April 2014

REF PF1121-K IVD For in vitro diagnostic use

PATHFAST® CK-MB-Ⅱ

<REAGENT FOR PATHFAST>

60 Tests

Intended Use

PATHFAST® CK-MB-Ⅱ is a product for in vitro diagnostic use with the in vitro diagnostic system PATHFAST® for the quantitative measurement of CK-MB in heparinized or EDTA whole blood and plasma. Measurements of CK-MB are used in the diagnosis of acute myocardial infarction. PATHFAST® CK-MB-Ⅱ is for use in clinical laboratory or point of care (POC) settings.

Summary

Creatine kinase (CK) is a key enzyme of energy metabolism in muscle that catalyzes the reversible phosphorylation of creatine. This dimeric enzyme has two subunits, M and B, which associate to form three isoenzymes, 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 accounting for approximately 10 – 40% of myocardial CK. Damage to the myocardium results in a transient and progressive release of CK-MB into the circulation. The CK-MB concentration increases at 2.5- 5 hours after onset of chest pain reaching a peak at 12 to 24 hours and then returning to normal levels within 48 to 72 hours. This characteristic temporal pattern is diagnostic for AMI. The low concentration of CK-MB in serum of healthy subjects and non-cardiac tissues contributes to its widely accepted use as an aid for diagnosing and monitoring of myocardial injury.

The PATHFAST® CK-MB-Ⅱ is an assay for measurement of CK-MB in the format of a chemiluminescent enzyme immunoassay (CLEIA). All required components for performing the testing are packed in one reagent cartridge. By loading PATHFAST® CK-MB-Ⅱ into the in vitro diagnostic system PATHFAST®, CK-MB can be quantified within 17 min.

Package composition

Reagent cartridge 6 cartridges x 10 trays

The reagent cartridge consists of 16 wells. All wells with exclusion of sample well (#1) and counting well (#10) are covered with an aluminum seal having bar codes on it. All reagents for the test are filled in each well of the reagent cartridge.

Wells	Form	Ingredient	Quantity	Source
# 2	Liquid	Alkaline phosphatase conjugated anti CK-BB MoAb*	50µL	Calf intestine Mouse
		MES Buffer		
		Na azide	< 0.1%	
# 7	Liquid	Anti CK-MB MoAb* coated magnetic particles	50µL	Mouse
		MOPS Buffer		
#13	Liquid	Chemiluminescent substrate CDP-Star®	100µL	
# 11	Liquid	Sample Dilution Buffer	50µL	
		Na azide	< 0.1%	
# 3, 4, 5	Liquid	Washing Buffer	400µL	
		MOPS Buffer		
		Na azide	< 0.1%	

*MoAb = monoclonal antibody

#1,6,8,9,10,12,14,15,16 are empty wells.

CDP-Star® is a registered trademark of Applied Biosystems, LLC.

Calibrators

1.	Calibrator 1 (CAL-1)	2.0 mL x 1 bottle Na azide(0.05%)	(liquid saline solution)
2.	Calibrator 2 (CAL-2)	For 1.0 mL x 2 vials	(lyophilized)
3.	Calibrator diluent	1.0 mL x 2 bottles Na azide (0.05%)	(liquid)

MC ENTRY CARD 1 sheet

Instruction for use 1 sheet

Materials Needed But Not Provided

PATHFAST®. Analyzer and consumables

CK-MB Quality Control Materials

Assay Principle

The PATHFAST® CK-MB-Ⅱ procedure is based on CLEIA and MAGTRATION®. In this procedure, alkaline phosphatase labeled anti CK-BB monoclonal antibody and anti CK-MB monoclonal antibody coated magnetic particles are mixed with sample. CK-MB contained in the specimen binds to the CK-MB antibodies forming an immunocomplex with enzyme labeled antibody and antibody coated magnetic particles. After removing the unbound enzyme labeled antibody, a chemiluminescent substrate is added to the immunocomplex. After a short incubation, the luminescence generated by enzyme reaction is detected. The intensity of the measured luminescence is in relationship with the CK-MB concentration in the specimen which is calculated by means of a standard curve.

