CRP LATEX

OSR6199	**4 x 30 mL**	**R1**
	4 x 30 mL	**R2**

Intended Use
System reagent for the quantitative determination of C-Reactive Protein in human serum and plasma on Beckman Coulter AU Analyzers. Measurement of CRP is useful for the detection and evaluation of infection, tissue injury, inflammatory disorders and associated diseases. Measurements may also be useful as an aid in the identification of individuals at risk for future cardiovascular disease. High sensitivity CRP (hsCRP) measurements, when used in conjunction with traditional clinical laboratory evaluation of acute coronary syndromes, may be useful as an independent marker of prognosis for recurrent events, in patients with stable coronary disease or acute coronary syndromes.[1,2]

Summary
C-reactive protein (CRP) is one of the most sensitive acute-phase reactants. With the Beckman Coulter AU System CRP Latex reagent, CRP can be measured down to very low concentrations. Depending on the application used (different instrument settings) two measuring ranges are available:

1. Normal Application (1.0 - 480 mg/L): C-reactive protein levels in serum can rise dramatically after myocardial infarction, stress, trauma, infection, inflammation, surgery, or neoplastic proliferation. The increase occurs within 24 to 48 hours, and the level may be 2000 times normal. Because the increase is non-specific, however, it cannot be interpreted without a complete clinical history, and even then only by comparison with previous values.

2. Highly Sensitive (Cardiac / Neonatal) Application – Beckman Coulter AU400/400^{e}/480, AU600/640/640^{e}/680 (0.2 - 160 mg/L), AU2700/5400 (0.2 – 80 mg/L):
Studies have also shown that the detection of much lower CRP levels can provide valuable information. The typical CRP concentration for healthy adults is (depending on the specific level of the individual patient) < 1 mg/L[3]. Slightly higher values can indicate an increased risk for coronary heart disease in asymptomatic patients.[1,2] CRP concentrations above 3 mg/L, at the time of hospital admission, predict a precarious outcome after a myocardial infarct.[4] The following relative risk categories in relation to average CRP level have been recommended[5]: Low < 1mg/L, Average 1.0 to 3.0 mg/L and High > 3.0 mg/L. Increases in C-Reactive Protein values are not specific and should not be interpreted without a complete clinical history since CRP is an acute phase protein which can rise non-specifically due to other inflammatory conditions. For cardiac risk analysis, other cardiac disease-specific testing must be done, such as Total cholesterol, HDL cholesterol, and LDL cholesterol. When being used for risk assessment, levels of CRP > 10 mg/L should be evaluated for other non-cardiovascular origins. Testing for any risk assessment should not be performed while there is indication of infection, systemic inflammation, or trauma. **This assay is not meant for management of acute coronary syndrome and is not a substitute for traditional cardiovascular risk factors.** Screening the entire adult population for hsCRP is not recommended. The average of hsCRP levels determined two weeks apart should be used in performing risk assessment on metabolically stable patients. hsCRP is considered to be a Class IIa marker for acute coronary syndrome in addition to Troponin I.[6]

Cord blood normally has very low CRP concentrations (median 0.12 mg/L[7]). In the diagnostic evaluation of neonates with suspected infection, measurements of serial CRP levels are useful. Two low CRP levels obtained 24 hours apart indicate that bacterial infection is highly unlikely.[8] Thus, CRP Latex reagent is a valuable tool for the early diagnosis of infection in preterm infants and neonates. It assesses both the need for, and the effectiveness of, antibiotic treatment. However, CRP values alone should not be used as a basis for early discontinuance of antibiotic therapy.

Methodology
Immune complexes formed in solution scatter light in proportion to their size, shape, and concentration. Turbidimeters measure the reduction of incidence light due to reflection, absorption, or scatter. In this procedure, the measurement of the rate of decrease in light intensity transmitted (increase in absorbance) through particles suspended in solution is the result of complexes formed during the immunological reaction between the CRP of the patient serum and rabbit anti-CRP-antibodies coated on latex particles.

System Information
For AU400/400^{e}/480, AU600/640/640^{e}/680 and AU2700/5400 Beckman Coulter Analyzers.

Reagents
Final concentration of reactive ingredients:

Glycine buffer	100 mmol/L
Latex coated with anti-CRP Antibodies	< 0.5 %

Also contains preservatives.

Precautions
1. For *in vitro* diagnostic use.
2. Do not ingest. Harmful if swallowed.
3. Contains sodium azide as a preservative, which may react with lead joints in copper plumbing to form explosive compounds. Even though the reagent contains minute quantities of sodium azide, drains should be well flushed with water when discarding the reagent.

Preparation of reagents
The CRP Latex reagents are ready for use. No preparation is required. Agitate gently before use to ensure a uniform suspension of particles. Repeat at weekly intervals thereafter.

