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CE 0123

PATHFAST™ hs-cTnI

<REAGENT FOR PATHFAST>
60 Tests

English

▪ Intended use

PATHFAST hs-cTnI is a product for in-vitro diagnostic use with the in vitro diagnostic (IVD) automated analyser PATHFAST for the quantitative measurement of cardiac Troponin I (cTnI) in human heparinized or EDTA whole blood and plasma. PATHFAST hs-cTnI is intended to be used:

- as an aid in the diagnosis of acute coronary syndromes (ACS),
- in the risk stratification in patients presenting with suspected ACS,
- by laboratory technician, nurse or physician,
- in hospital including emergency room, doctor's office and clinical laboratory.

PATHFAST hs-cTnI is a device for near patient testing (NPT).

▪ Summary

Troponin complex consisting of three subunits I, T and C transmits intercellular calcium signal to actin myosin interaction and regulates the contraction of striated muscle (1). Cardiac isoforms of troponin I/T are expressed only in myocardium, differs from skeletal troponin I/T and are a very sensitive and specific marker for myocardial cell damage. An increasing number of patients with suspected ACS is a burden for emergency departments worldwide (2). In 2015 European Society of Cardiology (ESC) Guidelines recommended the use of cTn for diagnosis of NSTEMI with 99th percentile as the cut off and for more accurate measurement of low troponin concentrations, the use of high sensitivity cTn (hs-cTn), if the hs-cTn assay that cleared the high sensitivity criteria recommended by IFCC is available (3, 4). The introduction of hs-cTn assays enabled to develop the algorithm of fast triage of patients presenting with suspected ACS (5). 2020 ESC guidelines advise a Class I recommendation, two serial measurements of hs-cTn, on admission (0 h) and after 1 h (2 h), if there are the validated cut offs for 0 h/1 h (0 h/2 h) algorithm of the assay, with which a large proportion of the patients can be safely triaged for either rule-out for discharge or rule in for lifesaving management (6).

▪ Test principle

The PATHFAST hs-cTnI procedure is based on chemiluminescent enzyme immunoassay (CLEIA) and MAGTRATION. All required components for performing the testing are packed in one reagent cartridge. By loading PATHFAST hs-cTnI into the in vitro diagnostic system PATHFAST, cTnI can be accurately quantified within 17 min. In this procedure, alkaline phosphatase labelled anti-cTnI monoclonal antibody (MoAb) and anti-cTnI MoAb coated magnetic particles are mixed with the sample. cTnI contained in the specimen binds to the anti-cTnI antibodies forming an immunocomplex with enzyme labelled antibody and antibody coated magnetic particles. After removing the unbound enzyme labelled antibody, a chemiluminescent substrate is added to the immunocomplex. After a short incubation, the luminescence generated by enzyme reaction is detected. The cTnI concentration in the specimen is calculated by means of a standard curve.

*"MAGTRATION" is technology of B/F separation where magnetic particles are washed in a pipette tip and is a trademark or registered trademark of Precision System Science Co., Ltd.

▪ Package composition of materials provided

Reagent cartridge 6 cartridges x 10 trays

The reagent cartridge consists of 16 wells. All wells with the exclusion of the sample well (# 1) and counting well (# 10) are covered with an aluminium seal having a bar code. All reagents for the test are filled in each well of the reagent cartridge. Do not reuse a reagent cartridge. This is designed for single use only.

Wells	Form	Ingredient	Quantity	Source
# 1	Empty	Sample well	-	-
# 2	Liquid	Alkaline phosphatase conjugated anti-cTnI MoAb, Na azide (< 0.1%)	50 µL	Calf intestine Mouse
# 7	Liquid	anti-cTnI MoAb coated magnetic particles	50 µL	Mouse
# 13	Liquid	Chemiluminescent substrate, CDP-Star	100 µL	-
# 11	Liquid	Sample dilution buffer Na azide (< 0.1%)	50 µL	-
# 3, 4, 5	Liquid	Washing buffer Na azide (< 0.1 %), Triton X-100 (< 0.1%)	400 µL	-

1, 6, 8, 9, 10, 12, 14, 15, 16 are empty wells.
"CDP-Star" is a trademark or registered trademark of Applied Biosystems, LLC.

