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REF PF1201-K IVD For in vitro diagnostic use

PATHFAST™ Presepsin

<REAGENT FOR PATHFAST>

60 Tests

Intended Use

PATHFAST Presepsin is a chemiluminescent enzyme immunoassay for the quantitative measurement of presepsin (sCD14-ST) concentration in whole blood or plasma. PATHFAST Presepsin is used as an aid in the diagnosis and prognosis of sepsis, in the assessment of the degree of septic severity and to aid in the risk stratification of critically ill septic patients.

Summary

CD14 is a glycoprotein expressed on the surface membrane of monocytes/macrophages (mCD14) and serves as a receptor for complexes of lipopolysaccharides (LPS) and LPS binding protein (LPBP). mCD14 co-localizes with toll-like receptor 4 (TLR4). Upon binding of the LPBP complex CD14 activates the TLR4-specific proinflammatory signaling cascade thereby starting the inflammatory reaction of the host against infectious agents. The complex of LPS-LPBP-CD14 is released into circulation by shedding of CD14 from the cell membrane yielding soluble CD14 (sCD14). However, plasma protease activity generates also another sCD14 molecule called sCD14 subtype (sCD14-ST) or presepsin[1]. The levels of presepsin were significantly higher in septic patients than in patients with SIRS (systemic inflammatory response syndrome) or appearently healthy individuals[1][2]. The one of the mechanism of presepsin secretion is related to phagocytosis process and cleavage with lysozomal enzymes of microorganism[3]. Presepsin levels were elevated earlier than IL-6 and D-dimer along with occurrence of blood bacteria in a rabbit cecal ligation and puncture (CLP) model[4].

The determination of the presepsin concentration can be used not only for diagnosis and prognosis of sepsis, but also to monitor the course of the disease and the responses to therapeutic interventions[5][6].

PATHFAST Presepsin is a chemiluminescent enzyme immunoassay (CLEIA) for the quantitative measurement of presepsin concentration. Monoclonal antibodies and polyclonal antibodies recognizing presepsin are used in the assay. All required components are packed in one reagent cartridge. Using the PATHFAST analyzer presepsin concentration can be determined within 17 minutes.

Important Notes:

Results obtained from this assay should always be judged in combination with the clinical examination, patient's medical history, and other findings before momentous actions will be prefaced.

Package composition

Reagent cartridge 6 cartridges x 10 trays

The reagent cartridge consists of 16 wells. All wells except the sample well (#1) and the counting well (#10) are covered with aluminum seal containing bar codes. All reagents required 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 presepsin PoAb*	50 μL	Microorganism Rabbit
		MES*** Buffer		
		Na azide	< 0.1%	
# 7	liquid	anti presepsin MoAb** coated magnetic particles	50 μL	Mouse
		MES*** Buffer		
#13	liquid	Chemiluminescent substrate CDP-Star	100 μL	
# 11	liquid	Sample Dilution Buffer	50 μL	
		Tris Buffer		
		Na azide	< 0.1%	
# 3,4,5	liquid	Washing Buffer	400 μL	
		MOPS**** Buffer		
		Na azide	< 0.1%	

*PoAb = Polyclonal antibody
**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 trademark of Applied Biosystems, LLC.

Calibrators

1.	Calibrator 1 (CAL-1)	1 x 2.0 mL (solution ready to use)
2.	Calibrator 2 (CAL-2)	2 x lyophilized for 1.2 ml
3.	Calibrator diluent	2 x 1.2 mL (liquid) Na azide (0.05%)

MC ENTRY CARD	1 sheet
Instruction for use	1 sheet
Control data sheet	1 sheet

Materials Needed But Not Provided

PATHFAST™ Analyzer and consumables
Presepsin Quality Control Materials

Assay Principle

The test principle of PATHFAST Presepsin is based on non-competitive CLEIA combined with *MAGTRATION® technology. During incubation of the sample with alkaline phosphatase labeled anti presepsin polyclonal antibody and anti presepsin monoclonal antibody coated magnetic particles, the presepsin of the sample binds to the anti presepsin antibodies forming an immunocomplex with enzyme labeled antibody and antibody coated magnetic particles. After removing the unbound substances by *MAGTRATION® technology, a chemiluminescent substrate is added. After a short incubation, the luminescence intensity generated by the enzyme reaction is measured. The luminescence intensity is related to the presepsin concentration of the sample 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

Reagents cartridge

1. Do not reuse a reagent cartridge. This is designed for single use only.
2. Do not peel off the aluminum seal.
3. Handle the reagent cartridge by plucking at edge of it and do not touch the aluminum seal and the black well with the fingers.
4. Avoid contamination of saliva in the black well.
5. When the reagent cartridge is dropped and damaged, do not use it.
6. After certain terms of 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.
7. Avoid contamination and exposure to direct sunlight.
8. Used reagent cartridges contain human body fluids. Handle with appropriate care to avoid skin contact and injection.
9. Do not use reagents beyond its indicated expiration date.
10. Do not use any reagent cartridge, which was kept at room temperature.
11. 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.

