ECA RUO Assay

Instructions for Use

REF 1010GL | IFU_1010GL Rev.1 | For Research Use Only

■ FOR RESEARCH USE ONLY — Not for use in diagnostic procedures

1. Intended Purpose

The ECA RUO is a research use only assay for viscoelastometry that allows the assessment of coagulation activation, clot formation and clot stability in whole blood triggered by the snake venom ecarin with the addition of the heparin inhibitor polybrene and without recalcification.

2. Principle of the Assay

In the ECA assay ecarin, an enzyme from the venom of the snake Echis carinatus, activates prothrombin to meizothrombin (which is a form of thrombin). Meizothrombin activates blood platelets in the sample, and converts fibrinogen to fibrin, leading to the clotting of the sample.

When a thrombin antagonist is present, the formed meizothrombin is antagonized and therefore the clotting time (CT) is prolonged. The use of ecarin for the detection of direct thrombin antagonists was introduced by Nowak et al [1] and was also used in viscoelastometry analysis [2][3]. Recent publications have shown a high sensitivity for ecarin-activated viscoelastometry for the detection of dabigatran [4][5]. In addition, it was shown that ecarin-induced viscoelastometry shows an increased sensitivity for fibrinolysis [6]. This is likely due to the low levels of free calcium ions in the test, as the assay reagent does not recalcify the citrated blood sample.

3. Materials Provided

10 sealed single-use pouches containing one pipet tip with reagent each, providing a dry chemistry reagent composed of ecarin, polybrene (a heparin antagonist), buffer and stabilizers. Each pouch further contains one desiccant bag.

4. 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

5. Reagent Preparation

The reagent is ready to use.

6. 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.

7. 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:** 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 specimen 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.

8. Sample Collection

■ **CAUTION:** Collect the sample according to the recommended procedures [8][9]. Samples should be analyzed within 3 hours from blood collection. Always ensure blood collection tubes are filled to the indicated fill volume to avoid excessive citrate levels.

9. Test Procedure

1. Check the expiry date of the device. Expiry date format is yyyy-mm-dd. Do not use expired product.

■ **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.
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.

10. Quality Control

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

11. Result Interpretation and Expected Values

Due to the presence of polybrene in the assay, heparin does typically not affect the test results. Direct FXa antagonists do not influence the assay results, as their inhibitory action acts upstream of the activation by ecarin in the coagulation cascade.

Deficiencies of platelets or fibrinogen can reduce the blood clot firmness in the assay as determined by the A5, A10, A20 and MCF parameters.

The presence of t-PA in the sample can lead to fibrinolysis. According to literature [6] ecarin-triggered viscoelastometry shows an accelerated fibrinolysis compared to tissue factor-triggered viscoelastometry, which is likely due to the lack of recalcification in the ECA assay [10].

Direct thrombin inhibitors prolong the clotting time in the ECA assay.

The clot curve determined during the analysis should be smooth and not noisy. Repeat measurements with irregular curves.

12. Manufacturer

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13. Symbols

Symbol Name	Meaning
Manufacturer	Identifies the manufacturer of the device
Use-by date	Expiry date after which the device must not be used
Batch code	Identifies the manufacturing batch/lot
Catalogue number	Manufacturer's catalogue reference number
Country of manufacture	Country where the device was manufactured
Do not use if package is damaged	Consult instructions for use if packaging is damaged
Temperature limit	Storage temperature range for the device
Do not re-use	Device is intended for single use only

Consult IFU	Consult instructions for use or electronic IFU
Contains sufficient for tests	Indicates the number of tests the package contains
Contains biological material of animal origin	Device contains material of animal origin

14. References

[1] Nowak G, Bucha E. Quantitative determination of hirudin in blood and body fluids. Semin Thromb Hemost. 1996;22(2):197-202.

[2] Schaden E et al. Ecarin modified rotational thrombelastometry: a point-of-care applicable alternative to monitor the direct thrombin inhibitor argatroban. Wien Klin Wochenschr. 2013;125(5-6):156-9.

[3] Körber MK et al. In vitro and ex vivo Measurement of Prophylactic Dabigatran Concentrations with a New Ecarin-Based Thromboelastometry Test. Transfus Med Hemother. 2017;44(2):100-105.

[4] Fong AYY et al. Effect of Dabigatran on Clotting Time in the Clotpro Ecarin Clotting Assay. Clin Appl Thromb Hemost. 2020;26:1076029620972473.

[5] Oberladstätter D et al. A prospective observational study of the rapid detection of clinically-relevant plasma direct oral anticoagulant levels following acute traumatic injury. Anaesthesia. 2021;76(3):373-380.

[6] Zátroch I et al. New under the sun: ClotPro's ECA-test detects hyperfibrinolysis in a higher number of patients, more frequently and 9 min earlier. Blood Coagul Fibrinolysis. 2023;34(2):99-104.

[7] Biosafety in microbiological and biomedical laboratories; U.S. Department of Health and Human Services, Washington, 5th Edition.

[8] CLSI/NCCLS H03-A6; Procedures for the collection of diagnostic blood specimens by venipuncture.

[9] CLSI H21-A5 Collection, transport, and processing of blood specimens for testing plasma-based coagulation assays and molecular hemostasis assays.

[10] Bruce S. The effect of calcium of fibrinolysis in vitro. J Clin Pathol. 1964;17(3):282-6.

15. Version History

Date	Version	Change Description
2025-03-26	1	Initial version