Calibrator 1 (CAL-1)	2.0 mL x 1 bottle (liquid, Na azide < 0.1%)
Calibrator 2 (CAL-2)	For 1.0 mL x 2 vials (lyophilized)
Calibrator diluent	1.0 mL x 2 bottles (liquid, Na azide < 0.1%)
MC ENTRY CARD	1 sheet
Instruction for use	1 sheet
CONTROL DATA SHEET	2 sheets

Materials required but not provided

PATHFAST Analyser (Product #: 300929) and consumables
PATHFAST TIP (Product #: 300936)
PATHFAST WASTE BOX (Product #: 300950)
hs-cTnI Quality Control Materials
PATHFAST SAMPLE DILUENT 2 (Product #: PF02D)

▪ Precautions and warnings

1. Do not peel off the aluminium seal of the reagent cartridge.
2. Handle the reagent cartridge by holding the edge of it and do not touch the aluminium seal or the black well with your fingers.
3. When the reagent cartridge is dropped and damaged, do not use it.
4. Avoid contamination of saliva in the black well.
5. Avoid contamination of foreign substances such as fungi, bacteria and detergent into the specimen.
6. After a certain period of storage or shipment, there may be some reagents adhered to the aluminium seal. If such a condition is observed, gently tap the cartridge on the table before use.
7. Store the reagent cartridges in an upright position at all times.
8. CAL-2 contains human serum. Although the used raw materials were negative for HBs antigen, HIV antibody and HCV antibody, it should be handled as infectious due to a risk of infections.
9. Used reagent cartridges contain bodily fluids. Handle with appropriate care to avoid skin contact and injection.
10. Azide can react with copper and lead used in some plumbing systems to form explosive salts. When disposing of azide-containing materials, they should be flushed away with large volumes of water.
11. Dispose of all measured reagents and materials according to the standard disposal method. For example, autoclave at 121 °C for 20 minutes. Follow general precautions and handle all components as if capable of transmitting infectious agents.
12. The PATHFAST reporting system contains error codes to warn the operator of specific malfunctions. Any reports containing such error codes should be held for follow-up. See the PATHFAST operator's manual.
13. Patient samples may contain heterophilic antibodies that could react in immunoassay to give a falsely high or low result. This assay has been designed to minimize interference from heterophilic antibodies. Nevertheless, complete elimination of this interference from all patient specimens cannot be guaranteed. A test result that is inconsistent with the clinical picture and patient history should be interpreted with caution.
14. The results should be evaluated in context of all laboratory findings and the total clinical status of the patient. In cases where the laboratory results do not match the clinical picture or history, additional tests should be performed.
15. When any serious incident occurs in relation to the product, report to the manufacturer and the competent authority in which the user and/or the patient is located.

Storage and expiration

1. Store at 2 - 8 °C.
2. Store the cartridge tray with the label side up.
3. Avoid water damage during storage.
4. Do not open the cartridge tray until just before use.
5. Avoid contamination and exposure to direct sunlight.
6. CAL-1 can be used until the expiration date after opening.
7. CAL-2 is stable for 3 days at 2 - 8 °C and 1 month at -20 °C or lower after reconstitution.
8. The expiration date is listed on each reagent cartridge and kit box label.
9. Do not use reagents beyond the indicated expiration date.

Sample collection

Use whole blood or plasma collected with qualified collection tube containing Na-heparin, Li-heparin or EDTA.

Sample stability

Whole blood sample must be stored at 2 to 25 °C and analysed within 4 hours after collection.

Heparinized plasma samples are stable under the conditions bellow:

2 to 25 ˚C:	24 hours
-20 ˚C or lower:	1 month (freeze only once)

EDTA plasma samples are stable under the conditions bellow:

2 to 8 ˚C:	24 hours
15 to 25 ˚C:	6 hours
-20 ˚C or lower:	2 months (freeze only once)

Sample volume: 100 µL

▪ Preparation and procedure

Refer to the PATHFAST operator's manual for detailed information of the analyser operation.

Reagent preparation

1. Reagent cartridge: Ready to use.
2. CAL-1: Ready to use. (Limited to use with reagent of the same lot.)
3. CAL-2: Transfer the whole volume of one bottle of calibrator diluent into one vial of CAL-2. Do not use different lots of calibrator diluent to dissolve CAL-2. Stand for 15 minutes at room temperature after the reconstitution. Mix gently and ensure that calibrator is completely dissolved. (Limited to use with reagent of the same lot.)

