PENTAPHARM GmbH

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in-TEM®

Intended Use:

For in Vitro Diagnostic Use Only

In-TEM® is a ready-to-use liquid ROTEM® system reagent for the examination of the intrinsic coagulation system and its interaction with thrombocytes in citrated blood.

Reagents:

Product Name: in-TEM®
Reference Number: REF 503-02
Package Size: 10 x 1 vials in-TEM® for 10 x 10 tests
Constituents:
Partial thromboplastin phospholipid made of rabbit brain (chloroform extract), ellagic acid, buffer, preservatives in small glass vials.
Preparation of the ready-to-use reagent:
The reagent is ready-to-use after being mixed carefully but thoroughly.
Storage and Stability:
Store at +2 to +8 °C. The unopened reagent is stable until the expiry date indicated on the label.
Stability after Initial Use:
Opened vials must be used within 8 days after opening. Always enter the expiry date of the opened reagent in the field intended for this on the reagent vial. Store at +2 to +8 °C. Avoid contamination and always close the vials again after each use to avoid evaporation.
Additional Material:
ROTEM® device; blood collection tubes (~0.106 M or ~0.129 M sodium citrate) for coagulation testing; cup and pin (measurement cells; REF 700005 / REF 400050); pipette tips (REF 400041 / REF 400040 / REF 400044), star-TEM® reagent (REF 503-01 or REF 503-10) for recalcification reagent.
Specimen:
Freshly prepared citrated blood. Carefully mix 9 vol. of venous blood with 1 vol. sodium citrate (~0.106 M or ~0.129 M sodium citrate) (1, 2).

Method:

Analytical Principle:
in-TEM® contains an optimum concentration of ellagic acid, which leads to a standardised mild activation of the contact phase through the negatively loaded surface. The sample is recalcified with star-TEM®.
In the thromboelastometric measurement with ROTEM® the clotting process is started after adding in-TEM® and star-TEM® to the sample and continuously monitored by the ROTEM® analyser. There is an automatic calculation of the Clotting Time (CT), Clot Formation Time (CFT), Maximum Clot Firmness (MCF) and other parameters. By these parameters the full haemostasis through the clot activation, clot formation, clot polymerisation and clot stability and fibrinolysis can be registered as well as the inhibition of the clotting cascade by anti-coagulants, fibrin deficiency, fibrin polymerisation defects, thrombocytopenia, defects in platelet function and a hyperfibrinolysis and the effects of antifibrinolytica (3,4). Deviation of the parameters from the established reference ranges indicate a potential coagulation disturbance.
The reagent may also be used for a heparin insensitive clotting analysis with the additional use of the hep-TEM® reagent (REF 503-09) (refer to package insert hep-TEM®).
Measurement Calculation:
The ROTEM® device offers numerous parameters. Their mathematical and medical backgrounds are explained in the ROTEM® user manual.
Limitation of the Procedure:
Always use freshly prepared citrated blood specimens. In tests using in-TEM® of blood samples from healthy subjects, sample storage time has not been found to influence the parameters measured for up to 4 hours after blood sampling. Store citrated blood at room temperature, NOT at +2 to +8 °C (5). Before analysis bring citrated blood samples to 37 °C and immediately prior to use, mix carefully and thoroughly to eliminate storage sedimentation. Avoid foaming!
Aspirin, clopidogrel and von Willebrand Factor have a very weak influence in this method. Abnormal patterns in the INTEM test can also be caused by the inhibitory influence of aprotinin on the contact phase.
Quality Control:
Use of control materials for regular quality control is recommended (e.g. REF 503-21 RO-TROL N / REF 503-24 ROTROL N / REF 503-25 ROTROL P). Further information may be found in the respective instructions for using these materials.
Expected Values:
Following reference ranges have been obtained in blood samples from healthy Central European blood donors for in-TEM® with the ROTEM® (n=155): CT 100-240 sec, CFT 30-110 sec, α-angle 70-83 und MCF 50-72 mm. These values are for orientation only! They are not binding and may vary from lab to lab, depending, as known from other clotting tests, on blood sampling technology and other pre-pre-analytical factors. It is recommended to confirm these by studying an own reference group in each hospital/laboratory.
Pathological Results:
Pathological results are often obtained with INTEM under the following clinical conditions:

- → Deficiency of coagulation factors (congenital or acquired)
- → Fibrinogen deficiency and / or fibrin polymerisation disorders (differentiation to platelet disorder possible via FIBTEM, refer to package insert fib-TEM®)
- → Platelet function disorders and / or thrombocytopenia (differentiation to fibrinogen disorder possible via FIBTEM, refer to package insert fib-TEM®)
- → Anti-coagulants heparin, LMWH or pentasaccharide, Hirudin, argatroban or other direct thrombo inhibitors (in higher dosage)
- → Hyperfibrinolysis (confirmation possible with APTEM, refer to package insert ap-TEM®)
- → Dilution effects (dilution coagulopathy)
- → Aprotinin in higher dosage

Research applications:
Information on the use of the in-TEM® reagent in combination with native blood for research purposes can be requested from Pentapharm GmbH.
Warnings: For in vitro diagnostic use only
Precautions:
Human blood should be handled with care, following the precautions recommended for biohazardous material (6).

