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CE 0123

PATHFAST™ hsCRP

<REAGENT FOR PATHFAST>

60 Tests

English

▪ Intended use

PATHFAST hsCRP is a product for in-vitro diagnostic use with the in vitro diagnostic (IVD) automated analyser PATHFAST for the quantitative measurement of C-reactive protein (CRP) in human serum, heparinized or EDTA whole blood and plasma. PATHFAST hsCRP is intended to be used:

- as an aid to diagnose inflammatory disorders and associated disorders in patients with suspected inflammation-related diseases,
- as an aid to assess the risk of cardiovascular disease,
- as an aid to determine the probability of recurrence of cardiac events in patients with stable coronary heart disease (CHD) and acute coronary syndrome (ACS),
- by laboratory technician, nurse or physician,
- in hospital including emergency room, doctor's office and clinical laboratory.

PATHFAST hsCRP is a device for near patient testing (NPT).

▪ Summary

C-reactive protein (CRP) is an acute phase protein, belonging to the pentraxin family. CRP is composed of five identical subunits and each of the subunits (23 kDa) are non-covalently bound to form the pentamer with the center hole. CRP is synthesized in the liver in response to inflammatory cytokines produced by inflammation, infection, trauma, etc. In the acute phase, the secretion of CRP is greatly accelerated, followed by the increased CRP concentration in blood. CRP may intervene in atherosclerosis by directly activating the complement system and inducing apoptosis, vascular cell activation, monocyte recruitment, lipid accumulation, thrombosis, and more (1-3).
Conventionally, CRP have been used as a biomarker of inflammatory events. On the other hand, it is suggested that hsCRP assay may be used as an aid to assess the risk of cardiovascular disease and as an aid to determine the probability of recurrence of cardiac events in patients with stable CHD and ACS (4-9).

▪ Test principle

The PATHFAST hsCRP 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 hsCRP into the in vitro diagnostic system PATHFAST, CRP can be accurately quantified within 17 min. In this procedure, alkaline phosphatase labelled anti-CRP monoclonal antibody (MoAb) and anti-CRP MoAb coated magnetic particles are mixed with the sample. CRP contained in the specimen binds to the anti-CRP 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 CRP 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-CRP MoAb, Triton X-100 (< 0.1%)	50 µL	Micro-organism Mouse
# 7	Liquid	anti-CRP MoAb coated magnetic particles	50 µL	Mouse
# 13	Liquid	Chemiluminescent substrate, CDP-Star	100 µL	-
# 11	Liquid	Sample dilution buffer	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)	2.0 mL x 1 bottle (liquid)
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)
hsCRP 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 ℃ 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. Calibrators can be used until the expiration date after opening.
7. The expiration date is listed on each reagent cartridge and kit box label.
8. Do not use reagents beyond the indicated expiration date.

Sample collection

Use serum, 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.
Serum and 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, CAL-2: Ready to use. (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 CRP.
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 serum and plasma samples, 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 serum and plasma because of increasing CRP 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. When collecting plasma with certain EDTA tubes, some differences might be shown between the measured CRP values in the EDTA plasma and in the corresponding serum.
6. Samples with result above 30 mg/L 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 > 30 mg/L.

▪ Specific performance data

Representative performance data on the PATHFAST are given below.

Metrological traceability
The calibrators for PATHFAST hsCRP are traceable to the reference material IRMM・CRM 470.

Trueness (bias)
CRM470 sample (certified value: 39.2 mg/L) was diluted 20-fold with non-CRP serum and the diluted sample was measured in triplicate on 3 instruments. The assayed mean values were compared to the expected value.
The mean bias of 3 instruments was -1% (expected value: 1.96 mg/L).

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

Whole blood	Mean (mg/L)	S.D. (mg/L)	C.V. (%)
Level-1	0.827	0.040	4.8
Level-2	4.79	0.250	5.2
Level-3	8.73	0.537	6.2

Plasma	Mean (mg/L)	S.D. (mg/L)	C.V. (%)
Level-1	1.29	0.068	5.3
Level-2	7.25	0.289	4.0
Level-3	13.0	0.485	3.7

Serum	Mean (mg/L)	S.D. (mg/L)	C.V. (%)
Level-1	0.912	0.047	5.2
Level-2	8.99	0.364	4.0
Level-3	13.6	0.688	5.1

Precision (reproducibility)
Serum samples at 4 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 (mg/L)	Within-run precision		Total precision	
		S.D. (mg/L)	C.V. (%)	S.D. (mg/L)	C.V. (%)
Level-1	0.916	0.069	7.5	0.070	7.6
Level-2	4.63	0.279	6.0	0.391	8.4
Level-3	15.1	1.19	7.9	1.29	8.5
Level-4	25.6	1.24	4.8	1.37	5.4

Analytical sensitivity
Limit of detection (LoD): 0.002 mg/L
Limit of quantitation (LoQ): 0.007 mg/L (C.V. 10%)

Linearity
CRP antigen was spiked into serum at 3 concentration levels (0.354, 3.51, 32 mg/L). The samples were serially diluted with 10-fold using saline and assayed.
The recovery rate against the theoretical value was within 91 - 110% up to 32 mg/L.