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

Precautions

Reagent cartridge

1. Do not use reagents beyond its labeled expiration date.
2. Do not reuse a reagent cartridge. This is designed for single use only.
3. Do not peel off the aluminum seal.
4. Handle the reagent cartridge by holding the edge of it and do not touch the aluminum seal and the black well with your fingers.
5. When the reagent cartridge is dropped and damaged, do not use it.
6. Store the reagent cartridges in an upright position at all times.
7. Avoid contamination of saliva in the black well.
8. Avoid contamination and exposure to direct sunlight.
9. During storage or shipment, there may be some reagents adhering to the aluminum foil. If such a condition is observed, gently tap the cartridge on table before use.
10. Dispose in accordance with applicable national and local institution's regulations. Follow general precautions, and handle all components as if capable of transmitting infectious agents.
11. Used reagent cartridges contain human body fluids. Handle with appropriate care to avoid exposure to these fluids.
12. Do not use any reagent cartridge which was left at room temperature over 8 hours.
13. Reagent cartridge contains <0.1% sodium azide, which may react with copper and or led plumbing to form potentially explosive metal azides. When disposing of such reagents and buffers, always flush the disposal area e.g. plumbing with large volumes of water to prevent possible formation of such explosive compounds.

Storage Instructions

Store at +2 to +8 ℃. Do not open the cartridge tray prior to use.

Expiration

The expiration date is listed on each reagent cartridge, cartridge tray and package.

Sample Collection

1. Use whole blood or plasma collected with qualified collection tube containing heparin-Na, heparin-Li, EDTA-Na or EDTA-K.
2. Whole blood samples must be analyzed within 4 hours of collection. Immediately before dispensing a whole blood sample into a sample well of a cartridge, the whole blood sample contained in a blood collection tube should be mixed gently (do not use vortex mixer). After dispensing the whole blood sample and loading the cartridge on the PATHFAST®, the assay must be started immediately.
3. Plasma samples may be kept for 4 weeks at -20 ℃ or lower until testing is performed. However frozen samples should not be repetitively frozen and thawed prior to testing.
4. It should be ensured that fibrin clots and other insoluble materials are not present in the sample, otherwise such material should be removed by centrifugation or filtrations.
5. All plasma samples stored longer than 8 hours must be recentrifuged prior to testing.

Preparation and procedure

Reagent preparation

1. Reagent cartridge
   Ready to use.
2. Calibrator 1 (CAL-1)
   Ready to use.
   After opening calibrators are stable for the expire date which is printed on the bottle label, if they are stored at +2-+8℃.
3. Calibrator 2 (CAL-2)
   Reconstitute each vial of CAL-2 with one bottle of Calibrator diluent. Stand for 15 minutes at room temperature after the reconstitution. Mix gently and ensure that the calibrator is completely dissolved. Reconstituted calibrators are stable for 3 days at +2 to +8 ℃ or 1 month at –20 ℃ or lower. Do not exceed five freeze/thawing cycles.

   NOTE: Use the same lot of CAL-1, CAL-2, Calibrator diluent and PATHFAST® reagent cartridge. Never mix different lots of CAL–1, CAL-2, Calibrator diluent and PATHFAST® reagent cartridge.

Installation of master calibration curve

1. Installation of the 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 the PATHFAST®. Refer to the PATHFAST® operator's manual for more information.

User calibration

1. User calibration is necessary when a new reagent lot is used after installation of the master calibration curve on 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 four reagent cartridges, two for CAL-1 and two for CAL-2 are necessary for user calibration.
4. Set the reagent cartridges in the cartridge rack, then dispense approximately 100 µL of CAL-1 and CAL-2 into the appropriate sample wells and load the cartridge rack onto the PATHFAST®.
5. Carry out user calibration in accordance with the PATHFAST® operator's manual.

Quality Control Assay (QC Assay)

1. Refer to the PATHFAST® operator's manual. A QC Assay is performed after every calibration to check the calibration curves and store data from QC samples for quality control. A QC Assay is indispensable for assuring validity of sample results. After each calibration, with each new shipment of previously calibrated test kit, or whenever the institution wishes to verify the performance of the system, analyze two levels of quality control material with known levels of CK-MB.
2. Good laboratory practices recommend the use of appropriate quality controls. It is recommended that federal, state and local guidelines for quality control be followed. If controls do not perform as expected, do not use the test results. Repeat the controls and test or call your authorized PATHFAST® distributor for technical service.

