Instructions for Use

HI Reagent · REF 1041EU–HI · UDI 59998629921041EU_1UA

Intended Purpose

The HI is a ready-to-use reagent for in-vitro diagnostic professional use, intended for confirmation 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 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

None identified.

Intended Users

- Trained healthcare professionals
- Trained laboratory professionals

Environment of Use

Indoors in a typical laboratory setting, equipped and designed to ensure standard electrical connections, adequate lighting, and standard environmental settings regarding temperature, humidity, and pressure to ensure the functionality of typical electrical devices including electrical medical devices and personal computers.

Intended Patient Population

Adult patients suspected to be exposed to unfractionated heparin.

Principle of the Assay

The HI assay is a functional whole-blood based assay to be used on viscoelastometry analyzers which uses the contact activator ellagic acid for activation of the intrinsic coagulation pathway and the enzyme heparinase I for inactivation of heparin present in the sample.

Viscoelastometry allows detection of whole blood clot formation and thus detects: coagulation initiation (clotting time, CT), blood clot firmness (maximum clot firmness, MCF, or A20 — amplitude 20 minutes after CT), and clot stability or fibrinolysis (maximum lysis, ML).

Unfractionated heparin (UFH) is an anticoagulant that binds to antithrombin through a specific pentasaccharide sequence, causing a conformational change that enhances antithrombin's ability to inactivate clotting factors, mainly thrombin (factor IIa) and factor Xa, and to a lesser extent factors IXa, XIa, and XIIa. Heparinase I digests the heparin polymer into smaller fragments that no longer increase antithrombin's anticoagulant action.

In the HI assay, thrombin generation is mediated via coagulation factors XII, XI, IX, VIII, X, and V. The reagent also contains calcium chloride for recalcification of the citrated blood sample — a combination commonly used in viscoelastometry tests such as the hep-tem® or HI-test assays.

When no UFH is present, the CT of both the IN and HI assays is short. In the presence of UFH, the CT of the IN assay is prolonged or abolished entirely, while the HI test remains largely unaffected. The combination of IN and HI assays therefore confirms the presence of heparin, or detects that prolongation of the IN assay has a cause other than heparin.

Materials Provided

10 sealed single-use pouches, each containing one pipet tip with reagent providing a dry chemistry reagent composed of ellagic acid, recombinant heparinase I, 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-second aspiration/dispensing cycles
- Blood collection tube (3.2% sodium citrate) for coagulation testing
- IN assay

Reagent Preparation

The reagent is ready to use.

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

For professional use by trained personnel only.

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 must 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 minimise 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 risk management activities for the device:

- In case of off-label use, test results may be incorrectly interpreted by the user.
- In case of device handling errors, patient coagulation may be incorrectly reflected.
- In case of use of an expired product, patient coagulation may be incorrectly reflected.
- In case of unacceptable transport and storage conditions, patient coagulation may be incorrectly reflected.

Warnings related to residual risks are provided throughout this document. No undesirable side-effects were identified during 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 recommended procedures using a blood collection tube with 3.2% sodium citrate. Samples should be analysed within 3 hours from blood collection. Store 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. Use of an 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), allow the sample to warm up for 5 minutes on the heated position of the viscoelastometry analyzer.
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 reagents with the blood sample. Ensure sample pipetting is performed without interruption.
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 HI assay over time.

Result Interpretation and Expected Values

The effect of unfractionated heparin on the HI assay is detected by the clotting time (CT). The HI test is applied in combination with the IN test and a ratio is formed:

IN/HI ratio: CT-IN / CT-HI

The reference range was determined in a clinical study including 123 healthy individuals (aged 18.9–79.2 years; 51.2% female, 48.8% male). Reference ranges were determined using the 95% central interval (2.5^{th} – 97.5^{th} percentile):

Parameter	Mean	2.5th – 97.5th Percentile
IN/HI Ratio	0.97	0.88 – 1.09
HI CT [sec]	137.7	110 – 167

The ability of the HI assay to detect UFH at therapeutic level (≥0.3 anti-Xa U/mL) was evaluated in a clinical study including 101 samples from patients exposed to UFH. In 54 patients, the anti-Xa level was >0.3 U/mL.

