Instructions for Use

IN Assay · REF 1031EU · UDI 59998629921031EU

IFU_1031EU_EN Rev.1

Intended Purpose

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.

CE IVD
Notified Body 2265

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 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 (direct oral anticoagulants), 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 laboratory setting.

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 using the contact activator ellagic acid for activation of the intrinsic pathway of blood coagulation on viscoelastometry analyzers.

Viscoelastometry detects coagulation initiation (CT), blood clot firmness (MCF/A20) and clot stability/fibrinolysis (ML).

Unfractionated heparin (UFH) binds to antithrombin through a specific pentasaccharide sequence, inhibiting activated clotting factors (mainly thrombin and factor Xa). In the IN assay, thrombin generation is mediated via coagulation factors XII, XI, IX, VIII, X and V.

When no UFH is present, CT is short. When UFH is present, CT is prolonged or abolished. The reagent contains ellagic acid and calcium chloride.

Materials Provided

10 sealed single-use pouches, each containing one pipet tip with reagent (dry chemistry: 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)

Storage and Stability

Condition	Detail
Refrigerated (unopened)	Store at +2 to +8 °C. Stable until expiration date on pouch label.
Room temperature (unopened)	Unopened pouches may be stored at room temperature for up to 1 month.
After opening	For immediate use within 1 minute of opening the pouch.

CAUTION: Incorrect storage conditions may affect reagent stability and lead to wrong test results.

Warnings and Precautions

CAUTION: A use of the device outside of its intended purpose may lead to the test results being incorrectly interpreted by the user.

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.

CAUTION: General precautions (e.g., wear gloves and minimize skin exposure to specimens and reagents) should be followed when handling all materials.

CAUTION: Incorrect storage conditions may affect reagent stability and lead to wrong test results.

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 risk management activities for the device:

- Off-label use: test results may be incorrectly interpreted by the user.
- Device handling errors: the patient's coagulation may be incorrectly reflected.
- Use of expired product: the patient's coagulation may be incorrectly reflected.
- Unacceptable transport and storage conditions: the patient's coagulation may be incorrectly reflected.

No undesirable side-effects were identified. No information for the patient is required.

Sample Collection

CAUTION: Collect a venous blood sample 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. Format yyyy-mm-dd.

   CAUTION: Do not use expired product.

2. Allow the reagent tip pouch to reach room temperature.
3. If sample is cold (<22 °C), allow to warm up for 5 min on the heated position of the analyzer.
4. Create the test in the analyzer software.
5. Place the Cup and Pin into the analyzer.
6. Tear open the reagent tip pouch, attach to electronic pipette, aspirate 340 µL sample.
7. Dispense the blood sample into the Cup.
8. Aspirate and dispense once again for thorough mixing. Ensure pipetting is performed without interruption.
9. Start the test.
10. The test will stop, or you can stop it 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 over time.

Result Interpretation and Expected Values

The effect of UFH is detected by the clotting time (CT). The reference range for CT was determined in a clinical study including 123 healthy individuals (aged 18.9–79.2 years; 51.2% female, 48.8% male; 95% central interval):

CT Reference Range: 113 – 164 sec

A clinical study evaluated 101 samples from 52 patients exposed to UFH. A control group of n=103 was included. CT grouped by anti-Xa activity:

Anti-Xa Group	N	Mean ± SD [sec]	Range [sec]
<0.1 U/mL	17	150 ± 28	102 – 210
0.1 – 0.3 U/mL	12	165 ± 16	136 – 196
0.3 – 1 U/mL	30	256 ± 76	149 – 389
>1 U/mL	42	2181 ± 1361	332 – 3600

Sensitivity and Specificity at Cut-off 190 sec:

Measure	Value
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

Reference Range — Other Viscoelastometry Parameters (n=123):

Parameter	Mean	2.5th – 97.5th Percentile
A5 (mm)	44.1	37 – 52
A10 (mm)	52.7	46 – 60
A20 (mm)	57.1	50 – 63
MCF (mm)	57.6	50 – 64
ML (%)	6.3	2 – 13

Precision

Precision study: 3 runs, 3 analyzers, 3 operators, 3 lots (n=54 per sample). Results for IN clotting time [sec]:

Sample	Mean [sec]	SD [sec]	CV (%)
Citrated blood	158.2	4.7	3.0%
CB + 0.5 IU/mL UFH	269.6	20.9	7.8%

Limitations and Interferences

Every in-vitro diagnostic method can deliver incorrect results under certain circumstances. Precautions should be taken to avoid misinterpretations.

Measurements with noisy curves or irregular shapes should be discarded and repeated. The clinical context and other laboratory values should be considered when interpreting the IN assay.

The IN assay mechanism is not specific to UFH. A deficiency in clotting factors (e.g., due to liver dysfunction, haemophilia, or vitamin K antagonist therapy) can prolong the clotting time. Other anticoagulants (argatroban, dabigatran, FXa antagonists) can also prolong CT.

ECMO: Extensive contact with artificial surfaces can alter clotting times and affect specificity of UFH detection.

Interference Testing Results (CT in sec, 6-fold determinations):

The following substances were tested with in-vitro addition to citrated blood. Measurements were performed in 6-fold determinations:

Substance / Condition	Mean CT [sec]	SD [sec]	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	11.5	+89%
Dabigatran 300 ng/mL	384.8	5.4	+177%
40% Hemodilution	153.8	2.8	+11%

NOTE: LMWH and dabigatran showed marked effects on the IN assay clotting time. Apixaban and hemodilution had lesser effects. Consider clinical context when interpreting prolonged CT results.

Summary of Safety and Performance

Summary of safety and performance is provided in electronic format and is available for download at www.apiro.eu/eIFU.

Manufacturer

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Australian Distributor	Haemoview Diagnostics, Australia

Symbols

Symbol	Meaning	Symbol	Meaning
Manufacturer icon	Manufacturer	Hourglass icon	Use-by date
LOT	Batch code	REF	Catalogue number
Country of manufacture (HU)	Country of manufacture	Crossed box icon	Do not use if package is damaged
2°C – 8°C	Temperature limit	No-reuse icon	Do not re-use
IFU book icon	Consult instructions for use or eIFU	Σ n	Contains sufficient for <n> tests
Not for near-patient testing	Not intended for near-patient testing	■ Caution/Warning	Caution / Warning
UDI	Unique device identifier	CE 2265	CE marking of conformity (NB 2265)
IVD	In vitro diagnostic medical device	Biohazard icon	Biological risks

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Version History

Date	Version	Change Description
2025-10-29	1	Initial version

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