May 2014

REF PF1101-K **IVD** **For in vitro diagnostic use**

PATHFAST® cTnI-Ⅱ

<REAGENT FOR PATHFAST>

60 Tests

Intended Use

PATHFAST® cTnI-II is an in vitro diagnostic test for the quantitative measurement of cardiac Troponin I (cTnI) in heparinized or EDTA whole blood and plasma. Measurements of cardiac Troponin I are used as an aid in the diagnosis of acute myocardial infarction (AMI). PATHFAST® cTnI-II is for use in clinical laboratory or point of care (POC) settings.

Summary

Cardiac troponin I (cTnI) is a cardiac muscle protein with a molecular weight of 22,500 daltons. In the heart, troponin I (TnI) together with troponin T (TnT) and troponin C (TnC) forms a troponin complex that plays a fundamental role in the transmission of intracellular calcium signal of actin-myosin interaction. Troponin I is released rapidly to blood circulation 4-6 hours after the onset of AMI and remains elevated for several days thereafter.

The PATHFAST® cTnI-II is an assay for measurement of cTnI 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® cTnI-II into the in vitro diagnostic system PATHFAST®, cTnI 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 cTnI MoAb*	50µL	Calf intestine Mouse
		MES Buffer		
		Na azide	< 0.1%	
# 7	Liquid	anti cTnI MoAb* coated magnetic particles	50µL	Mouse
		MOPS Buffer		
#13	Liquid	Chemiluminescent substrate ;CDP-Star®	100µL	
# 11	Liquid	Sample Dilution Buffer	50µL	
		Tris Buffer		
		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
Control Data Sheet 1 sheet
Instruction for use 1 sheet

Materials Needed But Not Provided

PATHFAST® Analyzer and consumables
cTnI Quality Control Materials

Assay Principle

The PATHFAST® cTnI-II procedure is based on CLEIA and *MAGTRATION®. In this procedure, alkaline phosphatase labeled anti cTnI monoclonal antibody and anti cTnI monoclonal antibody coated magnetic particles are mixed with sample. cTnI contained in the specimen binds to the anti cTnI 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 cTnI 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 only heparin-Li, heparin-Na, or EDTA-K_2.
2. Heparinized whole blood samples must be analyzed within 4 hours after 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. Heparinized 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. When EDTA whole blood or EDTA plasma is used, the assay must be performed within two hours of collection. The values of cTnI in EDTA plasma are approximately 15% lower than those in heparinized plasma after 6 hours of collection.
5. 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.
6. 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 three 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 detailed information.

User calibration
Refer to the PATHFAST® operator's manual.
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 cTnI.
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 anticoagulated whole blood and plasma sample (heparin-Na, heparin-Li, or EDTA-K_2).
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 result above 50.0 ng/mL can be diluted with sample diluent (Product # PF02D) and retested if a quantitative result is desired or alternatively, the result can be reported as > 50.0 ng/mL.

High Dose Hook Effect
Patient samples with high troponinI levels can cause a paradoxical decrease in the chemiluminescent intensity (high dose hook effect). In this assay, the PATHFAST® results were reported as >50 ng/mL when samples above the range of the test were prepared by spiking troponin I antigen purified from human heart muscle into a buffer solution to 44900 ng/mL.

Expected values
1. The expected/reference values may vary across laboratories and locations depending on various factors. It is therefore recommended that each institution establish its own expected/reference values.
2. The reference interval for the PATHFAST® cTnI-II assay was determined by testing 490 healthy individuals from Northeastern, Southeastern, and Southwestern US. The 99th percentile of the reference interval is 0.029 ng/mL.

Specific performance data of the test
Representative performance data on the PATHFAST® are given below.
1. Assay range: 0.019 – 50 ng/mL.
2. Methods comparison (plasma samples)
   slope=0.947 (95% CI = 0.891 – 1.004); intercept = –0.005 (95% CI = –0.025 to 0.019), r=0.994, n=57 (y : PATHFAST® cTnI-II, x : Dade Behring Stratus® CS cTnI) Range of samples: 0.100 to 43.45 ng/mL.
   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.
   Stratus® CS is a registered trademark of Siemens Corporation.
3. Bias between lithium heparin plasma and other sample matrices on 83 samples.

Comparison to lithium heparin plasma:	**Slope (95% CI*)**	**Intercept (95% CI)**
lithium heparin whole blood	0.97 (0.92 – 1.01)	0.003 (0.000 – 0.006)
sodium heparin plasma	1.01 (0.99 – 1.03)	0.001 (-0.002 – 0.004)
sodium heparin whole blood	0.96 (0.92 – 0.99)	0.004 (0.000 – 0.010)
EDTA-K_2 plasma	0.99 (0.98 – 1.02)	0.000 (-0.004 – 0.010)
EDTA-K_2 whole blood	1.00 (0.96 – 1.04)	0.003 (-0.002 – 0.010)

*CI = Confidence Interval

4. Traceability
   PATHFAST® cTnI-II calibrators are prepared from human troponin ITC complex and assigned using internal working calibrators.
   The internal working calibrators' values are based on internal reference materials.
5. Imprecision
   Precision was determined using 4 whole blood and plasma samples representing low (n=2), (n=1), and high (n=1) cTnI levels. The samples were testing in 20 replicates using 3 reagent lots on 1 instrument, and 1 reagent lot on 3 instruments, for a total of 6 different types of runs. The low sample was tested twice, so there were a total of 8 runs. The within-run and total standard deviations were calculated according to the CLSI EP5-A2 [ISBN 1-56238-542-9]. The following results were obtained:

		Whole Blood (ng/mL)			
Precision		**LL**	**L**	**M**	**H**
Across Instruments/ within lot	mean	0.096	0.930	12.6	38.5
	SD	0.005	0.056	0.427	0.210
	CV	4.7%	6.0%	3.4%	0.5%
Across lots/ within instrument	mean	0.096	1.01	12.4	41.3
	SD	0.007	0.092	0.241	2.64
	CV	7.0%	9.1%	1.9%	6.4%
		Plasma (ng/mL)			
Precision		**LL**	**L**	**M**	**H**
Across Instruments/ within lot	mean	0.146	0.924	11.6	30.4
	SD	0.006	0.062	0.762	2.447
	CV	4.3%	6.8%	6.6%	8.0%
Across lots/ within instrument	mean	0.157	0.972	12.4	29.7
	SD	0.017	0.069	0.810	0.48
	CV	10.8%	7.1%	6.5%	1.6%

Precision was determined using 4 plasma samples near the assay's two cutoffs, tested 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 standard deviations were calculated according to the CLSI EP5-A2 [ISBN 1-56238-542-9]. The following results were obtained:

		Plasma (ng/mL)			
Precision		**#1**	**#2**	**#3**	**#4**
	mean (ng/mL)	0.022	0.029	0.086	0.251
Within-run	SD (ng/mL)	0.001	0.001	0.004	0.009
	CV (%)	6.2	5.1	4.3	3.7
Total	SD (ng/mL)	0.002	0.002	0.005	0.010
	CV (%)	7.1	6.1	5.4	3.9

6. Analytical specificity
   Cross reactivity with cardiac troponin T and skeletal troponin I was 0.08% and 0.09%, respectively. Cross reactivity with cardiac troponin C was not detected.
7. Recovery
   Plasma samples containing high and low levels of cTnI 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	cTnI Plasma Part 1 (ng/mL)	cTnI Plasma Part 2 (ng/mL)	cTnI Mix Part 1:Part 2 (ng/mL)	cTnI Recovery (%)
A	0.016	4.69	2.23	94.9
B	0.120	0.863	0.459	93.3
C	0.120	4.58	2.31	98.4
D	9.32	36.6	22.4	97.7

8. Limit of quantitation: < 0.019 ng/mL
   The precision profile for the PATHFAST® cTnI-II methods demonstrates a coefficient of variation (C.V.) of ≤10% at 0.019 ng/mL of cTnI concentration.
9. Whole Blood linearity: n=44; range=0.0013 – 50.1 ng/mL; slope = 1.01 (95% CI= 1.00 – 1.02), intercept = 0.00 (95% CI = 0.00 – 0.00);
   Plasma linearity: n=44, range=0.0007-55.9 ng/mL; slope = 1.00 (95% CI= 0.99 – 1.01), intercept = 0.00 (95% CI = 0.00 – 0.00).

Possible interferences

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)
Triglyceride	(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 1.32 ng/mL of cTnI 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)

Diagnosis of AMI – Serial Samples

An additional study was performed on prospectively collected banked serial plasma samples (heparin anticoagulant) from 333 consecutively eligible consented patients presenting to the ER with suspicion of myocardial infarction (MI). The study yielded 72 MI patients and 261 non-MI patients. The blood sample collection times were at 0-2 hours, at 2 to 6 hours, and at 6 to 12 hours of presentation to the ER. The clinical diagnosis of MI was performed by an adjudication panel according to ESC/ACCF/AHA/WHF 2007 guidelines. The adjudication panel used existing clinical data including ECG and other clinical information to make the diagnosis, but not the final discharge diagnosis or the PATHFAST® cTnI-II Test results. Clinical sensitivity and clinical specificity were calculated by comparing the PATHFAST® cTnI-II Test results to clinical diagnosis assigned by the adjudication panel. The calculations were performed with both the 99th percentile cutoff (≥0.029 ng/mL) and the ROC cutoff (≥0.264 ng/mL) of the assay.

			#/TOTAL	95% CI
Cutoff 0.029 ng/mL	0 to 2 h	Sens	73.6% (53/72)	(61.9% - 83.3%)
		Spec	92.7% (242/261)	(88.9% - 95.6%)
	2 to 6 h	Sens	93.1% (66/72)	(82.7% - 96.9%)
		Spec	93.1% (243/261)	(89.3% - 95.9%)
	6 to 12 h	Sens	91.7% (66/72)	(91.6% - 100%)
		Spec	91.6% (239/261)	(87.5% - 94.6%)

			#/TOTAL	95% CI*
Cutoff 0.264 ng/mL	0 to 2 h	Sens	23.6% (17/72)	(14.4% - 35.1%)
		Spec	99.2% (259/261)	(97.3% - 99.9%)
	2 to 6 h	Sens	62.5% (45/72)	(50.3% - 73.6%)
		Spec	98.1% (256/261)	(95.6% - 99.4%)
	6 to 12 h	Sens	80.6% (58/72)	(69.5% - 88.9%)
		Spec	97.7% (255/261)	(95.1% - 99.2%)

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. When using the 99th percentile cutoff, the PATHFAST® cTnI-II Test should be interpreted with at least 2 serial samples. When using the ROC cutoff, the test should be interpreted with 3 serial samples. When samples are collected in the early hours, it is not advisable to use the higher cutoff.
3. Patient samples may contain heterophilic antibodies that could react in immunoassays 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.

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
	: 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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