Installation of master calibration curve

1. Installation of a master calibration curve is necessary when a new reagent lot is used.
2. Install the master calibration curve by reading the bar code on MC ENTRY CARD, which is enclosed in each package, with the hand-held bar code reader of PATHFAST.

User calibration

1. User calibration is necessary when a new reagent lot is used after installation of the master calibration curve from MC ENTRY CARD.
2. User calibration is also necessary every 4 weeks after the first user calibration. (MC ENTRY CARD is not required.)
3. The calibrators, CAL-1 and CAL-2, must be tested both in duplicate. Therefore, 4 reagent cartridges, two for CAL-1 and two for CAL-2 are necessary for user calibration.
4. Place the reagent cartridges in the cartridge rack, and then dispense approximately 100 µL of CAL-1 and CAL-2 in sample wells to load onto PATHFAST.
5. Push the START button of PATHFAST and perform assay for the calibration.

Quality Control assay (QC assay)

1. QC assay is indispensable for assuring validity of sample results. QC assay is performed after every calibration to check the calibration curves and to obtain data from QC samples for quality control. After each calibration, with each new shipment of previously calibrated test kit, or whenever the institution wishes to verify the performance of the system, analyse two levels of quality control material with known concentrations of cTnI.
2. Good laboratory practice recommends the use of appropriate quality controls. It is recommended to follow national, federal, and local guidelines for quality control. If controls do not perform as expected, do not use the test results. Repeat the test or call your authorized PATHFAST distributor for technical service.

Sample assay

1. Place the reagent cartridge in the cartridge rack, then dispense approximately 100 µL of sample into a sample well of a cartridge.
2. Load the cartridge rack onto PATHFAST and push the START button of PATHFAST to perform sample assay.

Note

1. When a whole blood sample is used, the whole blood contained in a blood collection tube should be mixed gently just before dispensing. (Do not use a vortex mixer.) After dispensing the whole blood sample and loading the cartridge on PATHFAST, the assay must be started immediately.
2. When fibrin threads or clots and other insoluble materials are present in the plasma sample, such material must be removed by centrifugation or filtration.
3. When samples are left for more than 5 minutes after dispensing into a sample well, a lower result will be obtained analysing whole blood because of blood sedimentation and a higher result will be obtained analysing plasma because of increasing cTnI concentration by evaporation.
4. When a whole blood sample is used, input of an individual haematocrit value of the sample in PATHFAST is optional.
5. Samples with results above 50000 ng/L should be diluted with sample diluent (Product#: PF02D) and retested if a quantitative result is desired or alternatively, they can be reported as > 50000 ng/L.

▪ Specific performance data

Representative performance data on the PATHFAST are given below.

Metrological traceability

The calibrator for PATHFAST hs-cTnI is traceable to the reference material NIST Standard Reference Material for Human Cardiac Troponin Complex SRM2921 of the National Institute of Standard and Technology in USA which certified concentration for human cTnI.

Precision (repeatability)

Precision was assessed with whole blood and plasma samples at each 5 concentration levels. The samples were tested in 20 replicates. The following results were obtained.

Whole blood	Mean (ng/L)	S.D. (ng/L)	C.V. (%)
Level-1	20.8	1.73	8.3
Level-2	168	10.0	6.0
Level-3	713	49.5	6.9
Level-4	12180	781	6.4
Level-5	43447	2433	5.6

Plasma	Mean (ng/L)	S.D. (ng/L)	C.V. (%)
Level-1	21.6	1.87	8.7
Level-2	176	7.45	4.2
Level-3	578	23.8	4.1
Level-4	14188	604	4.3
Level-5	42034	1974	4.7

Precision (reproducibility)

Plasma samples at 8 concentration levels within the measurement range were assayed in duplicate in each run, 2 runs per day, for 20 days with 1 reagent lot on 1 instrument, for a total of 40 runs. The within-run and total coefficient of variations (C.V.) were calculated with standard deviations (S.D.) according to the CLSI EP5-A2 protocol. The following results were obtained.