Storage Instructions

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

Expiration

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

Sample Collection

1. Use whole blood or plasma collected with qualified collection tubes containing heparin or EDTA. Do not use serum.
2. 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 PATHFAST, the assay must be started immediately.
3. It should be ensured that fibrin threads or clots and other insoluble materials are not present in the sample, otherwise such material should be removed by centrifugation or filtrations.
4. Plasma samples are stable for 3 days at +2 to +8 °C and 9 months at -20 °C or lower. If testing is not performed beyond 3 days, plasma samples may be kept at -20 °C or lower. However frozen samples should not be repetitively frozen and thawed prior to testing.
5. Avoid vigorous mixing, including vortex mixing and long gentle mixing.
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.
3. Calibrator-2 (CAL-2):
   Reconstitute every vial of CAL-2 each with one bottle of Calibrator diluent. The reconstituted calibrator is stable for 24 hours at +2 to + 8°C and 6 months at -20°C or lower. Avoid more than six freeze/thaw cycles.

NOTE:
1. 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.
2. CAL-2 contains bovine serum albumin. Handle with appropriate care to avoid skin contact.

Installation of master calibration curve
1. Installation of 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. 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 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, then dispense approximately 100 μL of CAL-1 and CAL-2 in sample wells to load on 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 presepsin (sCD14-ST).
2. Good laboratory practices recommend the use of appropriate quality controls. It is recommended to follow federal, state 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. Use heparinized or EDTA whole blood or plasma as sample.
2. Set a reagent cartridge in cartridge rack, then pipette approximately 100 μL of the sample into a sample well of a cartridge and load the cartridge rack on PATHFAST.
3. Carry out the assay in accordance with the PATHFAST operator's manual.

Note:
1. When whole blood is used, the whole blood contained in a blood collection tube should be mixed gently just before dispensing (do not use vortex mixer). After pipetting the whole blood sample and loading the cartridge on PATHFAST, the assay must be started immediately.
2. When whole blood or plasma samples are left for more than 5 minutes after pipetting into a sample well, a lower result will be obtained analyzing whole blood because of blood sedimentation and a higher result will be obtained analyzing plasma because of increasing presepsin concentration by evaporation.
3. When whole blood is used, input of individual hematocrit value of the sample in the PATHFAST is optional. Refer to the PATHFAST operator's manual.
4. Samples with result above 20,000 pg/mL should be diluted with sample diluent (Product#PF02D) and retested if a quantitative result is desired or alternatively, they can be reported as > 20,000 pg/mL. The recommended dilution is 1 : 5.

Expected values

Reference range

Study-1: The reference range of presepsin is independent from age and gender. Presepsin concentration was determined in EDTA plasma samples from 230 apparently healthy individuals.

Age

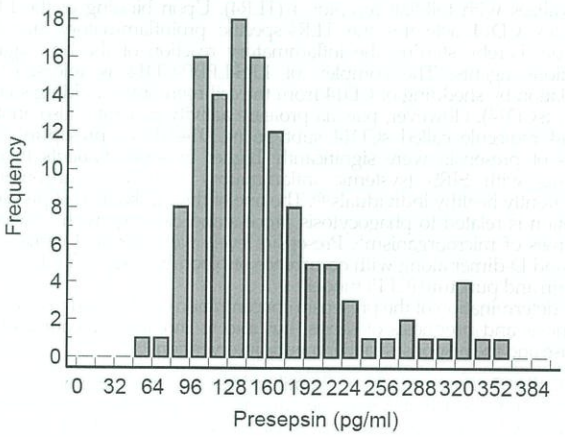
	All	< 30yrs	30-39 yrs	40-49 yrs	50+ yrs
Mean	155	152	158	146	164
SD	54.2	54.5	38.7	48.4	66.7
Median	145	141	150	136	152
95th percentile	327	332	270	265	346
N	230	55	46	63	66

Gender

	All	Males	Females
Mean	155	152	159
SD	54.2	54.4	54.1
Median	145	142	148
95th percentile	327	328	318
N	230	126	104

Study-2: A study performed using 119 EDTA plasma samples from apparently healthy individuals. The presepsin values ranged from 60 to 365 pg/mL with an arithmetric mean of 160 pg/mL; 95% CI (148 -171 pg/mL). The 5 th percentile was 92pg/mL (95% CI 79-100 pg/mL) and the 95th percentile 320pg/mL 95 % CI (233-363 pg/mL). ROC analysis revealed a cut off value of 337 pg/mL for discrimination between healthy normal individuals and patients with sepsis.

