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REF PF1071-KUS IVD For in vitro diagnostic use

PATHFAST® hsCRP

<REAGENT FOR PATHFAST®>

60 Tests

Intended Use

PATHFAST® hsCRP is a product for in-vitro diagnostic use with the in vitro diagnostic system PATHFAST® for the quantitative measurement of C-reactive protein (CRP) in human heparinized or EDTA whole blood or plasma, and serum, as an aid in the detection and evaluation of the infection, tissue injury, inflammatory disorders and associated disorders. PATHFAST® hsCRP is for use in clinical laboratory or POC settings.

Summary

C-reactive protein (CRP) is an "acute phase" protein and rises non-specifically in response to inflammation which is β-globulin with a molecular mass of approximately 118,000 daltons. CRP is highly conserved, composed of five identical cyclic globular subunits, and is classified as a member of the pentraxin superfamily of proteins.

As elevated CRP values are always associated with pathological changes, the CRP assay provides useful information for the diagnosis, therapy, and monitoring of inflammatory conditions and associated diseases.[1-4] Additionally, measurement of CRP by high sensitivity CRP assay may add to the predictive value of other markers used to assess the risk of cardiovascular and peripheral vascular diseases.[5-10]

It is recommended to follow-up elevated CRP values with repeat testing and detailed clinical evaluations. It is reported that intra-individual variations of the CRP levels are from 30 to 60% and serial measurements may be required to estimate true mean of CRP depending on the intended use in any specific individual.[11]

The PATHFAST® hsCRP is an assay for measurement of CRP – also at low concentrations - 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® hsCRP into the in vitro diagnostic system PATHFAST®, CRP 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 a bar code. All reagents for the test are filled in each well of the reagent cartridge.

Wells	form	Ingredient	Quantity	Source
# 1	Empty	sample well		
# 2	liquid	Alkaline phosphatase conjugated anti CRP MoAb* MES** Buffer	50µL	Micro-organism Mouse
# 7	liquid	anti CRP MoAb* coated magnetic particles MOPS*** Buffer	50µL	Mouse
#13	liquid	Chemiluminescent substrate CDP-Star®	100µL	
# 11	liquid	Sample Dilution Buffer MOPS Buffer	50µL	
# 3,4,5	liquid	Washing Buffer MES Buffer	400µL	
		Na azide	< 0.1%	

*MoAb = Monoclonal antibody;
**MES = 2-Morpholinoethanesulfonic acid, monohydrate
***MOPS = 3-Morpholinopropanesulfonic acid
#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)

MC ENTRY CARD 1 sheet
Instruction for use 1 sheet

Materials Needed But Not Provided

PATHFAST® Analyzer and consumables
hsCRP Quality Control Materials

Assay Principle

The PATHFAST® hsCRP procedure is based on CLEIA and MAGTRATION®. In this procedure, alkaline phosphatase labeled anti CRP monoclonal antibody and anti CRP monoclonal antibody coated magnetic particles are mixed with sample. CRP contained in the specimen binds to the CRP 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 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 pipette tip and is a registered trademark of Precision System Science.

Precautions

Reagents 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 until immediately prior to use.

Expiration

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

Sample Collection

1. Use serum, whole blood or plasma collected with qualified collection tube containing heparin-Na, heparin-Li or EDTA – 2Na, EDTA-2K.
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 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. It should be ensured that fibrin clots and other insoluble materials are not present in sample, otherwise such material has to be removed by centrifugation or filtration.
4. Plasma samples are stable for 7 days at +2 to +8 ℃ and 29 days at –20 ℃ or lower. However frozen samples should not be repeatedly frozen and thawed prior to testing.
5. Serum samples are stable for 7 days at +2 to +8 ℃ and 60 days at –20 ℃ or lower. However frozen samples should not be repeatedly frozen and thawed prior to testing.

6. Before processing, mix, and then centrifuge at 2,500 - 3,000 x g for 10 minutes all previously frozen specimens and those stored longer than 12 hours.

Preparation and procedure

Reagent preparation

1. Reagent cartridge: Ready to use.
2. Calibrator-1 (CAL-1): Ready to use.
   Calibrator-2 (CAL-2): 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 to +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 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 four 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, and 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 to obtain 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 concentrations of CRP.
2. Good laboratory practices recommend the use of appropriate quality controls. It is recommended that national, federal, 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 or plasma (heparin-Na, heparin-Li or EDTA–2Na, EDTA-2K), or serum as sample.
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. Plasma or serum samples with results > 30 mg/L can be tested after diluting the sample by 10-fold with sample diluent (Product # PF01D) if an exact result is desired. Sample containing above 150 mg/L of CRP antigen will be still reported as > 30 mg/L on PATHFAST® even after diluting 10-fold with the sample diluent.

