Instructions for use – IN

REF 1031EU UDI 59998629921031EU_1TV

Intended purpose

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

Contra-indications for use

The assay shall not be used in patients treated with DOACs, as this can lead to false-positive results in the IN assay.

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 unfractionated heparin.

Principle of the assay

The IN assay is a functional whole-blood based assay to be used on viscoelastometry analyzers [1] which uses the contact activator ellagic acid for the activation of the intrinsic pathway of blood coagulation. Viscoelastometry allows for the detection of whole blood formation in whole blood, and

thus detects coagulation initiation (by the clotting time, CT), blood clot firmness (by the maximum clot firmness, MCF, or related parameters, such as the A20, amplitude 20 minutes after CT) and clot stability or fibrinolysis (by the maximum lysis, ML).

Unfractionated heparin (UFH) is an anticoagulant that binds to antithrombin (which is a natural anticoagulant present in the blood) through a specific pentasaccharide sequence on the heparin molecule [2]. This binding causes a conformational change in antithrombin that significantly enhances its ability to inactivate certain clotting enzymes. The heparin–antithrombin complex inhibits several activated clotting factors, mainly thrombin (factor IIa) and factor Xa, and to a lesser extent factors IXa, XIa, and XIIa.

In the IN assay thrombin generation is mediated via the coagulation factors XII, XI, IX, VIII, X and V [3]. In addition to ellagic acid the reagent also contains calcium chloride for the recalcification of the citrated blood sample. The use of a combination of ellagic acid and calcium chloride is commonly used in viscoelastometry tests such as the in-tem® or IN-test assays [1] (tem® is a registered trademark by CA Casyso, Switzerland).

When no unfractionated heparin is present, the initiation of thrombin generation is fast and therefore the clotting time (CT) of the IN assay is short. Unfractionated heparin inhibits the coagulation activation via the intrinsic pathway. Therefore, in the presence of UFH the CT is prolonged or abolished entirely. This allows to detect unfractionated heparin by the CT prolongation.

The detection of unfractionated heparin using functional coagulation assays triggered via the contact pathway is common practice. Such assays are the aPTT (activated partial thromboplastin time) [4], the ACT (activated clotting time) [5], or viscoelastometry-based tests such as the in-tem®, IN-test or the IN assay presented in these instructions for use [6]. Due to their mode of action such assays are sensitive to unfractionated heparin but provide a relatively modest correlation of the clotting time / aPTT / ACT vs. the anticoagulant concentration as determined using chromogenic substrate-based assays (such as the anti-Xa test) [4-5].

Materials provided

10 sealed single-use pouches containing one pipet tip with reagent each, providing a dry chemistry reagent composed of ellagic acid and calcium chloride. Each pouch contains one desiccant bag.

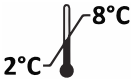
Additional materials and devices required

- 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 [7].

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, storage and transport

CAUTION: Collect a venous blood sample according to the recommended procedures [8-9] 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 [9]. Always ensure blood collection tubes are filled to the indicated fill volume to avoid excessive citrate levels [8].

Sample stability for at least 3 hours has been demonstrated for whole blood coagulation testing by viscoelastometry [17–18]. This was confirmed using citrated whole blood and citrated whole blood spiked with unfractionated heparin (0.4 U/mL), both stored for up to 3 hours. Comparison of samples analyzed after 30 and 180 minutes of storage showed only minimal differences in the IN clotting time, with mean changes of −2.9% for native citrated blood and −0.9% for heparin-spiked blood (n = 6 replicates).

Pneumatic tube transport has been reported not to have a relevant impact on viscoelastometry test results [19–20]. However, as transport conditions may differ between institutions, it is recommended that each laboratory verify the effect of its pneumatic tube transport system on IN assay results before routine use.

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 IN assay over time. Recommended quality control materials are:

- QC1 (REF: 2011EU)
- QC2 (REF: 2021EU)

Metrological traceability

For viscoelastometry measurement procedures, no internationally recognized reference measurement procedure, primary reference material, or internationally recognized conventional calibrator is available for establishing metrological traceability. Consequently, metrological traceability to SI units cannot be established.

