April 2014

REF PF1111-K IVD For in vitro diagnostic use

PATHFAST® Myo-Ⅱ

<REAGENT FOR PATHFAST>

60 Tests

Intended Use

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

Summary

Myoglobin (Myo) is a low molecular weight, heme-protein found in both cardiac and skeletal muscle. Following myocardial necrosis associated with acute myocardial infarction (AMI), Myoglobin is one of the first markers to rise above normal levels, increasing measurably above baseline within 1-3 hours post infarction, peaking at 6-12 hours and returning to baseline within 24-36 hours. In the absence of skeletal muscle trauma or other situations associated with a non cardiac related increase in circulating Myoglobin (e.g. renal failure), measurement of Myoglobin in blood has been used as an early marker of AMI. Several studies proved that the measurement of Myoglobin can be used as a rapid and sensitive test in the early phase of AMI for its diagnosis in conjunction with the ECG and for exclusion of myocardial infarction in patients presenting with acute chest pain, especially after a further negative test one hour later.

The PATHFAST® Myo-Ⅱ is an assay for measurement of Myoglobin 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® Myo-Ⅱ into the in vitro diagnostic system PATHFAST®, Myoglobin can be accurately 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 Myo MoAb*	50µL	Calf Intestine Mouse
		MES Buffer		
		Na azide	< 0.1%	
# 7	Liquid	Anti Myo 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	
		MES 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 (liquid) Na azide (0.05%)
2. Calibrator 2 (CAL-2) 2.0 mL x 1 bottle (liquid) Na azide (0.05%)

MC ENTRY CARD 1 sheet
Instruction for use 1 sheet

Materials Needed But Not Provided

PATHFAST® Analyzer and consumables
Myo Quality Control Materials

Assay Principle

The PATHFAST® Myo-Ⅱ procedure is based on CLEIA and *MAGTRATION®. In this procedure, alkaline phosphatase labeled anti Myo monoclonal antibody and anti Myo monoclonal antibody coated magnetic particles are mixed with sample. Myo contained in the specimen binds to anti Myo 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 Myoglobin 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. Calibrators:
   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℃.

   NOTE: Use the same lot of CAL-1, CAL-2 and PATHFAST® reagent cartridge. Never mix different lots of CAL-1, CAL-2 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 Myo.
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 1,000 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 >1,000 ng/mL.

High Dose Hook Effect

Patient samples with high myoglobin levels can cause a paradoxical decrease in the chemiluminescent intensity (high dose hook effect). In this assay, the PATHFAST® results were reported as >1000 ng/mL when samples above the range of the test were prepared by spiking myoglobin antigen purified from human heart muscle into a buffer solution to 107850 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® Myo-Ⅱ assay, the reference intervals for Myo in 98 healthy individuals were determined.

Mean	31.7 ng/mL
Min	10.2 ng/mL
Max	80.4 ng/mL
2.5th percentile	11.8 ng/mL
97.5th percentile	75.3 ng/mL

Specific performance data of the assay

Representative performance data on the PATHFAST® is given below.

1. Assay range: 5 – 1000 ng/mL
2. Methods comparison (plasma samples)
   y=1.00x + 1.16, r=0.992, n=126
   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 = 1.046x + 5.54, r = 0.996, n = 60
   (y: this method; x: Dade Behring Stratus® CS Myo)
   Stratus® is a registered trademark of Siemens Corporation.
3. Correlation between lithium heparin plasma and other sample matrices
   y=0.967x +0.210, r=0.993, n=67
   (y: lithium heparin whole blood; x: lithium heparin plasma)
   y=1.009x +0.825, r=0.996, n=67
   (y: sodium heparin plasma; x: lithium heparin plasma)
   y=0.957x +1.394, r=0.994, n=67
   (y: sodium heparin whole blood; x: lithium heparin plasma)
   y=1.002x - 0.543, r=0.995, n=67
   (y: EDTA-2Na plasma; x: lithium heparin plasma)
   y=0.968x +1.769, r=0.992, n=67
   (y: EDTA-2Na whole blood; x: lithium heparin plasma)
   y=0.997x - 0.116, r=0.994, n=67
   (y: EDTA-2K plasma; x: lithium heparin plasma)
   y=0.980x +2.330, r=0.991, n=67
   (y: EDTA-2K whole blood; x: lithium heparin plasma
4. Traceability
   PATHFAST® Myo-Ⅱ calibrators are prepared from purified myoglobin from human heart tissue and assigned using internal working calibrators.
5. Imprecision
   Precision was determined using the present method, three control materials in a protocol are 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.

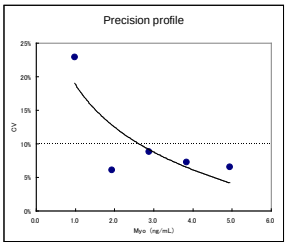
		Within-run precision		Total precision	
Sample	Mean (ng/mL)	S.D. (ng/mL)	C.V. (%)	S.D. (ng/mL)	C.V. (%)
QC-L	33.8	1.01	3.0	1.45	4.3
QC-M	102	2.85	2.8	3.86	3.8
QC-H	688	9.31	1.4	16.8	2.4

6. Recovery
   Plasma samples containing high and low levels of Myo 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	Myo Plasma Part 1 (ng/mL)	Myo Plasma Part 2 (ng/mL)	Myo Mix Part 1:Part 2 (ng/mL)	Myo Recovery (%)
A	200	333	290	108.8
B	101	185	155	108.6
C	52.9	146	103	103.7
D	263	958	615	100.7

7. Limit of quantitation: < 1.93 ng/mL
   Limit of quantitation were evaluated according to NCCLS EP17-A

[ISBN 1-56238-551-8].
The precision profile for the PATHFAST® Myo-Ⅱ methods demonstrates a coefficient of variation (C.V.) of below 10% at > 1.93 ng/mL of Myo concentration.



Possible interferences

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

Free bilirubin	(36 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 173 ng/mL of Myo 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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Symbols

CE	: EU Conformity
IVD	: For in vitro Diagnostic Use
Lot	: Batch Code
REF	: Product Number
	: Manufactured by
EC REP	: Authorized Representative
Σ	: 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

LSI Medience Corporation
13-4, Uchikanda 1-chome, Chiyoda-ku,
Tokyo 101-8517, Japan

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