Sample assay

1. Use heparinized or EDTA whole blood and plasma sample (heparin-Na, heparin-Li, EDTA-Na or EDTA-K) .
2. Set the reagent cartridge in the cartridge rack, then dispense approximately 100 µL of sample into the appropriate sample well of a cartridge and load the cartridge rack onto the PATHFAST®.
3. Carry out sample assay in accordance with the PATHFAST® operator's manual.

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 vortex mixer). After dispensing the whole blood sample and loading the cartridge rack on the PATHFAST®, the assay must be started immediately.
2. When samples are left for more than 5 minutes after dispensing into a sample well, a lower result may be obtained analyzing whole blood because of blood sedimentation and a higher result may be obtained analyzing plasma because of possible evaporation.
3. Samples with above 250 ng/mL can be diluted with sample diluent (Product# PF01D) or saline and retested if an exact result is desired or alternatively, the result can be reported as >250 ng/mL.

High Dose Hook Effect

Patient samples with high CK-MB levels can cause a paradoxical decrease in the chemiluminescent intensity (high dose hook effect). In this assay, the PATHFAST® results were reported as >250 ng/mL when samples above the range of the test were prepared by spiking CK-MB antigen purified from human heart muscle into a buffer solution to 19452 ng/mL.

Expected values

1. The expected values / reference values may vary from laboratory to laboratory and from country to country depending on various factors. It is therefore recommended that each institution establish its own reference values.
2. Using the PATHFAST® CK-MB-Ⅱ assay, the reference interval for CK-MB in 302 healthy individuals was determined.

Mean	0.969 ng/mL
Min	0.116 ng/mL
Max	4.59 ng/mL
2.5th percentile	0.189 ng/mL
97.5th percentile	3.01 ng/mL

Specific performance data of the assay

Representative performance data on the PATHFAST® is given below.

1. Assay range: 1 - 250 ng/mL.
2. Methods comparison (plasma samples)
   y=0.997x – 0.423., r=0.996, n=76
   Method comparison study (plasma samples) was performed by physician assistants and medical office personnel in three non-laboratory sites. Data was combined with three sites.
   y = 1.007x – 0.274, r = 0.988, n = 60
   (y : this method x : Dade Behring Stratus® CS CKMB)
   Stratus® is a registered trademark of Siemens Corporation.
3. Correlation between lithium heparin plasma and other sample matrices
   y=0.982x + 0.014, r=0.989, n=59
   (y: lithium heparin whole blood; x: lithium heparin plasma)
   y=1.005x - 0.072, r=0.997, n=59
   (y: sodium heparin plasma; x: lithium heparin plasma)
   y=0.964x -0.012, r=0.992, n=59
   (y: sodium heparin whole blood; x: lithium heparin plasma)
   y=1.024x +.0.240, r=0.998, n=59
   (y: EDTA-2Na plasma; x: lithium heparin plasma)
   y=1.003x + 0.433, r=0.989, n=59
   (y: EDTA-2Na whole blood; x: lithium heparin plasma)
   y=1.017x + 0.116, r=0.995, n=59
   (y: EDTA-2K plasma; x: lithium heparin plasma)
   y=1.002x + 0.151, r=0.990, n=59
   (y: EDTA-2K whole blood; x: lithium heparin plasma)
4. Traceability
   PATHFAST® CK-MB-Ⅱ calibrators are prepared from purified CK-MB from human heart tissue and assigned using internal working calibrators.
   The internal working calibrators' values are based on internal reference materials.
5. Imprecision
   Precision was determined using the present method, three control materials in a protocol as follows: Three plasma samples were assayed in duplicate for 20 non-consecutive days. The within-run and total standard deviations were calculated according to the NCCLS EP5-A2 [ISBN 1-56238-542-9]. The following results were obtained.

		Within-run precision		Total precision	
Sample	Mean (ng/mL)	S.D. (ng/mL)	C.V. (%)	S.D. (ng/mL)	C.V. (%)
QC-L	2.69	0.147	5.4	0.225	8.4
QC-M	36.0	1.42	3.9	2.29	6.4
QC-H	211	6.76	3.2	14.6	6.9

6. Analytical specificity
   Cross reactivity with CK-BB and CK-MM was 0.07% and 0.00%, respectively.

7. Recovery
Plasma samples containing high and low levels of CK-MB were mixed at one to one ratio to test recovery. The observed values were then compared to the expected values for each mixture.