   A dilution procedure of plasma sample is as follows:
   a. Pipette 900 µL of the sample diluent (Product # PF01D) into a test tube.
   b. Pipette 100 µL of plasma or serum sample (patient) to test tube. Mix gently.
   c. Transfer approximately 100 µL of the contents of the test tube into a sample well of a PATHFAST® reagent cartridge.
   d. Multiply by 10, the dilution factor, to the result reported on PATHFAST® to obtain the original CRP concentration of sample.

Expected Value

1. The expected value may vary from laboratory and 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® hsCRP assay, the calculated value for the 97.5th percentile for CRP in serum samples of 275 apparently healthy individuals (160 males and 115 female – age range 16-77 years) was 4.16 mg/L.

Specific performance data of the test

Representative performance data on the PATHFAST® are given below.

1. Assay range: 0.05 - 30 mg/L
2. Methods comparison (serum samples):
   （y：this method; x：Siemens, Immulite® hsCRP）
   y = 0.98x + 0.024; r = 0.995; n = 139
   Range of samples: 0.268 -28.4 mg/L
   y = 0.98x + 0.037; r = 0.991; n = 119
   Range of samples: 0.268 -9.58 mg/L
   Method comparison study (serum samples) was performed by physician assistants and medical office personnel in three non-laboratory sites. Data was combined with three sites.
   y = 1.01x – 0.056, r = 0.990, n = 60
   Range of samples: 0.330 – 28.4 mg/L
   IMMULITE® is a registered trademark of Siemens Medical Solutions Diagnostics.
3. Correlation between lithium heparin plasma and other sample matrices
   y=0.99x + 0.333, r=0.990, n=48
   (y: lithium heparin plasma; x: serum)
   y=0.98x – 0.213, r=0.996, n=49
   (y: lithium heparin whole blood; x: lithium heparin plasma)
   y=1.03x – 0.025, r=0.991, n=65
   (y: sodium heparin plasma; x: lithium heparin plasma)
   y=0.97x – 0.034, r=0.994, n=54
   (y: sodium heparin whole blood; x: lithium heparin plasma)
   y=0.98x – 0.217, r=0.993, n=56
   (y: EDTA-2K plasma; x: lithium heparin plasma)
   y=1.02x – 0.235, r=0.995, n=48
   (y: EDTA-2K whole blood; x: lithium heparin plasma)
   y=0.98x – 0.233, r=0.992, n=49
   (y: EDTA-2Na plasma; x: lithium heparin plasma)
   y=0.95x + 0.143, r=0.987, n=53
   (y: EDTA-2Na whole blood; x: lithium heparin plasma)
4. Traceability
   The calibrators for PATHFAST® hsCRP are traceable to the reference material IRMM･CRM 470.
5. Imprecision
   The imprecision was determined using the present method, 4 control materials in a protocol as follows: Four serum samples were assayed in duplicate for 20 non-consecutive days. The within-run and total standards deviations were calculated according to the NCCLS EP-5A2 [ISBN 1-56238-542-9]. The following results were obtained:

		Within-run precision		Total precision	
Sample	Mean (mg/L)	S.D. (mg/L)	C.V. (%)	S.D. (mg/L)	C.V. (%)
QC-LL	0.916	0.069	7.5	0.068	7.4
QC-M	4.63	0.279	6.0	0.374	8.1
QC-M	15.1	1.19	7.8	1.29	8.5
QC-H	25.6	1.24	4.8	1.37	5.3

Imprecision study (serum based control samples) was performed in duplicate 2 times a day for 5 days by physician assistants and medical office personnel in three non-laboratory sites. Data was combined with three sites.

