HIGH SENSITIVE C-REACTIVE PROTEIN

Diagnostic reagent for determination of CRP concentration with higher sensitivity.
Liquid. Dual Reagents. Store at +2/+8°C.For in Vitro Diagnostic Use. **Do not freeze.**

Ref No	Package	Ref No	Package	Ref No	Package	Ref No	Package
LM275	240 mL	L2110	240 mL	TAB240	550 mL	ZA48	125 mL

Changes made in the instructions for use are marked as grey.

INTENDED USE

Test for the quantitative determination of C-reactive protein in human serum and plasma.

GENERAL INFORMATION

In 1930, Tillet and Francis identified a substance in the sera of acutely ill patients that bound cell wall C-polysaccharide of Streptococcus pneumoniae and agglutinates the organisms.[1] In 1941 the substance was shown to be a protein and named C-reactive protein (CRP).[2] CRP is composed of five identical, non-covalently associated, non-glycosylated subunits of 23 kDa to form a disk-shaped structure with radial symmetry and total mass of approximately 115 kDa.[3] It is an acute phase reactant produced by the liver in response to inflammatory cytokines, most prominently interleukin-6.[4,5,6] It also helps non-specific host defense against infectious organisms by activating the classical complement pathway[3] and can bind to phagocytic cells.[6]

CRP is one of the most potent acute phase reactants, and plasma concentrations increase up to 1000-fold after myocardial infarction, stress, trauma, infection, inflammation, surgery or neoplastic proliferation.[7] and is normally present in plasma at a concentration below 5 mg/L. Concentrations higher than 5 to 10 mg/L suggest the presence of an infection or inflammatory process. Concentrations are usually higher in bacterial infection than in viral infection, but concentrations greater than 100 mg/L can be seen in uncomplicated influenza and infectious mononucleosis. The increase with inflammation occurs within 6 to 12 hours and peaks at about 48 hours and is usually proportional to the extent of tissue damage. However, since the increase is non-specific, it cannot be interpreted without other clinical information.[3]

A systematic review and meta-analysis reported that CRP has an estimated diagnostic sensitivity of 75% and specificity of 67% in distinguishing bacterial infection from non-infectious inflammation causes.[5]

Another study found that in pediatric patients with a systemic inflammatory response, the use of a panel of eight biomarkers, including CRP and procalcitonin, had a negative predictive value of 90% in identifying patients without bacterial infection.[8]

CRP is frequently used to diagnose bone and joint infections in both children and adults.[9,10] It is also used as a marker for the timing of transition to oral antibiotic therapy in the treatment of bone and joint infections in children.[11,12] Although some studies have concluded that CRP can be used to safely guide and reduce antibiotic use in the treatment of acute exacerbation of chronic obstructive pulmonary disease, these results have not yet come into use in clinical practice outside of these study settings.[13,14,15] There are also some studies on the appropriateness of deciding on the continuation of antibiotic use during bacteremia treatment guided by CRP results.[12,16]

Epidemiologic studies show that mildly elevated CRP concentrations are associated with cardiovascular disease (CVD) risk.[17] Increased concentrations may reflect low-grade, chronic intimal inflammation; but the use of CRP in CVD requires the use of a test with detection limits below 0.3 mg/L, often referred to as a high-sensitivity CRP assay.[3]

TEST PRINCIPLE

Immunoturbidimetric method

The test aims to detect an antigen-antibody reaction between a polyclonal, a monoclonal and specific Anti-CRP antibody with the CRP antigen in the samples.

The CRP concentration in the sample is quantified by turbidimetric measurement of the turbidity of this antigen-antibody complex at a wavelength of 572 nm.

REAGENT COMPONENTS

Reagent 1:

Glycine Buffer	≤ 0.12 mol/L
Sodium azide	≤ %0.1

Reagent 2:

Anti-CRP antibody	
Sodium azide	≤ %0.1

REAGENT PREPARATION

Reagents are ready for use.

REAGENT STABILITY AND STORAGE

Reagents are stable at +2/+8°C till the expiration date stated on the label which is only for closed vials.

Once opened vials are stable for 30 days at +2/+8°C in optimum conditions. On board stability is strongly related

to auto analyzers' cooling specification and carry-over values.

Reagent stability and storage data have been verified by using Clinical and Laboratory Standards Institute (CLSI) EP25-A protocol.[18]

SAMPLE REQUIREMENTS

Serum and plasma collected by standard procedure can be used. For plasma, specimen collection tubes with Li heparin, K2 EDTA, K3 EDTA can be used. Multiple sample freezing and thawing should be avoided.

