ClotPro EN

RVV-test

REF 113012 CE IVD

enicor GmbH
Reichenbachstr. 27 - 80469 Munich - Germany
Phone: +49 89 997 428 181 - www.clot.pro - info@clot.pro

PN 113012-IE (AA) 2021-07

For in vitro diagnostic use.

Intended Purpose

RVV-test is a ready to use system reagent for the detection of direct factor-Xa (FXa) inhibitors in citrated blood for the ClotPro® analyzer [1]. RVV-test in combination with the ClotPro analyzer is a semi-automated and semi-quantitative test.

Intended User

For use by trained healthcare professionals. Near patient and laboratory professional use.

Principle

ClotPro is a new generation viscoelastometry system for detecting blood coagulation using a continuous measurement of clot firmness [1]. The parameters clotting time (CT), maximum clot firmness (MCF), maximum lysis (ML), and others are automatically calculated and further described in the ClotPro user manual [2].

In RVV-test $CaCl_2$ recalcifies the sample and a reagent derived from the Russell's viper venom (RVV) activates factor X (FX) from the sample to activated FX (FXa). Produced FXa activates prothrombin to thrombin, which in turn cleaves fibrinogen to fibrin. This leads to the clotting of the sample, which is detected via the CT.

Direct FXa inhibitors in the sample inactivate formed FXa and thus delay thrombin formation. This leads to a prolonged CT in the RVV-test.

Other anticoagulants (heparins, thrombin inhibitors) also prolong the CT in RVV-test. The assay is therefore not specific towards FXa inhibitors.

Unlike the anti-FXa analysis using chromogenic substrates, RVV-test determines the effect of FXa inhibitors in the undiluted citrated whole blood sample, including FX, FV, prothrombin and fibrinogen from the sample. In the anti-FXa analysis, a strongly diluted plasma sample is mixed with FXa (of usually bovine origin) and an artificial dye. In this respect, differences in the effect of FXa inhibitors between the RVV-test and the anti-FXa analysis are to be expected.

Materials provided

10 sealed single-use pouches containing one active tip each, providing a dry chemistry reagent composed of Russell's viper venom reagent, $CaCl_2$ and stabilizers. Each pouch contains one desiccant bag.

Additional materials required

- ClotPro analyzer
- Blood collection tube (~0.109 M sodium citrate) for coagulation testing
- ClotPro Cups & Pins
- Pipette

Reagent preparation

The reagent is ready to use.

Storage and stability

Store the product at +2 to +8 °C. The unopened active tips are stable until the expiration date printed on the pouch label. Unopened pouches may be stored at room temperature for up to 1 month. Opened pouches are for immediate use without delay (testing within one minute after opening).

Warnings and precautions

For use by trained healthcare professionals.

Tips from damaged pouches must not be used!

Human blood samples and control materials are **potentially infectious** and should be handled with care, following general precautions recommended for bio-hazardous materials [3].

General precautions (e.g., wear gloves and minimize skin exposure to specimen and reagents) should be followed when handling all materials. Dispose of waste according to the local regulations. A material safety data sheet is available upon request.

Residual Risks

Sources of reagent error:

- Improper use of reagents can lead to wrong test results and cause an incorrect evaluation of the patient's coagulation status.

Sources of procedural error:

- A defective electronic pipette or its improper use can lead to incorrect pipetting volumes and cause an incorrect evaluation of the patient's coagulation status.
- Blood aspired into the Active tip must not be returned into the blood tube as the blood in the tip is contaminated with reagents. In addition, an Active tip which has come into contact with blood must not be used again.
- Poor sample quality due to pre-analytic problems can lead to wrong test results and an incorrect evaluation of the patient's coagulation status.
- Poor sample quality due to improper storage (e.g., the sample is stored for too long before use) can lead to wrong test results and an incorrect evaluation of the patient's coagulation status.
- Wrong sample temperature can lead to impaired test results and an incorrect evaluation of the patient's coagulation status.
- Excessive time elapsed between pipetting steps can lead to wrong test results and an incorrect evaluation of the patient's coagulation status.