Sample	Mean (ng/L)	Within-run precision		Total precision	
		S.D. (ng/L)	C.V. (%)	S.D. (ng/L)	C.V. (%)
Level-1	21.3	1.25	5.9	1.55	7.3
Level-2	25.9	1.27	4.9	1.31	5.1
Level-3	34.9	1.56	4.5	1.72	4.9
Level-4	44.9	1.43	3.2	2.01	4.5
Level-5	180	9.18	5.1	11.0	6.1
Level-6	575	21.5	3.7	37.4	6.5
Level-7	14292	623	4.4	787	5.5
Level-8	41750	2153	5.2	2304	5.5

Analytical sensitivity

Limit of blank (LoB): 1.23 ng/L

Limit of detection (LoD): 2.33 ng/L

Limit of quantitation (LoQ): 14.2 ng/L (C.V. 10%)

The C.V. value at the 99th percentile concentration (29 ng/L) is 6.6%.

Linearity

cTnI antigen was spiked into plasma at 3 concentration levels (85.0, 7154, 55931 ng/L). The samples were serially diluted with 10-fold using PATHFAST SAMPLE DILUENT 2 and assayed.

The recovery rate against the theoretical value was within 92 - 103% up to 55931 ng/L.

Assay range: 2.33 - 50000 ng/L

The assay range was set from the results of LoD and linearity.

High dose hook effect

cTnI antigen (approximately 44900000 ng/L) was serially diluted and assayed. There was no high dose hook effect for the samples with their cTnI values up to 44900000 ng/L.

Analytical specificity

Interference of endogenous substances

The following factors were found to have an effect of less than 10% on the assay at the concentrations indicated in parentheses.

Free bilirubin	(60 mg/dL)
Conjugated bilirubin	(60 mg/dL)
Lipemia	(3000 FTU)

Triglyceride	(1000 mg/dL)
Haemoglobin (haemolysis)	(1000 mg/dL)
Rheumatoid Factor	(500 IU/mL)

Interference of exogenous substances

The following drugs which might be used in target patients were found to have an effect of less than 10% on the assay at the concentrations indicated in parentheses.

Acetaminophen	(20 mg/dL)
Acetylsalicylic acid	(0.3 ng/mL)
Allopurinol	(2.5 mg/dL)
Ampicillin	(5 mg/dL)
Ascorbic acid	(3 mg/dL)
Atenolol	(1 mg/dL)
Caffeine	(10 mg/dL)
Captopril	(5 mg/dL)
Digoxin	(5 ng/mL)
Dopamine	(65 mg/dL)
Erythromycin	(20 mg/dL)
Furosemide	(2 mg/dL)
Methyldopa	(2.5 mg/dL)
Nifedipine	(6 mg/dL)
Phenytoin	(10 mg/dL)
Theophylline	(25 mg/dL)
Verapamil	(16 mg/dL)

Cross-reactivity

The following substances have no significant cross-reactivity on the assay at the concentration indicated in parentheses.

cTnC	(1000 ng/mL)
cTnT	(1000 ng/mL)
sTnI	(1000 ng/mL)

Reactivity to various troponin forms was calculated to the result of cTnI-T-C complex. The assayed values of each troponin form listed below are within ±20% of ternary cTnI-T-C complex (21744 ng/L).

Free cTnI
Dephosphorylated cTnI
Phosphorylated cTnI

Correlation between samples of plasma and whole blood

x	y	Anticoagulants	n	Slope	Intercept	r
Plasma	Whole blood	Li-heparin	68	0.962	0.415	0.995
		Na-heparin	68	0.964	0.000	0.993
		EDTA-2K	68	0.985	0.018	0.990
		EDTA-2Na	68	0.972	0.000	0.992

The regression equation was calculated by Passing-Bablok fit.

Expected values

1. Reference limit
   Using the PATHFAST hs-cTnI assay, the calculated value for the 99th percentile for cTnI in heparin plasma samples of 490 apparently healthy individuals was 29 ng/L.
2. Measurable normal value and gender specificity
   The gender specific 99th percentile and the measurable number of healthy subjects between LoD and 99th percentile was identified. The measurable number of healthy subjects between LoD and 99th percentile was 487 (66.3%) from 734 (Males: 382, Females: 352) healthy subjects with exclusion criteria: age < 18, HbA1c ≥ 6.5%, NT-proBNP ≥ 125 ng/L < 75 years, NT-proBNP ≥ 450 ng/L ≥ 75 years, eGFR < 60 mL/min/1.73m^2. As the result of this study, PATHFAST hs-cTnI was classified as a high sensitivity cardiac troponin assay (level - 2) defined by IFCC (4, 7).

	n	Gender specific 99th percentile (ng/L)	Measurable concentrations > LoD (%)
Overall	734	27.9	66.3
Males	382	29.7	78.8
Females	352	20.3	52.8

The expected values/reference values may vary from laboratory to laboratory and from country to country depending on various factors. It is therefore recommended for each institution to establish corresponding expected/reference values.