Instructions for Use

Procedure (INTEM assay):

A. Mix the reagents carefully before use to return **any sporadically formed sediments gently to the suspension**. Let the reagents reach room temperature prior to use (approx. 15 minutes for reagents from the refrigerator).
B. Prepare a citrated blood specimen as recommended. Preheat the citrated blood if possible to measuring temperature.
C. NOTE: Follow the ROTEM® user manual for correct operation of the device
D. Choose a channel for measurement.
E. Remove cup & pin (measurement cell) together from the pack and place the pin (stamp) upright in the cuvette firmly onto the measurement axis (it is essential not to touch it).
F. Insert the cup (cuvette) into the pre-warmed cup holder and press it firmly into place with the MC-rod (REF 100017) up to the stop.

→ **Automatic Pipetting:**
Follow each on-screen instruction when performing the test using the automatic pipette.
→ **Manual Pipetting:**
Perform pipetting in a pre-warmed cuvette in the pre-warmed cuvette holder in the following sequence:

1. 20 µL star-TEM® reagent.
2. 20 µL in-TEM® reagent.
3. 300 µL citrated blood (pre- warmed; with new pipette tip).
4. Begin the measurement with the appropriate command (e.g. Manual) in the desired pre-selected channel.
5. Mix the sample and reagent by aspirating a 300 µL volume into the pipette once and slowly dispensing it.
6. Finally, place the cup holder containing the sample mixture carefully and immediately on the appropriate channel.
7. Stop the measurement at the desired time, remove the sample and dispose of it in conformity with local regulations.
8. The channels may then be released for the next measurement using the appropriate command.

Performance Data:

Precision:

	CT VC (%)	CFT VC (%)	α-angle VC (%)	A10 VC (%)	MCF VC (%)
Inter channel [1]	4	3	1	1	2
Inter device [2]	<4	<8	<2	<4	<3
In Series [3]	3	12	2	4	4
Day to day [4]	<8	<16	1	<4	4
Inter Lab [5]	9	7	<1	3	2

[1] Comparison of the 4 channels of a device
[2] Comparison of 5 independent devices
[3] 15 measurements in one channel of a device
[4] 1 measurement each over 10 days on one device
[5] Data from 3 centers

Blood samples from healthy donors or ROTROL N were used for the measurements. In case of strong pathological samples (CT 500-900 s, MCF<30 mm) the measurements are often less precise.

Heparin responsiveness:
From approx. 0.3 U/mL heparin (whole blood mixed with UFH) or in clinical samples from approx. 0.6 U/ml anti Xa activity in Plasma an extension of the initial CT value is seen in the test with in-TEM®. With > 1 U/mL UFH (whole blood mixed with UFH) or with strongly heparinised patients (e.g. vascular/ cardular surgery) the CT mostly reaches values around 600-900 sec or the sample cannot be clotted (CT>15 min).
As the strength of the heparin effect also depends for the specific patient on pre-analytical factors and the type of the heparin used a quantification of the heparin levels by means of thromboelastography is not recommended.

Bibliography:

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(2) DIN 58905-1; Blutentnahme: Teil 1: Gewinnung von venösem Citratplasma für hämostaseologische Analysen,
(3) Blutgerinnungsstudien mit der Thromboelastographie, einem neuen Untersuchungsverfahren. Hartert, H.: Klin. Wochenschrift 1948; 26: 577-583
(4) Thromboelastographic Coagulation Monitoring during Cardiovascular Surgery with the ROTEG Coagulation Analyzer, Calatzis, A. et.al.: Management of Bleeding in Cardiovascular Surgery edited by Roque Pifarre'; Hanley & Belfus, Inc. Philadelphia, PA, 2000
(5) NCCLS Document H21-A2. Collection, transport, and processing of blood specimens for coagulation testing and performance of coagulation assays, 3rd ed.Approved Guideline 1998
(6) Biosafety in Microbiological and Biomedical Laboratories, U.S. Department of Health and Human Services, Washington 1993 (HHS publication No. (CD) 93-8395)

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