CRP activity stability in serum and Li-heparinized plasma:

2 weeks at +20/+25°C
3 weeks at +2/+8ºC
1 year at -20°C

CRP activity stability in K2 EDTA and K3 EDTA plasma:

1 day at +20/+25°C,
3 weeks at +2/+8ºC,
1 year at -20°C.

CALIBRATION AND QUALITY CONTROL

Calibration: The assay requires the use of an CRP HS Calibrator.

CRP HS Calibrator - 2 Levels - Liquid
Ref.No: TA205

Calibration stability is 30 days. Calibration stability depends on the application characteristics and cooling capacity of the autoanalyzer used.

Control: Commercially available control material with established values determined by this method can be used. We recommend:

CRP HS Control Level I -Liquid
Ref.No: TA210

CRP HS Control Level II - Liquid
Ref.No: TA211

At least two level controls must be run once in every 24 hours. Each laboratory should determine its own quality control scheme and procedures. If quality control results are not within acceptable limits, calibration is required.

REFERENCE INTERVAL / MEDICAL DECISION LEVELS

Serum and plasma[19] : <5.0 mg/L

The CDC/AHA recommended the following hsCRP cut-off points for CVD risk assessment [20,21]:

hsCRP level (mg/L)	Relative risk
<1.0	low
1.0-3.0	average
>3.0	high

Each laboratory should investigate the transferability of the expected values to its own patient population and if necessary, determine its own reference range.

Reference interval data have been verified by using CLSI EP28-A3c protocol.[22]

PERFORMANCE CHARACTERISTICS

Measuring Interval

According to CLSI EP34-ED1:2018, "Measuring Interval" refers to the interval where the analyte concentration is measured with intended accuracy in terms of medical and laboratory requirements without dilution, concentrating or any kind of pre-treatment that is between the analyte's lower limit of quantitation (LLoQ) and upper limit of quantitation (ULoQ).[23]

The determined analytic measuring interval for CRP HS is 0.35-40 mg/L.

Detection Capability

Limit of Detection (LoD): 0.20 mg/L

Limit of Quantitation (LoQ): 0.35 mg/L

Note: LoQ values are based on Coefficient of Variation Percentage (CV) ≤ 20%.

LoD and LoQ values have been verified by using CLSI EP17-A2:2012 protocol.[24]

Linearity

This method shows measurement linearity in the activities up to 40 mg/L. Autoanaylzer's auto-dilution system can be used if the concentrations have higher values. See device manual for further information.

For the manual dilution procedure, dilute the sample 1:5 using 0.90% isotonic. After this process, multiply the result of the reworked sample by the dilution factor. Do not report the sample result after dilution if it is marked as lower than the linear lower limit. Rerun with a suitable dilution.

Linearity Studies data have been verified by using CLSI EP06-A:2003 protocol.[25]

Precision

Running system has been developed according to 20x2x2 "The Single Site" protocol. Repeatability and Within-Laboratory Precision/Within-Device values have been obtained according to the running results.

According to the protocol in use, 2 separate runs per day have been made for 20 days (no obligation for being consecutive days). This protocol has been applied to each low and high samples separately and 80 results have been obtained for each one. Statistically, the results have been obtained using 2-factor Nested-ANOVA model.[26]

Repeatability (Within Run) and Repeatability (Day to Day) SD and CV% values of CRP HS have been given in the table 1 and 2 respectively.

Table 1. CRP HS Repeatability (Within Run) results obtained from samples in two different concentrations

Mean Concentration	SD*	CV%	n
2.57 mg/L	0.06	2.48	80
6.42 mg/L	0.15	2.25	80

***SD: Standard Deviation**

Note: This working system has been named "Within-Run Precision" in the previous CLSI - EP05-A2 manual.[27]

Table 2. CRP HS Repeatability (Day to Day) results obtained from samples in two different concentrations

Mean Concentration	SD	CV%	n
2.57 mg/L	0.09	3.70	80
6.42 mg/L	0.19	3.04	80

Note: This working system has been named "Total Precision" in the previous CLSI - EP05-A2 manual.[27]

Method Comparison and Correlation:
Correlation with a reference reagent is r= 0.99
Regression analysis according to Passing-Bablok Fit**:**
Slope: 1.007
Intercept: 0.098

Prozone
No prozone effect was observed up to 100 mg/L tested for CRP HS.

Interference

Endogenous interferant and analyte concentrations that have been used in the Iron scanning tests has been determined according to "CLSI EP37-ED1:2018" and "CLSI EP07-ED3:2018" manuals.[28,29]

The total acceptable error rate, which is going to be used to detect whether the observed differential value obtained from CRP HS interference scanning test is appropriate, is determined as ±10%.[30]

In CRP HS test results, no significant interaction has been observed in the determined endogenous interferant and analyte concentrations or between interferants and analyte.