Diagnostic performance criteria

To identify cTnI cut off values for 0 h/1 h algorithm recommended by 2015 and 2020 ESC Guidelines (3,6), cTnI concentration were measured using the PATHFAST hs-cTnI assay in EDTA plasma samples obtained at 0 h and 1 h after admission to the chest pain unit (CPU) from 1221 patients with suspicion of NSTEMI (669 for derivation and 610 for validation). As the result of the combination of two cohorts the identified cut offs for 0 h rule-out and 0 h/1 h rule-out showed 100% and 99.7% NPV respectively, and for 0 h/1 h rule-in showed 80.1% PPV. In total more than 62% patients could be triaged (5).

0 h Rule-out algorithm of patients with symptom ≥ 3 h before presentation

Cut off	n	NPV (%)	Sensitivity (%)	Specificity (%)	Ruled-out (%)
< 3ng/L	792	100	100	46.5	37.2

0/1 h Rule-out algorithm

Cut off	n	NPV (%)	Sensitivity (%)	Specificity (%)	Ruled-out (%)
< 4 ng/L and Δ0 - 1h < 3ng/L	1221	99.7	99.1	58.1	47.2

0/1 h Rule-in algorithm

Cut off	n	NPV (%)	Sensitivity (%)	Specificity (%)	Ruled-in (%)
≥ 90 ng/L or Δ0 - 1 h ≥ 20 ng/L	1221	80.1	65.7	96.2	15.6

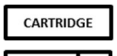
▪ References

1. Park KC, Gaze DC, Collinson PO, Marber MS. Cardiac troponins: from myocardial infarction to chronical disease. Cardiovascular Research 2017; 113:1708-1718.
2. Niska R, Bhuiya F, Xu J. National Hospital ambulatory medical care survey: 2007 emergency department summary. Natl Health Stat Report 2010;1-31.
3. Roffi M, Patrono C, Collet J-P, Mueller C, Valgimigli M, Andreotti F, et al. 2015 ESC Guidelines for the management of acute coronary syndromes in patients presenting without persistent ST-segment elevation. Eur Heart J 2016; 37:267-315.
4. Apple FS, Jaffe AS, Collinson P, et al. IFCC educational materials on selected analytical and clinical applications of high sensitivity cardiac troponin assays. Clin Biochem. 2015; 48:201-203.
5. Sörensen NA, Neumann JT, Ojeda F, et al. Diagnostic evaluation of a high-sensitivity troponin I point-of-care I assay. Clin Chem 2019; (65): 1592-1601
6. Collet J-P, Thiele H, Barbato E, et al., 2020 ESC Guidelines for the management of acute coronary syndromes in patients presenting without persistent ST-segment elevation. Eur Heart J 2020; 00:1-79.
7. Christenson et al., Validation of high-sensitivity performance for a United States Food and Drug Administration cleared cardiac troponin I assay. Clin Biochem. 2018; 56:4-10.

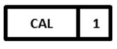
Symbols

LSI Medience Corporation uses the following symbols and signs in addition to those listed in the EN ISO 15223-1:2021 (Medical devices - Symbols to be used with information to be supplied by the manufacturer - Part 1: General requirements).

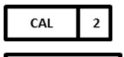
This symbol means "Device for near patient testing".
(Symbols for self-testing and near-patient testing under the IVD Regulation 2017/746/EU. MedTech Europe. Dec. 13, 2018)



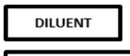
: Reagent cartridge



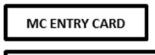
: Calibrator 1



: Calibrator 2



: Calibrator diluent



: Entry card for master calibration curve

: Datasheet for control

* PATHFAST: JP Registered Trademark No.5982733

Summary of safety and performance is available from:
European Database on Medical Devices (EUDAMED).

Contact for technical assistance
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