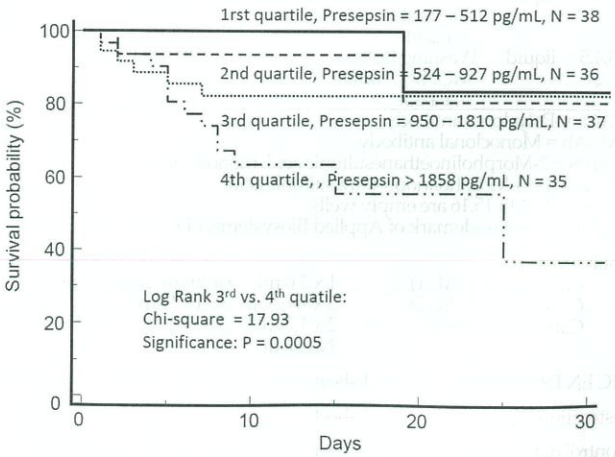
Presepsin distribution in normal indiviuals (N=119)



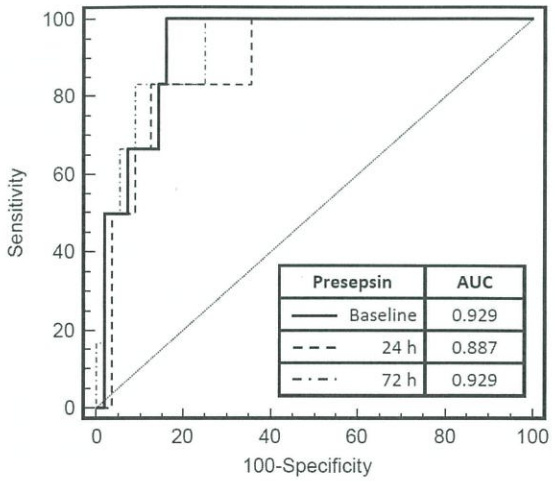
Note: The expected value/reference values may vary from laboratory to laboratory and from country to country depending on various factors. It is therefore recommendable for each institution to establish corresponding reference values. We recommend each laboratory to establish its own reference values. Presepsin levels are elevated in clinically relevant microbial infections and continue to rise with the increasing severity of the disease[3)5)]. However, as an expression of individual different immune responses and different clinical situations, the same focus of infection may be associated with varying individual elevations in presepsin concentrations. Therefore, clinicians should use the presepsin results in conjunction with the patient's other laboratory findings and clinical signs, and interpret the concrete values in the context of the patient's clinical situation. The reference ranges are therefore given for orientational purpose only.

Prognostic power of presepsin

In patients suspicious for sepsis at presentation who developed sepsis the presepsin values were determined at baseline and compared to the 30 days mortality. Kaplan-Meier analysis of survival within 30 days after admission to hospital showed that patients with presepsin values in the highest quartile (>1,858 pg/mL) revealed a mortality risk of > 60% (p=0.0005). Whereas patients with presepsin values in the lower quartiles reached a lower mortality of 20 %.



The figure below represents the results of the ROC analysis regarding the prognosis of mortality using the presepsin values at baseline, 24 hours and 72 hours after presentation. The prognostic power of presepsin at baseline and at 72 hours after presentation are comparable (AUC = 0.929).



Analytical performance data

Representative performance data on the PATHFAST Presepsin is given below.

1. Assay range: 20 – 20,000 pg/mL
2. Correlation between whole blood and plasma on PATHFAST.
   y = 1.04 x - 10.8 ; r = 0.986 ; n = 104 (y: EDTA whole blood, x: EDTA plasma)
   y = 1.02 x + 28.6 ; r = 0.980 ; n = 104 (y: heparinized whole blood, x: EDTA plasma)
   y = 1.01 x + 26.9 ; r = 0.983 ; n = 104 (y: heparinized plasma, x: EDTA plasma)
3. Standardization
   The calibrators for PATHFAST Presepsin are assigned by amino acid analysis[10].
4. Imprecision
   Reproducibility was determined using the present method, four control materials in a protocol as follows: Four plasma samples were assayed in duplicate for 20 non-consecutive days. The within-run and total standards deviations and coefficient of variations were calculated according to the NCCLS document EP5-A2 [ISBN 1-56238-542-9]. The following results were obtained;

		Within-run precision		Total precision	
Sample	Mean (pg/mL)	S.D. (pg/mL)	C.V. (%)	S.D. (pg/mL)	C.V. (%)
QC-LL	445	19.8	4.4%	19.4	4.4%
QC-L	882	25.9	2.9%	35.0	4.0%
QC-M	4,801	154	3.2%	183	3.8%
QC-H	19,292	753	3.9%	956	5.0%

5. Analytical sensitivity: < 20.0 pg/mL
   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 zero calibrator.
6. Limit of quantitation: < 57.1 pg/mL
   Limit of quantitation is the lowest analyte concentration that can be reproducibly measured with a between run coefficient of variation of 10 %.
7. High-dose hook effect
   None up to 4,000,000 pg/mL
8. Analytical specificity
   The following substances have no significant cross-reactivity on the assay at the concentration indicated in parentheses on PATHFAST Presepsin.

   sCD14 (4.0ug/mL) not detectable

Possible interferences

The following substances were found to interfere less than 10 % on the assay at the concentrations indicated in the following list.

