Instructions for use – TPA

REF 1021EU – TPA UDI 59998629921021EU_1TG

Intended purpose

CE 2265 IVD **The TPA is a ready to use reagent for in-vitro diagnostic professional use, intended for detection of tranexamic acid 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 tranexamic acid in the patient's blood is suspected.

Contra-indications for use

The TPA assay should not be used for patients that have been exposed to epsilon-amino-caproic acid or aprotinin, as these drugs could lead to false-positive results.

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 tranexamic acid.

Principle of the assay

The TPA is a functional whole-blood based assay for the detection of tranexamic acid on viscoelastometry analyzers. While a routine monitoring of tranexamic acid is not required, there are

clinical situations where its detection can be of relevance for the clinical management of the patient, such as when it is unknown whether an individual has received tranexamic acid or not.

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, fibrinolysis is fast. Tranexamic acid inhibits fibrinolysis, and therefore in the presence of tranexamic acid fibrinolysis is either delayed or blocked entirely.

The effect of tranexamic acid on the TPA assay is reflected by the lysis time (LT), which is the time from the clotting time (CT) until a maximum lysis (ML) of 50% is detected. When the ML does not reach 50%, no LT is displayed.

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 for viscoelastometry triggered by recombinant tissue factor, with the addition of a high dose of tissue plasminogen activator 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.

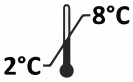
Additional materials and devices required

- 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.

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 [10].

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 [11][12] 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 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).

The reference range for the lysis time (LT) is 135-270 sec.

This was determined in a clinical study including 123 healthy individuals, aged 18.9 - 79.2 years, 51.2% female and 48.8% male, by the calculation of the 95% central interval (2.5° percentile – 97.5° percentile).

In a clinical study including 105 samples from 40 patients treated with tranexamic acid, the TPA assay was determined in citrated whole blood and the tranexamic acid concentration was determined in plasma after protein precipitation and phospholipid removal with liquid chromatography coupled to mass spectrometry [13]. Patients were 18.4 - 81.8 years old, 45.7% female and 54.3% male.

In all 69 patients with a TXA concentration ≥ 10 µg/mL maximum fibrinolysis was below 50% and therefore no lysis time was expressed. When no lysis time was reached, the lysis time was set as 3500 sec for the subsequent analysis.

When the patients treated with tranexamic acid (n=105) and the control group (reference patients, n=123) are analyzed together using a cut-off of ≥ 2100 sec for the lysis time, the sensitivity, specificity, positive predictive value and negative predictive values were as follows:

Sensitivity	100%
Specificity	81%
Positive predictive value (PPV)	70%
Negative predictive value (NPV)	100%
Positive likelihood ratio	5.3
Negative likelihood ratio	0

All individuals without exposure to tranexamic acid showed lysis times below the cut-off of 2100 sec. However, tranexamic acid at concentrations lower than 10 µg/mL already inhibited fibrinolysis in the sample.

The calculation of the lysis time (LT) in the TPA assay is based on the determination of the CT (clotting time), the MCF (maximum clot firmness) and the ML (maximum lysis).

The results for these parameters in the clinical trials were as follows:

Reference range (n=123):

	CT (sec)	MCF (mm)	ML (%)
2.5° percentile	22	17	91
97.5° percentile	50.8	41	96
mean	32.2	28.6	94.2
SD	7.2	6.9	2.7
min	20	9	71
max	58	47	97

Tranexamic acid study (n=105):

CT (sec)

	min	max	mean	SD
TXA 2.6 - 4.9µg/mL	21	55	36.9	11.1
TXA 5 - 9.6 µg/mL	27	58	40.3	8.2
TXA > 10 µg/mL	21	291	50.6	35.5

MCF (mm)

	min	max	mean	SD
TXA 2.6 - 4.9µg/mL	51	63	56.7	4.0
TXA 5 - 9.6 µg/mL	47	66	58.9	4.5
TXA > 10 µg/mL	43	71	59.2	5.4

ML (mm)

	min	max	mean	SD
TXA 2.6 - 4.9µg/mL	22	97	78.0	32.8
TXA 5 - 9.6 µg/mL	3	98	25.1	20.8
TXA > 10 µg/mL	0	18	6.5	4.3

Precision

In a precision study citrated blood with and without the addition of 10 µg tranexamic acid /mL was tested in 3 runs, on three analyzers, three operations and including 3 TPA reagent lots (54 determinations per sample). The resulting mean, standard deviation (SD) and coefficient of variation (CV) for the lysis time were as follows:

Citrated blood without the addition of tranexamic acid:

n	54
min	165 sec
max	285 sec
mean	206.7 sec
SD	24.0
CV	11.6%

Citrated blood with the addition of 10 µg/mL tranexamic acid:

All samples had fibrinolysis < 50% and therefore no lysis time.

n	54
mean	3500 sec
SD	0
CV	0%

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

Limitations and interferences

The mechanism of the TPA assay is not specific to tranexamic acid.

Other antifibrinolytic drugs such es epsilon-amino-caproic acid and aprotinin can also block TPA-triggered fibrinolysis in viscoelastometry and lead to false-positive results in the TPA assay [2][17].

Endogenous inhibition of fibrinolysis can also block fibrinolysis in the TPA assay and therefore lead to false-positive results [13-15].

Interference by the following substances was tested using citrated blood with and without tranexamic acid at a concentration of 20 µg/ml with the in vitro addition of the following substances:

- platelet inhibitors: Aspirin 0.1 mg/mL, Cangrelor 100 ng/mL,
- Anticoagulants: Apixaban 100 ng/mL, Apixaban 300 ng/mL, UFH 1 U/mL, UFH 3 U/mL, UFH 5 U/mL, LMWH 0.5 U/mL, LMWH 1 U/mL,
- Hemodilution: 20% dilution in NaCl 0.9% (in citrate 10%), 40% dilution in NaCl 0.9% (in citrate 10%).

All tested substances did not affect the detection of tranexamic acid in the assay using a cut-off of 2100 sec for the lysis time.

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

APIRO Diagnostics Kft.
Liget utca 3/2, HU-2040 Budaörs, Hungary
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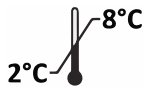
Symbols

Symbol	Meaning
	Manufacturer
	Batch code
	Country of manufacture
	Temperature limit
	Consult instructions for use or electronic instructions for use
	Contains human blood or plasma derivatives
	Not intended for near-patient testing

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

Symbol	Meaning
UDI	Unique device identifier
IVD	In vitro diagnostic medical device

Symbol	Meaning
	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] Fabresse N, Fall F, Etting I, Devillier P, Alvarez JC, Grassin-Delyle S. LC-MS/MS determination of tranexamic acid in human plasma after phospholipid clean-up. J Pharm Biomed Anal. 2017 Jul 15;141:149-156.

[14] 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.

[15] 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.

[16] 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.

[17] Nielsen VG, Cohen BM, Cohen E. Elastic modulus-based thrombelastographic quantification of plasma clot fibrinolysis with progressive plasminogen activation. Blood Coagul Fibrinolysis. 2006 Jan;17(1):75-81.

Version history of these instructions for use

Date	Version	Change description
2025-04-25	1	Initial version
2025-06-24	2	Sections “Sample collection” and “Limitations and interferences” supplemented with additional information.
2025-10-24	3	Switched symbols “Manufacturer” and “Country of manufacture”.