REF PF1031-K IVD

Issue Date: 01/11/2022
Revision: ver.2

CE 0123

PATHFAST™ CK-MB

<REAGENT FOR PATHFAST>

60 Tests

English

▪ Intended use

PATHFAST CK-MB is a product for in-vitro diagnostic use with the in vitro diagnostic (IVD) automated analyser PATHFAST for the quantitative measurement of Creatine kinase isozyme MB (CK-MB) in human heparinized or EDTA whole blood and plasma.
PATHFAST CK-MB is intended to be used:
- as an aid to the diagnosis of acute myocardial infarction (AMI),
- by laboratory technician, nurse or physician,
- in hospital including emergency room, doctor's office and clinical laboratory.

PATHFAST CK-MB is a device for near patient testing (NPT).

▪ Summary

Creatine kinase (CK) is a key enzyme of energy metabolism in muscle that catalyses the reversible phosphorylation of creatine. This dimeric enzyme has two subunits, M and B, which associate to form three isozymes, CK-MM, CK-MB and CK-BB. CK-MM and CK-BB are distributed primarily in the skeletal muscle and in the brain, respectively. CK-MB is found predominantly in cardiac muscle. Damage to the myocardium results in a transient and progressive release of CK-MB into the circulation. The CK-MB concentration increases at 3 - 12 hours after onset of chest pain reaching a peak at 24 hours and then returning to normal levels within 48 - 72 hours. This characteristic temporal pattern is useful in the diagnosis of myocardial infarction. Although the cardiac-specific troponins are now considered the biochemical markers of choice in the evaluation myocardial infarction, if troponin is not available, CK-MB can also be used as a marker to aid in the diagnosis of myocardial infarction. The low concentration of CK-MB in plasma of healthy subjects and non-cardiac tissues contributes to its widely accepted use as an aid for diagnosing and monitoring of myocardial injury (1-4).

▪ Test principle

The PATHFAST CK-MB 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 CK-MB into the in vitro diagnostic system PATHFAST, CK-MB can be accurately quantified within 17 min (5). In this procedure, alkaline phosphatase labelled anti-CK-BB monoclonal antibody (MoAb) and anti-CK-MB MoAb coated magnetic particles are mixed with the sample. CK-MB contained in the specimen binds to the anti-CK-BB and anti-CK-MB 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 CK-MB 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-CK-BB MoAb, Na azide (< 0.1%)	50 µL	Calf Mouse
# 7	Liquid	anti-CK-MB 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

Materials required but not provided
PATHFAST Analyser (Product #: 300929) and consumables
PATHFAST TIP (Product #: 300936)
PATHFAST WASTE BOX (Product #: 300950)
CK-MB Quality Control Materials
PATHFAST SAMPLE DILUENT 1 (Product #: PF01D)

▪ 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 4 days at 2 - 8 °C and 3 months 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 samples must be stored at 2 to 25 °C and analysed within 4 hours after collection.
Plasma samples are stable under the conditions below:

2 to 25 °C:	24 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 CK-MB.
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 CK-MB 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 result above 500 ng/mL should be diluted with sample diluent (Product #: PF01D) or saline solution and retested if a quantitative result is desired or alternatively, they can be reported as > 500 ng/mL.

▪ Specific performance data

Representative performance data on the PATHFAST are given below.

Metrological traceability
The calibrator for PATHFAST CK-MB is traceable to the reference material IRMM/IFCC-455 of the Institute for Reference Materials and Measurements (IRMM), Geel, Belgium to which values for CK-MB (mass) have been assigned.

Precision (repeatability)
Precision was assessed with whole blood and plasma samples at each 3 concentration levels. The samples were tested in 20 consecutive replicates. The following results were obtained.

Whole blood	Mean (ng/mL)	S.D. (ng/mL)	C.V. (%)
Level-1	16.6	0.831	5.0
Level-2	65.6	3.29	5.0
Level-3	274	13.7	5.0

Plasma	Mean (ng/mL)	S.D. (ng/mL)	C.V. (%)
Level-1	16.5	0.805	4.9
Level-2	65.1	2.73	4.2
Level-3	248	6.99	2.8

Precision (reproducibility)
Plasma samples at 3 concentration levels within the measurement range were assayed in duplicate in each run, 1 run per day, for 20 days with 1 reagent lot on 1 instrument, for a total of 20 runs. The within-run and total coefficient of variations (C.V.) were calculated with standard deviations (S.D.) according to the CLSI EP5-A protocol. The following results were obtained.