It should be noted that endogenous interferants, as well as various medicines and metabolites, anticoagulants (e.g. Heparin, EDTA, citrate, oxalate) and preservatives (e.g. sodium floride, iodoacetate, hydrochloride acide) such as additives, materials that may contact with samples during collection and processing (serum separator devices, sample collection containers and contents, catheters, catheter wash solutions, skin disinfectants, hand cleaners and lotions, glass washing detergents, powder gloves), dietary substances known to affect some specific tests (caffeine, beta-carotene, poppy seeds, etc.), or some substances present in a sample that cause foreign proteins (heterophilic antibodies, etc.), autoimmune response (autoantibodies, etc.), or due to malignancy (for example, interference by paraproteins with phosphate testing and indirect ion selective electrode methods) may show some negative effects that will cause various attempts and some misjudgements.[29]

These performance characteristics have been obtained using an autoanalyzer. Results may vary slightly when using different equipment or manual procedures.

WARNINGS AND PRECAUTIONS

IVD**:** For in Vitro Diagnostic use only.
Do not use expired reagents.
Reagents with two different lot numbers should not be interchanged.
For professional use.
Follow Good Laboratory Practice (GLP) guidelines.

CAUTION: Human source samples are processed with this product. All human source samples must be treated as potentially infectious materials and must be handled in accordance with OSHA standards.

Danger

EUH032	:Releases a very toxic gas if contacts with acid.
H317	:May cause allergic skin reaction.

Precaution

P280	:Use protective gloves / clothes / glasses / mask.
P264	:Wash your hands properly after using.
P272	:Contaminated work clothes should not be allowed to be used outside of the workplace.

Intervention

P302+P352	:Wash with plenty of water and soap if it contacts with skin.
P333+P313	:Seek medical help if it irritates your skin or develops rash.
P362+P364	:Remove contaminated clothes and wash properly before using.

Disposal

P501	:Dispose the vials and contents according to the local regulations.

REFERENCES

1. Tillett WS, F.T., Serological reactions in pneumonia with a non-protein somatic fraction of pneumococcus. J Exp Med 1930. 52: p. 561-571.
2. Abernathy TJ, A.O., The occurrence during acute infections of a protein not normally present in the blood. II. Isolation and properties of the reactive protein. J Exp Med 1941. 73: p. 183-190.
3. Rifai, N., Chiu, R. W., & Young, I., et al., (2023) Tietz Textbook of Laboratory Medicine (7th ed.), Chapter 31:

Amino Acids, Peptides, and Proteins, p.349-e42, Elsevier, St. Louis, Missouri 63043
4. Langsted A, Kamstrup PR and Nordestgaard BG. High lipoprotein(a) and high risk of mortality. Eur Heart J 2019;40:2760–70.
5. Kamstrup PR, Tybjaerg-Hansen A, Steffensen R, et al. Genetically elevated lipoprotein(a) and increased risk of myocardial infarction. JAMA 2009;301:2331–9
6. Rasmussen KL. Plasma levels of apolipoprotein E, APOE genotype and risk of dementia and ischemic heart disease: a review. Atherosclerosis 2016;255:145–55.
7. Gabay, C. and I. Kushner, Acute-phase proteins and other systemic responses to inflammation. N Engl J Med 1999. 340(6): p. 448-54.
8. Roberts WL, Moulton L, Law TC, et al. Evaluation of nine automated high-sensitivity C-reactive protein methods: implications for clinical and epidemiological applications. Part 2. Clin Chem 2001;47:418–25.
9. Hansen SEJ, Madsen CM, Varbo A, et al. Low-grade inflammation in the association between mild-to-moderate hypertriglyceridemia and risk of acute pancreatitis: a study of more than 115000 individuals from the general population. Clin Chem 2019;65:321–32.
10. Zacho J, Tybjaerg-Hansen A, Jensen JS, et al. Genetically elevated C-reactive protein and ischemic vascular disease. N Engl J Med 2008;359:1897–908.
11. Ridker PM, Danielson E, Fonseca FA, et al. Rosuvastatin to prevent vascular events in men and women with elevated C-reactive protein. N Engl J Med 2008;359:2195–207.
12. Ridker PM, Danielson E, Fonseca FA, et al. Reduction in C-reactive protein and LDL cholesterol and cardiovascular event rates after initiation of rosuvastatin: a prospective study of the JUPITER trial. Lancet 2009;373:1175–82.
13. Rifai, N., Chiu, R. W., & Young, I., et al., (2023) Tietz Textbook of Laboratory Medicine (7th ed.), Chapter 36: Lipids and Lipoproteins, p.354-414.e10, Elsevier, St. Louis, Missouri 63043
14. Imhof A, Frohlich M, Loewel H, et al. Distributions of C-reactive protein measured by high-sensitivity assays in apparently healthy men and women from different populations in Europe. Clin Chem 2003;49:669–72.
15. Rifai N and Ridker PM. Population distributions of C-reactive protein in apparently healthy men and women in the United States: implication for clinical interpretation. Clin Chem 2003;49:666–9.
16. DeFilippis AP, Young R, Carrubba CJ, et al. An analysis of calibration and discrimination among multiple cardiovascular risk scores in a modern multiethnic cohort. Ann Intern Med 2015;162:266–75.
17. Mora, S., K. Musunuru, and R.S. Blumenthal, The clinical utility of high-sensitivity C-reactive protein in cardiovascular disease and the potential implication of JUPITER on current practice guidelines. Clin Chem 2009. 55(2): p. 219-28.
18. Clinical and Laboratory Standards Institute (CLSI). Evaluation of Stability of In Vitro Diagnostic Reagents; Approved Guideline. CLSI Document EP25-A. Wayne, PA: CLSI; 2009
19. Dati F, Schumann G, Thomas L, et al. Consensus of a group of professional societies and diagnostic companies on guidelines for interim reference ranges for 14 proteins in serum based on the standardization against the IFCC/BCR/CAP reference material (CRM 470). Eur J Clin Chem Clin Biochem 1996;34:517-520.
20. Pearson TA, Mensah GA, Alexander RW, et al. Markers of inflammation and cardiovascular disease: application to clinical and public health practice: A statement for healthcare professionals from the Centers for Disease Control and Prevention and the American Heart Association. Circulation 2003 Jan 28;107(3):499-511.
21. Ridker PM. Clinical Application of C-Reactive Protein for Cardiovascular Disease Detection and Prevention. Circulation 2003;107:363-369.
22. Clinical and Laboratory Standards Institute (CLSI). Defining, Establishing and Verifying Reference Intervals in the Clinical Laboratory; Approved Guideline – Third Edition. CLSI Document EP28-A3c. Wayne, PA: CLSI; 2010.
23. Clinical and Laboratory Standards Institute (CLSI). Establishing and Verifying an Extended Measuring Interval Through Specimen Dilution and Spiking – 1st Edition. CLSI Document EP34. Wayne, PA: CLSI; 2018
24. Clinical and Laboratory Standards Institute (CLSI). Evaluation of Detection Capability for Clinical Laboratory Measurement Procedures; Approved Guideline – Second Edition. CLSI Document EP17-A2. Wayne, PA: CLSI; 2012
25. Clinical and Laboratory Standards Institute (CLSI). Evaluation of the Linearity of Quantitative Measurement Procedures: A Statistical Approach - 1st Edition. CLSI Document EP06-A. Wayne, PA: CLSI; 2003.
26. Clinical and Laboratory Standards Institute (CLSI). Evaluation of Precision of Quantitative Measurement Procedures; Approved Guideline – Third Edition. CLSI Document EP05-A3. Wayne, PA: CLSI; 2014.
27. Clinical and Laboratory Standards Institute (CLSI). Evaluation of Precision Performance of Quantitative Measurement Methods; Approved Guideline - Second Edition. CLSI Document EP05-A2. Wayne, PA: CLSI; 2004.
28. Clinical and Laboratory Standards Institute (CLSI). Supplemental Tables for Interference Testing in Clinical Chemistry - First Edition. CLSI Document EP37. Wayne, PA: CLSI; 2018.
29. Clinical and Laboratory Standards Institute (CLSI). Interference Testing in Clinical Chemistry - Third Edition. CLSI Document EP07. Wayne, PA: CLSI; 2018.
30. CLIA proficiency testing criteria for acceptable analytical performance, as printed in the Federal Register July 11, 2022;87(131:41194-242.

Archem Sağlık Sanayi ve Tic. A.Ş.
Mahmutbey Mah. Halkalı Cad. No:124 Kat:4
Bağcılar/İstanbul/Turkey
Tel: + 90 212 444 08 92
Fax: +90 212 629 98 89
info@archem.com.tr **www.archem.com.tr**

CE

SYMBOLS

IVD	In Vitro Diagnostic Medical Device
LOT	Lot number
R1	Reagent 1
R2	Reagent 2
GTIN	Global Trade Item Number
REF	Reference Number
GLP	Good Laboratory Practices
FOR USE WITH	Identifies products to be used together
PRODUCT OF TURKEY	Product of Turkey
	Manufacturer
	Expiration date
	Temperature limitation
	Consult instructions for use
	Caution
	Number of Test