Sample Mixture	CK-MB Plasma Part 1 (ng/mL)	CK-MB Plasma Part 2 (ng/mL)	CK-MB Mix Part 1:Part 2 (ng/mL)	CK-MB Recovery (%)
A	1.92	9.05	5.11	93.1
B	29.5	68.9	46.7	95.0
C	21.9	43.7	31.2	95.0
D	34.2	196	111	96.7

8. Limit of quantitation: < 0.192 ng/mL
Limit of quantitation was evaluated according to NCCLS EP17-A [ISBN 1-56238-551-8].
The precision profile for the PATHFAST® CK-MB-Ⅱ methods demonstrates a coefficient of variation (C.V.) of below 10% at >0.192 ng/mL of CK-MB concentration.

Possible interferences

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

Free bilirubin	(60 mg/dL)
Conjugated bilirubin	(60 mg/dL)
Triglyceride, lipemia in sample	(1000 mg/dL)
Hemoglobin (hemolysis)	(Hb 1000 mg/dL)
Rheumatoid Factor	(500 IU/mL)

The following substances were found to have no significant (less than 10%) when added to a plasma pool containing 14.2 ng/mL or 12.2 ng/mL of CK-MB at the concentrations indicated below.

Acetaminophen	20 mg/dL	(1320 µmol/L)
Acetylsaltic Acid	0.3 ng/mL	(1.67 nmol/L)
Allopurinol	2.5 mg/dL	(184 µmol/L)
Ampicillin	5 mg/dL	(143 µmol/L)
Ascorbic Acid	3 mg/dL	(170 µmol/L)
Atenolol	1 mg/dL	(37.6 µmol/L)
Caffeine	10 mg/dL	(515 µmol/L)
Captopril	5 mg/dL	(230 µmol/L)
Digoxin	5 ng/mL	(6.4 nmol/L)
Dopamine	65 mg/dL	(3.4 mmol/L)
Erythromycin	20 mg/dL	(273 µmol/L)
Furosemide	2 mg/dL	(61 µmol/L)
Methyldopa	2.5 mg/dL	(118 µmol/L)
Niphedipine	6 mg/dL	(173 µmol/L)
Phenytoin	10 mg/dL	(396 µmol/L)
Theophylline	25 mg/dL	(1390 µmol/L)
Verapamil	16 mg/dL	(0.33 µmol/L)

Limitations of procedure

1. The instrument reporting system contains error codes to warn the operator of specific malfunctions. Any report slip containing such error codes should be kept for follow-up. See the PATHFAST® operator's manual.
2. Patient samples may contain heterophilic antibodies that could react in immunoassays to give a falsely elevated or depressed result. This assay has been designed to minimize interference from heterophilic antibodies. Nevertheless, complete elimination of this interference from all patient specimens can not be guaranteed. A test result that is inconsistent with the clinical picture and patient history should be interpreted with cautions.

References

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2. Roberts R. Diagnostic assessment of myocardial infarction based on lactate dehydrogenase and creatine kinase isoenzymes. Heart Lung 1981; 10: 486-506
3. Galen RS and Gambino SR. Isoenzyme of CPK and LDH in myocardial infarction and certain other diseases. Pathobiol Annu 1975; 5: 283-315
4. Lott JA. Serum enzyme determination in the diagnosis of acute myocardial infarction: an update. Hum Pathol 1984; 15: 706-716

Symbols

: EU Conformity

IVD : For in vitro Diagnostic Use

Lot : Batch Code

REF : Product Number

: Manufactured by

EC REP : Authorized Representative

: Contains sufficient for

: Temperature Limitation

: Use by

: Caution, refer to accompanying documents

: Consult IFU

CAL 1 : Calibrator 1

CAL 2 : Calibrator 2

DILUENT : Diluent

CARTRIDGE : Reagent Cartridge

MC ENTRY CARD : Entry Card for Master Calibration Curve

*PATHFAST : US Registered Trademark No.3074207

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