In accordance with the principles of ISO 17511, target ranges for the quality control materials are assigned by the manufacturer using validated manufacturer-selected measurement procedures and representative production lots of the corresponding assay.

Result interpretation and expected values

The effect of unfractionated heparin on the IN assay is detected by the clotting time (CT).

The reference range for the clotting time (CT) is 113-164 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).

The ability of the IN assay to detect unfractionated heparin in a therapeutic level (>=0.3 anti-Xa U/mL) was evaluated in a clinical study which included 101 samples from 52 patients exposed to unfractionated heparin. The UFH level was determined in platelet poor plasma using anti-Xa analysis. Patients were 25.4 - 85.5 years old, 19.8% female and 80.2% male. In 54 samples the anti-Xa level was >0.3 U/ml. In 18 samples no clotting was detected and the clotting time was set with 3600 sec for the subsequent analysis. As a control group 103 control subjects were analyzed, which were not exposed to unfractionated heparin (19 – 78.6 years old, 49.5% female and 50.5% male.

When the IN assay CT results in the heparin-treated patients (n=101) are grouped according to the anti-Xa results, the following mean values and ranges are found:

anti-Xa group	N	Mean ± SD	Range (min–max)
< 0.1 U/mL	17	150 ± 28	102 – 210
0.1 – 0.3 U/mL	30	165 ± 16	136 – 196
0.3 – 1 U/mL	12	256 ± 76	149 – 389
> 1 U/mL	42	2181 ± 1361	332 – 3600

To assess the sensitivity and specificity for the IN assay for the detection of heparin, a cut-off of 0.3 U UFH / mL was applied, which represents the lower level of the therapeutic range [10].

When the patient group (n=101) and the control group (control subjects, n=103) are analyzed together using a cut-off of 190 sec [6] for the IN-test CT, the sensitivity, specificity, positive predictive value and negative predictive values were as follows:

Sensitivity	94%
Specificity	97%
Positive predictive value (PPV)	91%
Negative predictive value (NPV)	98%
Positive likelihood ratio (LR+)	28.3
Negative likelihood ratio (LR–)	0.06

The results of the other viscoelastometry parameters in the reference range study (n=123) was as follows:

	A5 [mm]	A10 [mm]	A20 [mm]	MCF [mm]	ML [%]
mean	44.1	52.7	57.1	57.6	6.3
2.5° - 97.5°	37 - 52	46 - 60	50 - 63	50 - 64	2 - 13

Precision

Intermediate precision

In an intermediate precision study citrated blood with and without the addition of 0.5 anti-Xa U /mL unfractionated heparin /mL was tested in 3 runs, on three analyzers, three operations and including 3 IN assay lots (54 determinations per sample). The resulting mean, standard deviation (SD) and coefficient of variation (CV) for the CT were as follows:

	mean	SD	CV
citrated blood (CB)	158.2	4.7	3.0%
citrated blood (CB) + 0.5 IU/mL UFH	269.6	20.9	7.8%

Between-day precision

The between-day precision of the IN assay was evaluated on 20 operating days using plasma-based quality control material (TECHNOCLOT Control N, Technoclone) with and without the addition of 0.5 anti-Xa U/mL unfractionated heparin. The resulting mean, standard deviation (SD) and coefficient of variation (CV) for the CT were as follows:

	quality control		quality control + UFH	
	replicate 1	replicate 2	replicate 1	replicate 2
mean	182.1	177.5	411.3	394.2
SD	8.3	5.9	31.3	33.1
CV	4.6%	3.3%	7.6%	8.4%

Limitations and interferences

Every in vitro diagnostic method can deliver wrong results under certain circumstances. It is therefore advisable to use certain precautions to avoid misinterpretations.

Measurements with noisy curves or irregular shapes should be discarded and repeated. The clinical context and other laboratory values (when available) should be considered when interpreting the IN assay. Generally, consider repeating a measurement that gives a very surprising or otherwise implausible result.