Sample collection

Collect the sample according to the recommended procedures [4]. Samples should be analyzed within 3 hours from blood collection. Always ensure blood collection tubes are filled to the indicated fill volume in order to avoid excessive citrate levels.

Test procedure

- Allow the active tip pouch to reach room temperature and place the blood sample into one of ClotPro´s pre-heating positions. If the sample is cold (< 22°C) it is advised to allow the sample to warm up for 5 min. In evaluations on the effect of pre-warming blood tubes which had room temperature little to no effect was observed compared to tubes which were not pre-warmed.
- Select the appropriate test in the ClotPro software according to the instructions in the ClotPro user manual.
- Take one Cup & Pin from the box (together) and insert the Pin onto the pin holder by firmly pushing the Cup until a definite stop is reached.
- Remove the Cup and place the pin holder in the parking position.
- Place the Cup into the test position for the respective channel.
- Tear open the active tip pouch, attach the active tip to the pipette and aspirate 340 µl sample from the blood tube using the electronic pipette provided with the ClotPro device.
- Dispense the blood sample into the Cup.
- Aspirate and dispense the sample once again to ensure thorough mixing of the reagents with the blood sample. Ensure sample pipetting is performed without interruption of process. Dispose the active tip according to local regulations.
- Take the pin holder from parking position and place it onto the Cup in the test position. The test will start automatically.
- Stop the channel when appropriate and turn the pin holder counter-clockwise (to the left) in order to release the Pin.
- Remove the pin holder and place it into the parking position.
- Remove Cup & Pin (together) and dispose according to local regulations.

Quality control

Plasma-based lyophilized quality control (QC) material is available in 2 levels (QC 1 / QC 2).

The use of control materials for regular QC is recommended. Common practice is to run QC using extrinsically and intrinsically activated viscoelastometry assays (i.e., EX-test and IN-test on the ClotPro analyzer) one level, once per week.

Further information for the use of QC material can be found in the respective product inserts.

Performance characteristics

Precision

Precision was determined with blood of a healthy donor tested on 4 ClotPro analyzers in 6 channels each (n=24).

	Reproducibility (inter-channel / inter-device)		
	Mean	SD	CV [%]
CT [s]	65.5	2.9	4.5%
A10 [mm]	55.8	0.8	1.4%
A20 [mm]	61.0	0.7	1.1%

Expected values

Expected values have been established analyzing a reference cohort (n=60) of apparently healthy donors.

CT [s]	A10 [mm]	A20 [mm]	MCF [mm]
48 - 77	47 - 63	53 - 67	54 - 68

In a study including patients under FXa inhibitor treatment (edoxaban, rivaroxaban or apixaban, n=90) plasma concentrations of FXa inhibitors ≥50ng/ml (n = 68) were associated with a CT ≥ 100s in RVV-test (range: 119-393 sec). 100 samples from individuals without anticoagulant therapy had all CTs <100 sec in RVV-test (range: 46-81 sec). Patients treated with FXa inhibitors who had a FXa inhibitor concentration of <50 ng/ml (n = 22) had CTs in RVV-test <100 sec (n = 9 / 41%) or >100 sec (n = 13 / 59%) (range: 54-178 sec).

The concentration of 50 ng/ml FXa inhibitor chosen as the cut-off for the detection of FXa inhibitors is based on the publication by Douketis et al. [5], where this cut-off has been used to define patients who had „undetectable or minimal residual anticoagulant levels ".

Patients treated with anticoagulants other than FXa inhibitors (dabigatran, unfractionated heparin, low molecular weight heparin, vitamin K antagonists, n = 65) had CTs in RVV-test of 55-425 sec, of which 18 patients (28%) had CTs of <100 sec and 47 patients (72%) CTs of ≥ 100 sec.

The sensitivity of RVV-test for the detection of FXa inhibitors at a plasma concentration of ≥50 ng/ml (n = 68) was 100% in the study, when using a cut-off of 100 sec. When patients with anticoagulants other than FXa

inhibitors were excluded, the specificity was 89%. When patients with anticoagulants other than FXa inhibitors were included, the specificity was 69%.