Interfering substance	No-interfering concentration
Free bilirubin	40 mg/dL
Conjugated bilirubin	40 mg/dL
Triglyceride	1,000 mg/dL
Hemoglobin (hemolysis)	600 mg/dL
Rheumatoid Factor	500 IU/mL
Acetaminophen	20 mg/dL
Acetylsaltic Acid	65.2 mg/dL
Allopurinol	4.0 mg/dL
Ampicillin	5.3 mg/dL
Ascorbic Acid	6.0 mg/dL
Atenolol	1.0 mg/dL
Caffeine	10 mg/dL
Captopril	5.0 mg/dL
Digoxin	0.61 ug/dL
Dopamine	65 mg/dL
Erythromycin	20 mg/dL
Furosemide	6.0 mg/dL
Methyldopa	2.5 mg/dL
Niphedipine	6.0 mg/dL
Phenytoin	10 mg/dL
Theophyline	25 mg/dL
Verapamil	16 mg/dL
Protein(Albumin)	6 g/dL
Imipenem	2.0 mg/dL
Cefotaxim	200 mg/dL
Vancomycin	4.0 mg/mL
Noradrenaline	4.0 ug/mL
Dobutamine	25 ug/mL
Heparin	100 IU/mL
EDTA	2.0 mg/mL

Limitations of Procedure

1. The instrument reporting system contains error codes to warn the operator of specific malfunctions. Any reports 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 influence 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 cautions.
3. The results of the PATHFAST Presepsin 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 agree with the clinical picture or history, additional tests should be performed.

References

1) Shirakawa K, Naitou K, Hirose J, Takahashi T, Furusako S. Establishment of one-step ELISA detecting Presepsin (sCD14-ST): a new marker for sepsis. Clin Chem Lab Med. 2010 Submitted for publication
2) Yaegashi Y, Shirakawa K, Sato N, Suzuki Y, Kojika M, Imai S, Takahashi G, Miyata M, Furusako S, Endo S. Evaluation of a newly identified soluble CD14 subtype as a marker for sepsis. J Infect Chemother. 2005 Oct; 11(5): 234-8
3) Naitoh K, Shirakawa K, Hirose J, Nakamura M, Takeuchi T, Hosaka Y, Furusako S, The new sepsis marker, sCD14-ST (PRESEPSIN), induction mechanism in the rabbit sepsis models. SEPSIS 2010: P19
4) Nakamura M, Takeuchi T, Naito K, Shirakawa K, Hosaka Y, Yamasaki F, Furusako S. Early elevation of plasma soluble CD14 subtype, a novel biomarker for sepsis, in a rabbit cecal ligation and puncture model. Critical Care 2008, 12 (Suppl 2) :P194 (doi: 10.1186/cc6415)
5) Kojika M, Takahashi G, Matsumoto N, Kikkawa T, Hoshikawa K, Shioya N, Shibata S, Suzuki Y, Aoki H, Shirakawa K, Endo S. Serum levels of soluble CD14 subtype reflect the APACHE II and SOFA Scores. Medical Postgraduates 2010 Jan; 48(1): 46-50
6) Takahashi G, Suzuki Y, Kojika M, Matsumoto N, Shozushima T, Makabe H, Yamada Y, Shioya N, Shibata S, Shirakawa K, Endo S. Evaluation of responses to IVIG therapy in patients with severe sepsis and septic shock by soluble CD14 subtype monitoring. Medical Postgraduates 2010 Jan; 48(1): 19-24
7) The results of Japanese clinical study for PATHFAST Presepsin
8) The results of Europian clinical sudy for PATHFAST Presepsin
9) Bone RC, Balk RA, Cerra FB, Dellinger RP, Fein AM, Knaus WA, Schein RM, Sibbald WJ. Definitions for sepsis and organ failure and guidelines for the use of innovative therapies in sepsis. The ACCP/SCCM Consensus Conference Committee. American College of Chest Physicians/Society of Critical Care Medicine. Chest. 1992 Jun;101(6):1644-55
10) The Japanese Pharmacopoeia Fifteenth Edition (JP15): Supplement II, Amino Acid Analysis of Proteins, 1814-1822

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
CONTROL DATA SHEET	: Data Sheet for control

* PATHFAST: JP Registered Trademark No.4685182