The mechanism of the IN assay is not specific to unfractionated heparin.

A deficiency in clotting factors (due to e.g. liver dysfunction, hemophilia, or vitamin K antagonist therapy) can prolong the clotting time in ellagic acid-triggered viscoelastometry [11-13]. The clotting time of ellagic acid-triggered viscoelastometry, can also be prolonged by the presence of anticoagulants other than unfractionated heparin, such as argatroban, dabigatran or FXa antagonists [14-15].

The extensive contact of blood with artificial surfaces during extracorporeal membrane oxygenation (ECMO) can lead to altered clotting times and therefore affect the specificity of the detection of unfractionated heparin using viscoelastometry [16].

Interference by the following substances was tested using citrated blood with the in vitro addition of low molecular weight heparin (LMWH), Apixaban (Eliquis ®, direct FXa antagonist) and Dabigatran (Pradaxa®, direct thrombin antagonist). In addition, hemodilution was tested using 40% of saline 0.9%, sodium citrate 3.2%.

Measurements were performed in 6-fold determinations. CT results were as follows:

	Mean	SD	change vs. control
Citrated blood (control)	139	3	n/a
LMWH 0.5 IU/mL	204	7.3	47%
LMWH 1 IU/mL	267.5	5.6	92%
Apixaban 100 ng/mL	148.8	4.8	7%
Apixaban 300 ng/mL	163.3	1.8	17%
Dabigatran 100 ng/mL	262.5	5.4	89%
Dabigatran 300 ng/mL	384.8	11.5	177%
40% hemodilution	153.8	2.8	11%

One can see that LMWH and dabigatran had a marked effect on the IN assay, while Apixaban and hemodilution had lesser effects in the experiment.

In summary, as a functional parameter the IN assay can be prolonged by factors other than heparin, and factor deficiencies, or other anticoagulants can lead to false-positive results.

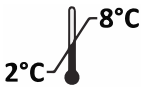
Summary of safety and performance

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
+36 30 203 3334 / info@apiro.eu / www.apiro.eu

Symbols

Symbol	Meaning
	Manufacturer
	Batch code
	Country of manufacture
	Temperature limit
	Consult instructions for use or electronic instructions for use
	Not intended for near-patient testing
	Unique device identifier
	In vitro diagnostic medical device

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
	Biological risks

References

[1] Volod O, Bunch CM, Zackariya N, Moore EE, Moore HB, Kwaan HC, Neal MD, Al-Fadhl MD, Patel SS, Wiarda G, Al-Fadhl HD, McCoy ML, Thomas AV, Thomas SG, Gillespie L, Khan RZ, Zamlut M, Kamphues P, Fries D, Walsh MM. Viscoelastic Hemostatic Assays: A Primer on Legacy and New Generation Devices. J Clin Med. 2022 Feb 7;11(3):860.

[2] Oduah EI, Linhardt RJ, Sharfstein ST. Heparin: Past, Present, and Future. Pharmaceuticals (Basel). 2016 Jul 4;9(3):38.

[3] Troisi R, Balasco N, Autiero I, Sica F, Vitagliano L. New insight into the traditional model of the coagulation cascade and its regulation: illustrated review of a three-dimensional view. Res Pract Thromb Haemost. 2023 Aug 7;7(6):102160.

[4] McLaughlin K, Rimsans J, Sylvester KW, Fanikos J, Dorfman DM, Senna P, Connors JM, Goldhaber SZ. Evaluation of Antifactor-Xa Heparin Assay and Activated Partial Thromboplastin Time Values in Patients on Therapeutic Continuous Infusion Unfractionated Heparin Therapy. Clin Appl Thromb Hemost. 2019 Jan-Dec;25:1076029619876030.

[5] Falter F, MacDonald S, Matthews C, Kemna E, Cañameres J, Besser M. Evaluation of Point-of-Care ACT Coagulometers and Anti-Xa Activity During Cardiopulmonary Bypass. J Cardiothorac Vasc Anesth. 2020 Nov;34(11):2921-2927.

