Instructions for use – TPA RUO assay
For research use only

REF 1020GL – TPA RUO

Intended purpose

The TPA 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 recombinant tissue factor, recombinant tissue plasminogen activator (TPA) and the heparin inhibitor polybrene.

Principle of the assay

In the TPA assay coagulation is activated by a combination of recombinant tissue factor, calcium chloride and polybrene as a heparin antagonist. Concurrently fibrinolysis is stimulated by a high dose of recombinant tissue plasminogen activator (TPA). This dose is functionally equivalent to 650 ng/ml.

When no tranexamic acid (or other fibrinolysis inhibition) is present, the high dose of recombinant TPA leads to a rapid fibrinolysis of the sample, which is detected by a short lysis time, and a high maximum lysis in the assay. The lysis time (LT) is the time that it takes from the CT (clotting time) until a lysis of 50% is detected, i.e. when the clot firmness is reduced by 50% after the MCF (maximum clot firmness) is reached. When fibrinolysis is blocked by tranexamic acid or another fibrinolysis inhibitor, the lysis time is prolonged or not reached anymore (when 50% fibrinolysis are not reached in the assay, no lysis time is detected). Therefore, by the prolongation of the lysis time (LT) and the decrease of maximum lysis (ML), the presence of fibrinolysis inhibition can be detected.

The use of a combination of tissue factor and tissue plasminogen activator (TPA) for the assessment of fibrinolysis inhibition was reported by Dirkmann et al [1], Kostousov et al [2] and Faraoni et al [3]. Recent publications have shown a high sensitivity to the method applied by the TPA assay for the assessment of tranexamic acid [4-9].

Materials provided

10 sealed single-use pouches containing one pipet tip with reagent each, providing a dry chemistry reagent composed of recombinant tissue factor, recombinant tissue plasminogen activator, polybrene (a heparin antagonist), calcium chloride, buffer and stabilizers. Each pouch contains one desiccant bag.

Additional materials and devices required

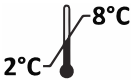
- Viscoelastometry analyzer and receptacles (Cups & Pins)
- Electronic pipette for 340 µl with 3 sec aspiration / dispensing cycles

- 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.

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 [10].

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 [11][12]. 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 TPA assay over time.

Result interpretation and expected values

The effect of fibrinolysis inhibitors on the TPA assay is detected by the lysis time (LT) and the maximum lysis (ML). In addition, the clotting time (CT) and the maximum clot firmness (MCF) can be regarded as process markers for the assay, indicating a regular coagulation activation and clot formation in the assay.

Fibrinolysis inhibitors such as tranexamic acid have been shown to prolong or abolish TPA-induced fibrinolysis in viscoelastometry [4-9]. In other conditions such as sepsis it has been shown that TPA-induced fibrinolysis may be compromised without the presence of exogenous fibrinolysis inhibitors [13-15].

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

Manufacturer

APIRO Diagnostics Kft.
Liget utca 3/2, HU-2040 Budaörs, Hungary
+36 30 203 3334 / info@apiro.eu / http://www.apiro.eu

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
	Biological risks

References

[1] Dirkmann D, Görlinger K, Gisbertz C, Dusse F, Peters J. Factor XIII and tranexamic acid but not recombinant factor VIIa attenuate tissue plasminogen activator-induced hyperfibrinolysis in human whole blood. Anesth Analg. 2012 Jun;114(6):1182-8

[2] Kostousov V, Wang YW, Cotton BA, Wade CE, Holcomb JB, Matijevic N. Influence of resuscitation fluids, fresh frozen plasma and antifibrinolytics on fibrinolysis in a thrombelastography-based, in-vitro, whole-blood model. Blood Coagul Fibrinolysis. 2013 Jul;24(5):489-97

[3] Faraoni D, Rozen L, Willems A, Torres CS, Pereira LM, Demulder A, Van der Linden P. Experimental model of hyperfibrinolysis designed for rotational thromboelastometry in children with congenital heart disease. Blood Coagul Fibrinolysis. 2015 Apr;26(3):290-7

[4] Groene P, Sappel SR, Saller T, Nitschke T, Sa PA, Paulus A, Chappell D, Schäfer ST. Functional testing of tranexamic acid effects in patients undergoing elective orthopaedic surgery. J Thromb Thrombolysis. 2021 May;51(4):989-996

[5] Kammerer T, Groene P, Sappel SR, Peterss S, Sa PA, Saller T, Giebl A, Scheiermann P, Hagl C, Schäfer ST. Functional Testing for Tranexamic Acid Duration of Action Using Modified Viscoelastometry. Transfus Med Hemother. 2021 Mar;48(2):109-117

[6] Yoshii R, Takahashi Y, Sawa T, Amaya F, Ogawa S. Long Duration of Action of Tranexamic Acid After Cardiac Surgery in a Hemodialysis Patient: A Case Report. A A Pract. 2023 May 5;17(5):e01676

[7] Coupland LA, Pai KG, Pye SJ, Butorac MT, Miller JJ, Crispin PJ, Rabbolini DJ, Stewart AHL, Aneman A. Protracted fibrinolysis resistance following cardiac surgery with cardiopulmonary bypass: A prospective observational study of clinical associations and patient outcomes. Acta Anaesthesiol Scand. 2024 Jul;68(6):772-780

[8] Dibiasi C, Ulbing S, Bancher-Todesca D, Ulm M, Gratz J, Quehenberger P, Schaden E. Concentration-effect relationship for tranexamic acid inhibition of tissue plasminogen activator-

induced fibrinolysis in vitro using the viscoelastic ClotPro® TPA-test. Br J Anaesth. 2024 Feb;132(2):343-351

[9] Yoshii R, Takahashi Y, Tanaka KA, Kawajiri H, Sawa T, Amaya F, Ogawa S. Point-of-care testing for tranexamic acid efficacy: a proof-of-concept study in cardiac surgical patients. Br J Anaesth. 2024 Jun;132(6):1211-1218

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

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

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

[13] Coupland LA, Rabbolini DJ, Schoenecker JG, Crispin PJ, Miller JJ, Ghent T, Medcalf RL, Aneman AE. Point-of-care diagnosis and monitoring of fibrinolysis resistance in the critically ill: results from a feasibility study. Crit Care. 2023 Feb 10;27(1):55.

[14] Heinz C, Miesbach W, Herrmann E, Sonntagbauer M, Raimann FJ, Zacharowski K, Weber CF, Adam EH. Greater Fibrinolysis Resistance but No Greater Platelet Aggregation in Critically Ill COVID-19 Patients. Anesthesiology. 2021 Mar 1;134(3):457-467.

[15] Bachler M, Bösch J, Stürzel DP, Hell T, Giebl A, Ströhle M, Klein SJ, Schäfer V, Lehner GF, Joannidis M, Thomé C, Fries D. Impaired fibrinolysis in critically ill COVID-19 patients.

Version history of these instructions for use

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