Exner T, Ellwood L, Rubie J, Barancewicz A. Testing for new oral anticoagulants with LA-resistant Russells viper venom reagents. An in vitro study. Thromb Haemost. 2013 Apr;109(4):762-5. doi: 10.1160/TH12-11-0842.

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## Version history of these instructions for use

| Date | Version | Change description |
|---|---|---|
| 2026-04-13 | 1 | Initial version |