Sample	Mean (ng/mL)	Within-run precision		Total precision	
		S.D. (ng/mL)	C.V. (%)	S.D. (ng/mL)	C.V. (%)
Level-1	4.27	0.232	5.4	0.322	7.5
Level-2	58.0	2.29	3.9	3.37	5.8
Level-3	338	10.7	3.2	22.0	6.5

Analytical sensitivity
Limit of blank (LoB): 0.012 ng/mL
Limit of detection (LoD): 0.106 ng/mL
Limit of quantitation (LoQ): 0.569 ng/mL (C.V. 10%)

Linearity
CK-MB antigen was spiked into plasma at 4 concentration levels (42.4, 137, 258, 584 ng/mL). The samples were serially diluted with 5-fold using saline and assayed. The recovery rate against the theoretical value was within 88 - 109% up to 500 ng/mL.

Assay range: 2 - 500 ng/mL
The assay range was set from the results of LoQ and linearity.

High dose hook effect
Sample with CK-MB antigen concentration of approximately 33000 ng/mL was serially diluted and assayed. There was no high dose hook effect for the samples with their CK-MB values up to 33000 ng/mL.

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.

CK-BB	(5000 ng/mL)
CK-MM	(50000 ng/mL)

Correlation between samples of Li-heparin plasma and other sample matrices

x	y		n	Slope	Intercept	r
Li-heparin Plasma	Li-heparin	Whole blood	58	0.982	0.036	0.989
	Na-heparin	Plasma	58	1.00	-0.149	0.997
		Whole blood	58	0.965	-0.052	0.992
	EDTA-2Na	Plasma	58	1.02	0.403	0.998
		Whole blood	58	1.00	0.747	0.989
	EDTA-2K	Plasma	58	1.02	0.192	0.995
		Whole blood	58	1.00	0.301	0.990

The regression equation was calculated by Passing-Bablok fit.

Method comparison
y= 1.69x − 0.825, r= 0.995, n= 105 (plasma samples, y: PATHFAST CK-MB, x: Stratus CS CKMB TestPak, Passing-Bablok fit).

Expected values
Reference interval
The reference interval for the CK-MB assay was determined by testing 302 apparently healthy individuals. The 95% interval ranging from the 2.5th to 97.5th percentile was determined to be < 2 - 5.12 ng/mL.

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.

▪ References

1. Elliott M. Antman, et al. ACC/AHA Guidelines for the Management of Patients With ST-Elevation Myocardial Infarction. Circulation. 2004; 110:e82–e292.
2. Russell V. Luepker, et al. AHA Scientific Statement; Case Definitions for Acute Coronary Heart Disease in Epidemiology and Clinical Research Studies. Circulation. 2003; 108: 2543-2549.
3. Kristian Thygesen, et al. Fourth universal definition of myocardial infarction (2018). European Heart Journal. 2019; 40: 237-269.
4. Adams JE, Abendschein DR, Jaffe AS. Biochemical markers of myocardial injury: is MB creatine kinase the choice for the 1990s? Circulation 1993;88:750-63.
5. Kurihara T, Yanagida A, Yokoi H, et al. Evaluation of cardiac assays on a benchtop chemiluminescent enzyme immunoassay analyzer, PATHFAST. Anal Biochem. 2008; 375(1): 144-146.

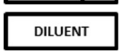
Symbols
LSI Medience Corporation uses the following symbols and signs in addition to those listed in the EN ISO 15223-1:2021 (Medical devices - Symbols to be used with information to be supplied by the manufacturer - Part 1: General requirements).

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)

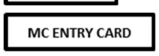
: Reagent cartridge

: Calibrator 1

: Calibrator 2



: Calibrator diluent



: Entry card for master calibration curve

* PATHFAST: JP Registered Trademark No.5982733

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

Contact for technical assistance
www.pathfast.eu/contact

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