Site	Mean	SD	%CV
Site 1	0.711	0.029	4.1%
Site 2	0.670	0.041	6.1%
Site 3	0.710	0.041	5.8%
Overall	0.697	0.043	6.2%

6. Analytical sensitivity (detection limit): 0.001 mg/L
   The analytical sensitivity represents the lowest analyte level that can be distinguished from zero. It is calculated as the value lying two standard deviations above that of plasma non-containing CRP.
7. Limit of quantitation: < 0.008 mg/L
   Limit of quantitation is the lowest analyte concentration that can be reproducibly measured with a between run coefficient of variation of 10 %
8. High-dose hook effect
   None up to 1250 mg/L
9. 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, lipemia in sample	(1000 mg/dL)
Hemoglobin (hemolysis)	(Hb 1000 mg/dL)
Rheumatoid Factor	(550 IU/mL)

10. Analytical specificity
    The following substances have no significant cross-reactivity on the assay at the concentration indicated in parentheses on PATHFAST® CRP assay

Human Albumin	1,000 mg/dL
Human IgG	1,000 mg/dL
Transferrin	1,000 mg/dL

Limitations of Procedure

1. For diagnostic purpose, CRP results should be used in conjunction with other test results, the overall clinical presentation to the physician, and all other appropriate information. When being used for risk assessment, the following precautions must be noted:
   - ✧ Increases in CRP are non-specific and should not be interpreted without a complete clinical history.
   - ✧ Screening the entire adult population is not recommended.
   - ✧ CRP measurements are not a substitute for traditional cardiovascular risk factors.
   - ✧ Acute coronary syndrome management should not depend on CRP measurements.
   - ✧ Patients with persistently unexplained CRP levels above 10 mg/L should be evaluated for other non-cardiovascular origins.
   - ✧ Testing for risk assessment should not be performed while there is an indication of infection, systemic inflammation, or trauma.
   - ✧ Secondary prevention measures should be based on global risk assessment and not depend on CRP.
   - ✧ Serial testing of CRP should not be used to monitor effects of treatment.
   - ✧ The average of CRP results repeated optimally over two weeks should be used in performing risk assessment on metabolically stable patients.
2. The instrument reporting system contains error codes to warn the operator of specific malfunctions. Any report slip containing such error codes should be held for follow-up. See the PATHFAST® operator's manual.
3. 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 cautions.

Interpretation of Results

It is recommended to follow-up elevated CRP values with repeat testing and detailed clinical evaluation. Intra-individual variations of CRP are significant and should be taken into account when interpreting values.

References

1) Dixon JS, Bird HA, Sitton NG, et al. C-reactive protein in the serial assessment of disease activity in rheumatoid arthritis. Scand J Rheumatol 1984;13:39-44.
2) Kushner DL,Rzewricki DL. The acute phase response :general aspects. Bailliere's Clin Rheumatol 1994;8:513-30.
3) van Leeuwen MA, van Rijswijk MH. Acute phase proteins in the monitoring of inflammatory disorders. Bailliere's Clin Rheumatol 1994;8:531-52.
4) Pulliam PN, Attia MW, Cronan KM. Undetectable serious bacterial infection C-reactive protein in febrile children 1 to 36 months of age with clinically undetectable serious bacterial infection. Pediatrics 2001; 108:1275-9
5) Ridker PM, Buring JE, Shih J et al. Prospective study of CRP and the risk of future cardiovascular events among apparently healthy women. Circulation 1998;98:731-3.
6) Ridker PM, Glynn RJ, Hennekens CH. CRP adds to the predictive value of total and HDL cholesterol in determining risk of first myocardial infarction. Circulation 1998;97:2007-11.
7) Ridker PM, Rifai N, Pfeffer MA, et al. Inflammation, pravastatin, and the risk of coronary events after myocardial infarction in patients with average cholesterol levels. Cholesterol and Recurrent Events (CARE) Investigators. Circulation 1998;98:839-44.
8) Tracy RP, Lemaitre RN, Psaty BM, et al. Relationship of CRP to risk of cardiovascular disease in the elderly. Results from the Cardiovascular Health Study and the Rural Health Promotion Project. Arterioscler Thromb Vasc Biol 1997;17:1121-7.
9) Macy EM, Hayes TE, Tracy RP. Variability in the measurement of CRP in healthy subjects: implications for reference intervals and epidemiological applications. Clin Chem 1997;43:52-8.
10) Ridker PM, Cushman M, Stampfer MJ, et al. Inflammation, aspirin, and the risk of cardiovascular disease in apparently healthy men. N Engl J Med 1997;336:973-9. Erratum: ibid 1997;337:356.
11) Guidance for Industry and FDA Staff, Review Criteria for Assessment of C-Reactive Protein (CRP), High Sensitivity C-Reactive Protein (hsCRP) and Cardiac C-Reactive Protein (cCRP) Assays, Document issued on: September 22, 2005

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
CARTRIDGE	: Reagent Cartridge
MC ENTRY CARD	: Entry Card for Master Calibration Curve

*PATHFAST : US Registered Trademark No.3074207

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