Instructions for use – HI

REF 1041EU – HI UDI 59998629921041EU_1UA

Intended purpose

CE 2265 IVD **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.**

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

None identified.

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 HI 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 and the enzyme heparinase I for the inactivation of heparin present in the sample.

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. Heparinase I digests the long, highly sulfated heparin polymer into smaller fragments that no longer can increase the anticoagulant action of antithrombin.

In the HI assay thrombin generation is mediated via the coagulation factors XII, XI, IX, VIII, X and V [3]. In addition to ellagic acid and heparinase the reagent also contains calcium chloride for the recalcification of the citrated blood sample. The use of a combination of ellagic acid, heparinase and calcium chloride is commonly used in viscoelastometry tests such as the hep-tem® or HI-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 both the IN and HI assays is short. Unfractionated heparin inhibits the coagulation activation via the intrinsic pathway. Therefore, in the presence of UFH the CT of the IN assay is prolonged or abolished entirely, while the HI test remains largely unaffected by the heparin. Using the combination of the IN and HI assays, one can therefore confirm the presence of heparin, or detect that the prolongation of the IN assay has a cause other than heparin.

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 [6]. Due to their mode of action such assays are sensitive for 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, recombinant heparinase I and calcium chloride. Each pouch contains one desiccant bag.

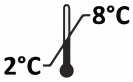
Additional materials and devices required

- Viscoelastometry analyzer and receptacles (Cups & Pins),
- Electronic pipette for 340 µL with 3 sec 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



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

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. 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. 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 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). In order to detect the effect of unfractionated heparin the HI test is applied in combination with the IN test and a ratio is formed between the CT of the IN assay and the CT of the HI assay:

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

The reference range for the HI assay and the IN/HI ratio was determined in a clinical study including 123 healthy individuals, aged 18.9 - 79.2 years, 51.2% female and 48.8% male. In 121 samples both the HI and IN assay results were available, and the reference ranges were determined using the 95% central interval (2.5° percentile – 97.5° percentile):

	IN/HI	CT [sec]
mean	0.97	137.7
2.5° - 97.5°	0.88-1.09	110-167

The ability of the HI assay to detect unfractionated heparin in a therapeutic level (>=0.3 anti-Xa U/ml) [10] when used in combination to the IN assay was evaluated in a clinical study which included 101 samples from 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 patients the anti-Xa level was >0.3 U/ml. In 18 samples no clotting was detected, and the clotting time was set as 3600 sec for the subsequent analysis.

When the HI assay CT results and the IN-CT / HI-CT results are grouped according to the anti-Xa results, the following mean values and ranges are found:

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

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

One can see in the first table (HI-CT) that the heparinase does not completely eliminate the effect of the heparin when heparin levels are high. This is due to the nature of heparinase as an enzyme which takes some time to inactivate large amounts of heparin. However, one can see that this does not disturb the detection of heparin by the IN-CT/HI-CT ratio.

In samples with heparin concentrations >=0.3 U/mL (n=54) the expected values for the HI-CT and IN-CT/HI-CT are as follows:

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

When the patient group (n=101) and the control group (reference subjects, n=123) are analyzed together using a cut-off of ≥ 1.1 for the ratio CT-IN / CT-HI (IN/HI ratio) [11], the sensitivity, specificity, positive predictive value and negative predictive values were as follows:

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

The results of the other viscoelastometry parameters in the reference range study was as follows:

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

Precision

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

	mean	SD	CV
citrated blood (CB)	171.8	7.2	4.2%
citrated blood (CB) + 3 IU/mL UFH	169.8	5.3	3.1%

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 HI assay. Generally, consider repeating a measurement that gives a very surprising or otherwise implausible result.

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 [12-14]. 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 [15-16].

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 [17].

The effect of 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)	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	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% hemodilution	157	4	6.9%

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

In summary, 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 on www.apiro.eu/eIFU

Manufacturer

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

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Version history of these instructions for use

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