[6] 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.

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

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

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

[10] Gouin-Thibault et al. Management of Therapeutic-intensity Unfractionated Heparin: A Narrative Review on Critical Points. TH Open. 2024 Oct 17;8(3):e297-e307).

[11] Gupta D, Arya V, Dass J, Gupta N, Kalra M, Sachdeva A, Kotwal J. Assessment of the phenotypic severity of hemophilia A: using rotational thromboelastometry (ROTEM) and APTT-clot waveform analysis. Blood Res. 2024 May 14;59(1):19.

[12] Schmidt DE, Chaireti R, Bruzelius M, Holmström M, Antovic J, Ågren A. Correlation of thromboelastography and thrombin generation assays in warfarin-treated patients. Thromb Res. 2019 Jun;178:34-40.

[13] Campello E, Zanetto A, Bulato C, Maggiolo S, Spiezia L, Russo FP, Gavasso S, Mazzeo P, Tormene D, Burra P, Angeli P, Senzolo M, Simioni P. Coagulopathy is not predictive of bleeding in patients with acute decompensation of cirrhosis and acute-on-chronic liver failure. Liver Int. 2021 Oct;41(10):2455-2466.

[14] Nygaard S, Hvas CL, Hvas AM, Adelborg K. In vitro Effect of Dalteparin and Argatroban on Hemostasis in Critically Ill Sepsis Patients with New-Onset Thrombocytopenia. TH Open. 2023 Jan 30;7(1):e42-e55.

[15] Henskens YMC, Gulpen AJW, van Oerle R, Wetzels R, Verhezen P, Spronk H, Schalla S, Crijns HJ, Ten Cate H, Ten Cate-Hoek A. Detecting clinically relevant rivaroxaban or dabigatran levels by routine coagulation tests or thromboelastography in a cohort of patients with atrial fibrillation. Thromb J. 2018 Feb 1;16:3.

[16] Prakash S, Wiersema UF, Bihari S, Roxby D. Discordance between ROTEM® clotting time and conventional tests during unfractionated heparin-based anticoagulation in intensive care patients on extracorporeal membrane oxygenation. Anaesth Intensive Care. 2016 Jan;44(1):85-92.

[17] Denessen EJS, Van Den Kerkhof DL, Jeurissen MLJ, Wetzels RJH, Verhezen PWM, Henskens YMC. Determining the optimal storage time and temperature for performing platelet function assays and global hemostasis assays. Platelets. 2022 Apr 3;33(3):416-424.

[18] Storm BS, Grønli RH, Pettersen K, Emblem ÅE, Joakimsen IS, Landsem A, Mollnes TE, Brekke OL. Impact of Preanalytical Storage Time and Air Exposure on ROTEM and Coagulation Sample Stability. Adv Hematol. 2026;2026:5145648. doi: 10.1155/ah/5145648.

[19] Mirus M, Buehrer E, Tiebel O, Schnabel C, Beyer-Westendorf J, Koch T, Thielecke L, Spieth PM, Heubner L. Effects of pneumatic tube systems on next-generation viscoelastic coagulation test devices in septic patients and healthy individuals: Results of the randomized controlled VETaPT trial. Sci Rep. 2026 May 28;16(1):16587.

[20] Lancé MD, Kuiper GJ, Sloep M, Spronk HM, van Oerle R, ten Cate H, Marcus MA, Henskens YM. The effects of pneumatic tube system transport on ROTEM analysis and contact activation assessed by thrombin generation test. Thromb Res. 2012 Sep;130(3):e147-50.

Version history of these instructions for use

Date	Version	Change description
2025-10-29	1	Initial version
Not released	2	Correction of REF (removal of device name next to it).
2026-07-28	3	Section “Sample collection” renamed to “Sample collection, storage and transport“ and supplemented with the information on sample stability, storage and transport. Section “Quality control” supplemented with the list of recommended QC materials and metrological traceability information. Section “Precision” supplemented with between-day precision results. Additional references were added to the section “References“.