Instructions for use – AP RUO assay
For research use only

REF 1050GL – AP RUO

Intended purpose

The AP RUO is a research use only assay for viscoelastometry that allows the examination of whole blood clot formation with fibrinolysis inhibition in citrated blood during viscoelastometry analysis.

Principle of the assay

The AP assay is a functional test for the assessment of coagulation activation and clot formation in citrated blood using viscoelastometry following an activation of the extrinsic pathway and a concurrent inhibition of fibrinolysis.

In the test blood coagulation is activated using a combination of recombinant tissue factor, calcium chloride, the heparin inhibitor polybrene and the fibrinolysis inhibitor tranexamic acid. Tissue factor stimulates the extrinsic coagulation cascade via the factors VII, X, V, and prothrombin, which leads to the activation of prothrombin to thrombin.

Thrombin converts fibrinogen to fibrin and activates platelets, which leads to the clotting of the sample. This is detected by the clotting time (CT) of the test. The clot formation is characterized by the A5, A10 and A20 parameters, as well as by the maximum clot firmness (MCF).

In the AP assay deficiencies of fibrinogen and / or platelets as well as disturbed blood clot polymerization can lead to a reduced clot formation, which leads to lower amplitudes in the A5, A10, A20 and MCF.

The use of tissue factor-activated viscoelastometry with fibrinolysis blockade was introduced by Calatzis et al [1]. The usefulness of this assay strategy for the assessment of coagulation in fibrinolysis was reported by different authors [2][3]. The use of viscoelastometry stimulated by tissue factor-activated + a fibrinolysis inhibitor in the context of suspected or manifest coagulopathy is a widely used standard procedure [4][5].

Materials provided

10 sealed single-use pouches containing one pipet tip with reagent each, providing a dry chemistry reagent composed of recombinant tissue factor, tranexamic acid, polybrene, calcium chloride, buffer and stabilizers. Each pouch further contains one desiccant bag.

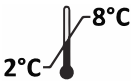
Additional materials and devices required

- 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.

Warnings and precautions

For professional use by trained personnel.

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

Intended for single use - do not reuse.

Human blood samples should be handled with care, following general precautions recommended for bio- hazardous materials [6].

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 local regulations. A material safety data sheet is available upon request.

Sample collection

Collect the sample according to the recommended procedures [7][8]. 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.

Test procedure

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

Result interpretation and expected values

The interpretation of the ap assay is performed using the A5, A10, A20 parameters for the evaluation of the clot formation. The CT and ML parameters can be regarded as process monitors, which show whether a proper coagulation activation and clot stability is found during the analysis.

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

Manufacturer

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Symbols

Symbol	Meaning
	Manufacturer
	Batch code

Symbol	Meaning
	Use-by date
	Catalogue number

Symbol	Meaning
	Country of manufacture
	Temperature limit
	Consult instructions for use or electronic instructions for use
	Contains human blood or plasma derivatives

Symbol	Meaning
	Do not use if package is damaged and consult instructions for use
	Do not re-use
	Contains sufficient for <n> tests

References

[1] Calatzis An, Fritzsche P, Kling M, Calatzis Al, Mielke L. A new technique for fast and specific coagulation monitoring. European Surgical Research. 1996; 28

[2] Levrat A, Gros A, Rugeri L, Inaba K, Floccard B, Negrier C, David JS. Evaluation of rotation thrombelastography for the diagnosis of hyperfibrinolysis in trauma patients. Br J Anaesth. 2008 Jun;100(6):792-7

[3] Barea-Mendoza JA, Terceros-Almanza LJ, García-Fuentes C, Bermejo-Aznárez S, Prieto Del Portillo IJ, Mudarra-Reche C, Valiente-Fernández M, Rodríguez-Biendicho A, Montejo González JC, Chico-Fernández M. Rotational thromboelastometry (ROTEM) profile in a cohort of asystole donors. Med Intensiva (Engl Ed). 2019 Oct;43(7):410-415

[4] Scala E, Marcucci C. Massive Hemorrhage: The Role of Whole Blood Viscoelastic Assays. Hamostaseologie. 2020 Nov;40(4):515-523

[5] Heubner L, Mirus M, Vicent O, Güldner A, Tiebel O, Beyer-Westendorf J, Fries D, Spieth PM. Point of care coagulation management in anesthesiology and critical care. Minerva Anestesiol. 2022 Jul-Aug;88(7-8):615-628

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

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

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

Version history of these instructions for use

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