Note: Reference ranges may not be identical to target ranges for specific clinical settings. Each center should examine the transferability of the reference areas to its own patient population and, if appropriate, determine its own reference areas.

Limitations and interferences

While the above study has shown a 100% sensitivity of the RVV-test (with a cut-off of 100 sec and a plasma concentration of the FXa inhibitors of ≥50 ng/ml), it has to be expected that in clinical practice discrepancies between laboratory tests to detect FXa inhibitors and the RVV-test may occur. Several studies have shown that laboratory tests for the detection of FXa inhibitors can yield variable results. In a study by Mani et al. [6], it was shown that laboratory tests for rivaroxaban can deliver results >50 ng/ml, even if no rivaroxaban is present in the sample according to mass spectrometry. Gouin-Thibault et al. [7] reported that a sample containing 40 ng rivaroxaban / ml was determined from 32 different laboratories to contain 29-77 ng rivaroxaban / ml. Tripodi et al. [8] reported that a sample containing no rivaroxaban was determined by 71 laboratories to contain 0-58 ng rivaroxaban / ml. Of course, viscoelastometry (like any other method) can also provide erroneous results. Therefore, implausible, very unexpected results or results deviating from other tests should be repeated.

RVV-test is not specific for direct FXa inhibitors. It is very sensitive to unfractionated heparin and also sensitive to direct thrombin inhibitors. RVV-test is usually prolonged in samples from patients treated with vitamin K inhibitors because two vitamin K-dependent factors are involved in the measurement (FX and prothrombin). Similarly, in samples containing low molecular weight heparin extended CTs in RVV-test are found when at least 0.4 anti-FXa U/ml are present.

It is expected that the RVV-test may be shortened in antithrombin deficiency, as well as prolonged when factor X or prothrombin are deficient. The validity of the RVV-test in such patients is probably limited. Furthermore, there is no experience with RVV-test in patients with significant hemodilution.

The effect of tranexamic acid and aspirin on the RVV-test was investigated in vitro and - as expected - no interference was found.

Packaging Symbols

Symbol	Description
	Near patient testing
	Remove Cups & Pins from packaging together
	Do not touch the Pins
	Use Cups & Pins together with Active tips™

Revision History

Version	Modification
AA	Initial version acc. to regulation (EU) 2017/746 (IVDR)

References

1. Calatzis A et al. ClotPro – a new generation viscoelastic whole blood coagulation analyser. Hämostaseologie 2018; 4a, A32, P013
2. ClotPro user manual
3. Biosafety in microbiological and biomedical laboratories; U.S. Department of Health and Human Services, Washington, 5th Edition
4. CLSI/NCCLS H03-A6; Procedures for the collection of diagnostic blood specimens by venipuncture
5. Douketis JD et al. Perioperative Management of Patients with Atrial Fibrillation Receiving a Direct Oral Anticoagulant. JAMA Intern Med. 2019 Aug 5
6. Mani H et al. Accurate determination of rivaroxaban levels requires different calibrator sets but not addition of antithrombin. Thromb Haemost. 2012;108(1):191-8
7. Gouin-Thibault I et al. Evaluation of dabigatran, rivaroxaban and apixaban target-specific assays in a multicenter French study. Thromb Res. 2017;158:126-133
8. Tripodi A et al. Interlaboratory variability in the measurement of direct oral anticoagulants: results from the external quality assessment scheme. J Thromb Haemost. 2018;16(3):565-570

Technical Assistance

You can contact us for technical assistance – please see contact details below.

Incident Reporting

Any serious incident that has occurred in relation to the device shall be reported to the manufacturer and the competent authority of the Member State in which the user and/or the patient is established.

Manufacturer

enicor GmbH - Reichenbachstr. 27 80469 Munich - Germany - Phone: +49 89 997 428 181
www.clot.pro - info@clot.pro