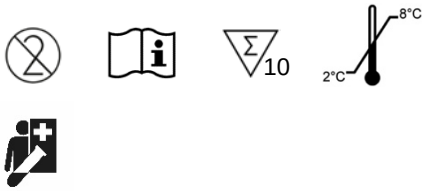
ClotPro **EN**

TPA-test

For viscoelastic coagulation testing

REF **113011** CE IVD



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

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For in vitro diagnostic use.

Intended purpose

TPA-test is a ready to use system reagent for the assessment of the fibrinolytic response to tissue plasminogen activator (t-PA) in citrated blood for the ClotPro® analyzer [1-2]. TPA-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 and fibrinolysis using a continuous measurement of clot firmness [1-2]. The parameters lysis time (LT), maximum lysis (ML), and others are automatically calculated and further described in the ClotPro user manual [3]. The LT is defined as the time from the detection of clotting (clotting time – CT) until a 50% fibrinolysis is detected. The ML is the fibrinolysis in % (in the relation to the maximum clot firmness).

In TPA-test [2], blood coagulation and fibrinolysis are triggered by recombinant tissue factor (TF, an activator of coagulation via the extrinsic pathway) and recombinant tissue plasminogen activator. In addition, the system reagent contains calcium chloride ($CaCl_2$) for the recalcification of the sample, and polybrene as a heparin inhibitor. By the action of TF and $CaCl_2$, thrombin is formed which splits fibrinogen to fibrin, activates blood platelets and factor XIII, which lead to the clot formation, which is detected on ClotPro by an increase of blood clot firmness.

In parallel, t-PA leads to the activation of plasminogen to plasmin, which cleaves fibrin and thus leads to fibrinolysis, which is detected by the reduction of clot firmness. In TPA-test a high dose of t-PA (equivalent to 650 ng/ml) normally triggers a rapid fibrinolytic effect, which is detected by a short LT and an ML of > 80%. However, various endogenous and exogenous factors can interfere with the activation or action of plasmin: plasminogen activator inhibitor 1 (PAI-1) as well as antifibrinolytic drugs such as tranexamic acid (TXA) block the activation of plasminogen to plasmin [4]. Direct plasmin antagonists, such as aprotinin, interfere with fibrinolysis in TPA-test. A reduced fibrinolytic response to t-PA in TPA-test is shown by a prolongation of the LT and a reduction of the ML.

Materials provided

10 sealed single-use pouches containing one active-tip each, providing a dry chemistry reagent composed of recombinant tissue factor, recombinant t-PA, polybrene 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 stated 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 [5].

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 [3, 6, 12]. 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 (or more). 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.
- Create the appropriate test in the ClotPro software according to the instructions in the ClotPro user manual [3].
- 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 and 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 with and without the addition of 10 µg TXA/ml on 4 ClotPro analyzers in 6 channels each (n=24).

	Reproducibility (inter-channel / inter-device)		
	Mean	SD	CV
Citrated blood:			
ML [%]	94.5	0.5	0.5%
LT [sec]	198.5	25.1	12.7%
Citrated blood + TXA:			
ML [%]	17.0	3.6	20.9%
LT [sec]	>3500		

Expected values

Expected values have been established analyzing a reference cohort (n=72) of apparently healthy donors (control groups in [2] and [7], calculated using the 95% central interval).

ML [%]	LT [sec]	MCF [mm]	CT [s]
88 - 96	151 - 411	20 - 44	29 - 58

In a study investigating patient samples under tranexamic acid (TXA) treatment (n=208 from 42 patients undergoing orthopedic surgery [8]) the following fibrinolytic response was found in relation to the determined TXA concentrations (mean±SD, range in parentheses):

TXA [µg/ml]	ML [%]	LT [sec]	n
0-1	96.4±0.7 (94.5 - 98)	394±176 (206 - 1289)	75
1-4	93.1±14.5 (22.5 - 98)	1248±923 (382 - 4500)	45
4-7	55.2±33.8 (11.5 - 98)	3366±1219 (1279 - 4500)	25
7-10	30.8±23.9 (5.5 - 96.5)	4209±733 (2149 - 4500)	26
>10	11.9±6.8 (2.5 - 36.5)	>4500	37

This shows that above a TXA concentration of 10 µg/ml a completely or almost completely blocked fibrinolytic response was recorded, while fibrinolysis was normal or close to normal at very low TXA concentrations of <1 µg/ml. Between 1 and 10 µg/ml a concentration-dependent decrease of fibrinolysis was recorded, with a significant variability between different samples.