Assay range: 0.05 - 30 mg/L
The assay range was set from the results of LoQ and linearity.

High dose hook effect
CRP antigen was spiked into Non-CRP serum at the concentration of approximately 1250 mg/L. The samples were diluted with Non-CRP serum and assayed. There was no high dose hook effect for the samples with their CRP values up to 1000 mg/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	(5000 FTU)
Triglyceride	(1000 mg/dL)
Haemoglobin (haemolysis)	(1000 mg/dL)
Rheumatoid Factor	(550 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.

Human serum albumin	(1000 mg/dL)
Human IgG	(1000 mg/dL)
Transferrin	(1000 mg/dL)

Correlation between samples of serum and other sample matrices

x	y		n	Slope	Intercept	r
Serum	Li-heparin	Plasma	76	1.07	0.004	0.985
		Whole blood	76	0.995	0.008	0.980
	Na-heparin	Plasma	76	1.06	0.000	0.983
		Whole blood	76	0.920	0.009	0.969
	EDTA-2K	Plasma	76	1.02	-0.005	0.979
		Whole blood	76	1.05	-0.002	0.986
	EDTA-2Na	Plasma	76	1.01	-0.003	0.978
		Whole blood	76	1.03	0.000	0.985

The regression equation was calculated by Passing-Bablok fit.

Method comparison

y= 0.994x – 0.052, r= 0.994, n= 100 (heparin plasma samples, y: PATHFAST hsCRP, x: BN II system, Passing-Bablok fit).

Expected values

1. Reference limit
   Using the PATHFAST hsCRP assay, the reference interval for CRP in 192 apparently healthy individuals (72 males and 120 females - age range 16 to 68 years) was determined to be: (97.5th percentile) 3.35 mg/L.
2. Expected value in Affected population
   The expected value in affected population was confirmed by American Heart Association/Centers for Disease and Control and Prevention Scientific Statement (4). The following values were shown as relative risk categories for cardiovascular disease.

Low risk:	< 1.0 mg/L
Average risk:	1.0 - 3.0 mg/L
High risk:	> 3.0 mg/L

   If the hsCRP level is ≥ 10 mg/L, the test should be repeated and the patient examined for sources of infection or inflammation.

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. Wu Y, Potempa LA, El Kebir et al. C-reactive protein and inflammation: conformational changes affect function. Biol Chem. 2015; 396(11): 1181-1197.
2. Schwedler SB, Filep JG, Galle J, et al. C-reactive protein: a family of proteins to regulate cardiovascular function. Am J Kidney Dis. 2006; 47(2): 212-222.
3. Salazar J, Martínez MS, Chávez-Castillo M, Núñez V, Añez R, Torres Y, Toledo A, Chacín M, Silva C, Pacheco E, Rojas J, Bermúdez V. C-Reactive Protein: An In-Depth Look into Structure, Function, and Regulation. Int Sch Res Notices. 2014 Dec 15; 2014: 653045.
4. Pearson TA, Mensah GA, Alexander RW, et al. AHA/CDC Scientific Statement: Markers of Inflammation and Cardiovascular Disease Application to Clinical and Public Health Practice. Circulation. 2003; 107: 499-511.
5. Rifai N, Tracy RP, Ridker PM. Clinical efficacy of an automated high-sensitivity C-reactive protein assay. Clin Chem. 1999; 45(12): 2136-2141.
6. Rifai N, Ridker PM. High-sensitivity C-reactive protein: a novel and promising marker of coronary heart disease. Clin Chem. 2001; 47(3): 403-411.
7. Speidl WS, Graf S, Hornykewycz S, et al. High-sensitivity C-reactive protein in the prediction of coronary events in patients with premature coronary artery disease. Am Heart J. 2002; 144(3): 449-455.
8. Delhaye C, Sudre A, Lemesle G, et al. Preprocedural high-sensitivity C-reactive protein predicts death or myocardial infarction but not target vessel revascularization or stent thrombosis after percutaneous coronary intervention. Cardiovasc Revasc Med. 2009; 10(3): 144-150.
9. Leu HB, Lin CP, Lin WT, et al. Risk stratification and prognostic implication of plasma biomarkers in nondiabetic patients with stable coronary artery disease: the role of high-sensitivity C-reactive protein. Chest; 126(4): 1032-1039.

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

: 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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