HI-CT grouped by anti-Xa value:

Anti-Xa Group	N	Mean ± SD	Range (min–max)
<0.1 U/mL	17	148.1 ± 25.5	110 – 206
0.1 – 0.3 U/mL	30	153.8 ± 12.3	135 – 186
0.3 – 1 U/mL	12	168.7 ± 23.3	136 – 210
>1 U/mL	42	189.1 ± 32.5	137 – 315

IN-CT/HI-CT grouped by anti-Xa value:

Anti-Xa Group	N	Mean ± SD	Range
<0.1 U/mL	17	1.01 ± 0.08	0.88 – 1.17
0.1 – 0.3 U/mL	30	1.07 ± 0.07	0.91 – 1.22
0.3 – 1 U/mL	12	1.51 ± 0.39	1.04 – 2.20
>1 U/mL	42	11.61 ± 7.45	2.10 – 24.8

Expected Values for Heparin Group (anti-Xa ≥0.3 U/mL, n=54)

In samples with heparin concentrations ≥0.3 U/mL (n=54), the expected values are as follows:

Parameter	N	Mean ± SD	Range (min–max)
HI-CT [sec]	54	184.59 ± 31.65	136 – 315
IN-CT/HI-CT Ratio	54	9.37 ± 7.81	1.04 – 24.8

Diagnostic Performance

When the patient group (n=101) and control group (n=123) are analysed together using a cut-off of ≥1.1 for the IN/HI ratio, the diagnostic performance is as follows:

Measure	Value
Sensitivity	96%
Specificity	91%
Positive predictive value (PPV)	78%
Negative predictive value (NPV)	99%
Positive likelihood ratio (LR+)	10.8
Negative likelihood ratio (LR–)	0.04

Reference Range — Other Viscoelastometry Parameters

Percentile	A5 [mm]	A10 [mm]	A20 [mm]	MCF [mm]	ML [%]
2.5th	37	45	50	50	2
97.5th	50	58	62	63	14

Precision

In a precision study, citrated blood with and without the addition of 3 anti-Xa U/mL UFH was tested in 3 runs, on three analyzers, three operations, and including 3 HI assay lots (54 determinations per sample). Results for HI clotting time [sec]:

Sample	Mean [sec]	SD [sec]	CV (%)
Citrated blood (CB)	171.8	7.2	4.2%
Citrated blood + 3 IU/mL UFH	169.8	5.3	3.1%

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 (when available) should be considered when interpreting the HI assay. Generally, consider repeating a measurement that gives a surprising or implausible result.

A deficiency in clotting factors (e.g., due to liver dysfunction, haemophilia, or vitamin K antagonist therapy) can prolong the clotting time in ellagic acid-triggered viscoelastometry. The clotting time can also be prolonged by anticoagulants other than UFH, such as argatroban, dabigatran, or FXa antagonists.

Extensive contact of blood with artificial surfaces during extracorporeal membrane oxygenation (ECMO) can lead to altered clotting times and therefore affect the specificity of UFH detection using viscoelastometry.

Interference Testing Results:

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)	146.8	4.1	n/a
LMWH 1 IU/mL	155.5	4.4	+5.9%
Apixaban 100 ng/mL	156.8	2.9	+6.8%
Apixaban 300 ng/mL	166.0	3.6	+13.1%
Dabigatran 100 ng/mL	255.2	15.1	+73.8%
Dabigatran 300 ng/mL	398.5	5.1	+171.4%
40% Haemodilution	157.0	4.0	+6.9%

NOTE: Dabigatran had a marked effect on the HI assay clotting time, while LMWH, apixaban, and haemodilution had lesser effects. The HI assay clotting time can be prolonged by non-heparin anticoagulants and factor deficiencies.

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

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