Samples obtained before TXA application from the same study [8] (n=24) resulted in LT of 245±68 sec (161-529) and ML of 95±1% (92.5-97), and thus in similar results compared to the control groups described previously.

Fibrinolytic response to t-PA in TPA-test was impaired in patients with severe COVID-19 infection in several studies:

COVID-19 patients (n=27) treated on the intensive care unit (ICU) showed significantly prolonged lysis times of 530±327 sec compared to a control group [2]. Critically ill COVID-19 patients (n=20) showed significantly prolonged lysis times of 508 sec (365-827) (median / 25°-75° percentile) compared to a control group [7]. COVID-19 patients treated on ICU (n=20) showed significantly prolonged lysis times (365.7±44.6 sec) compared to control individuals (n=10) (193.2±16.3 sec) [9]. In the latter study the lysis time was significantly correlated (r=0.7) to increasing PAI-1 levels in the COVID-19 patients.

These study results show that the fibrinolytic response to t-PA as determined by the TPA-test depends on both endogenous factors as well as on the presence of antifibrinolytic drugs.

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

Limitations and interferences

Direct FXa or thrombin antagonists, in particular thrombin antagonists can lead to shorter lysis times. This is likely due to the effect of TAFI (thrombin activatable fibrinolysis inhibitor) onto the fibrinolytic system. Unfractionated heparin is antagonized by the polybrene reagent present in TPA-test, and was shown to have no relevant effect up to a concentration of 5 U/ml.

Regarding the assessment of the fibrinolytic response to t-PA using TPA-test following the application of TXA, the determined effective concentrations of 5-10 µg TXA/ml for blocking fibrinolysis is in good agreement with the literature on the effectiveness of tranexamic acid in clinical investigations[10]. However, one should take into account that the assessment of TPA-test represents a snapshot under the currently present TXA concentration in the sample, and TXA has a relatively short half-life of approximately 2.3 h [(10)]. It may thus be advisable to repeat the TPA-test assessment if a sustained antifibrinolytic effect is aimed for.

The biological mechanisms assessed by TPA-test are complex and involve coagulation activation, clot formation, the activation of fibrinolysis, and the actual fibrinolytic process. Therefore, smaller variations determined between measurements might be due to baseline assay variability, while differences recorded by antifibrinolytic treatments or in severely ill patients are typically of larger magnitude.

The detection of the fibrinolysis response to t-PA in TPA-test depends on coagulation activation and clot formation in the assay. In clinical situations where coagulation activation and / or clot formation might be significantly altered, it may make sense to determine coagulation without fibrinolysis activation in parallel on ClotPro, which may support the interpretation of the ClotPro results. With the EX-test [(11)] an assay is available on ClotPro, which is equivalent to TPA-test with the exception that no stimulation of fibrinolysis by t-PA is present in this test.

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

References

1. Calatzis A et al. ClotPro – a new generation viscoelastic whole blood coagulation analyser. Hämostaseologie 2018; 4a, A32, P013
2. Heinz C et al. Greater Fibrinolysis Resistance but No Greater Platelet Aggregation in Critically Ill COVID-19 Patients. Anesthesiology. 2021 Mar 1;134(3):457-467.
3. ClotPro user manual
4. Medcalf RL et al. The Fibrinolytic System: Mysteries and Opportunities. Hemasphere. 2021 Jun 1;5(6):e570.
5. Biosafety in microbiological and biomedical laboratories; U.S. Department of Health and Human Services, Washington, 5th Edition
6. CLSI/NCCLS H03-A6; Procedures for the collection of diagnostic blood specimens by venipuncture
7. Bachler M et al. Impaired fibrinolysis in critically ill COVID-19 patients. Br J Anaesth. 2021 Mar;126(3):590-598.
8. Groene P et al. Functional testing of tranexamic acid effects in patients undergoing elective orthopaedic surgery. J Thromb Thrombolysis. 2021 May;51(4):989-996.
9. Hammer S et al. Severe SARS-CoV-2 Infection Inhibits Fibrinolysis Leading to Changes in Viscoelastic Properties of Blood Clot: A Descriptive Study of Fibrinolysis in COVID-19. Thromb Haemost. 2021 Feb 25.
10. Lanoiselée J et al. PeriOpeRative Tranexamic acid in hip arthrOplasty (PORTO) study investigators. Is tranexamic acid exposure related to blood loss in hip arthroplasty? A pharmacokinetic-pharmacodynamic study. Br J Clin Pharmacol. 2018 Feb;84(2):310-319.
11. EX-test, Instructions for Use
12. CLSI H21-A5 Collection, transport, and processing of blood specimens for testing plasma-based coagulation assays and molecular hemostasis assays

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