Storage and stability
1. The unopened reagents are stable until the expiration date printed on the label when stored at 2 - 8°C.
2. Opened bottles of reagent are stable for 90 days when stored in the refrigerated compartment of the analyzer.

Indications of Deterioration
Gross turbidity or precipitate in R1, or visible signs of microbial growth in the C-Reactive Protein reagents may indicate degradation and warrant discontinuation of use.

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Specimen Collection and Preparation

Serum, EDTA and Lithium heparin plasma may be used.
Comparison studies have shown no statistical significant difference between CRP recovery in serum and plasma within the accuracy and precision limits of the assay.
For specimen collection and preparation, only use suitable tubes or collection containers.
Centrifuge samples containing precipitates before performing the assay.

Sample Storage and Stability
C-reactive protein specimens are stable for 11 days at 20 - 25°C and 2 months at 4 - 8°C in serum and plasma. For longer storage, freeze serum to -20°C.[9]

Interfering Substances
Results of studies[10] show that the following substances may interfere with this C-reactive protein procedure.[11]
Inaccuracies due to bilirubin (40 mg/dL) are less than 5% at CRP concentrations of 1.0 mg/L
Inaccuracies due to hemolysate (500 mg/dL) are less than 5% at CRP concentrations of 1.0 mg/L
Inaccuracies due to intralipid* (1000 mg/dL) are less than 10% at CRP concentrations of 1.0 mg/L
* Intralipid, manufactured by Pharmacia, is a 20% fat emulsion used to emulate extremely turbid samples.

Samples containing heterophilic antibodies can cause falsely elevated results. Please note that oral contraceptives have been reported to affect results.[12]
The information presented is based on results from Beckman Coulter studies. Beckman Coulter Inc. makes no representation about the completeness or accuracy of results generated by future studies. For further information on interfering substances, refer to Young[13] for a compilation of reported interferences.

Procedure

A complete list of test parameters and operational procedures can be found in the User's Guide appropriate to the analyzer.
Because of the extended linearity range down to very low CRP concentrations, it is essential that the prozone settings are put in place for the AU2700/5400.

Materials provided
CRP Latex Reagent

Materials required but not provided
CRP Latex Normal Calibrator (Cat # ODC0026) for the Normal Application.
CRP Latex Highly Sensitive Calibrator (Cat # ODC0027) for the Highly Sensitive (Cardiac / Neonatal) Application.
0.9% Saline

Stability of Final Reaction Mixture
The Beckman Coulter AU analyzer automatically computes every determination at the same time interval.

Calibration
The frequency of calibration for the CRP Latex procedure is every 90 days for the Normal and Highly Sensitive (Cardiac / Neonatal) Applications.
Calibration of this CRP Latex procedure is traceable to IFCC (International Federation of Clinical Chemistry) standard CRM 470 (RPPHS).
The CRP Latex Normal / Highly Sensitive calibrators are 5-level calibrators. For the zero calibrator 0.9% saline should be used.
Recalibration of this test is required when any of these conditions exist:
1. A reagent lot number has changed or there is an observed shift in control values.
2. Major preventative maintenance was performed on the analyzer.
3. A critical part was replaced.

Quality Control
During operation of the Beckman Coulter AU analyzer, at least two levels of an appropriate quality control material should be tested a minimum of once a day for each of the applications used. In addition, controls should be run after calibration, with each new lot of reagent, and after specific maintenance or troubleshooting steps described in the appropriate User's Guide. Quality control testing should be performed in accordance with regulatory requirements and each laboratory's standard procedure.
Please note that recovery of non-Beckman Coulter controls may vary with reagent lots of immunoassay products, due to the use of non-human materials in the controls.

Results
Automatically printed out for each sample in mg/L at 37°C.

Dynamic Range

1. The CRP Latex reagent is linear from:
   1.0 to 480 mg/L Normal Application (All analyzers)

   Beckman Coulter AU400/400^{e}/480, AU600/640/640^{e}/680
   0.2 to 160 mg/L Highly Sensitive (Cardiac / Neonatal) Application

   Beckman Coulter AU2700/5400
   0.2 to 80 mg/L Highly Sensitive (Cardiac / Neonatal) Application

Beckman Coulter AU Analyzers	Assay Range Normal Application	Assay Range Highly Sensitive (Cardiac / Neonatal) Application
AU400/400^{e}/480	1.0 to 480 mg/L	0.2 to 160 mg/L
AU600/640/640^{e}/680	1.0 to 480 mg/L	0.2 to 160 mg/L
AU2700/5400	1.0 to 480 mg/L	0.2 to 80 mg/L

Samples outside of the lower or upper limit of linearity should be re-run in the appropriate application.
Samples exceeding the upper limit of linearity should be diluted, repeated, and multiplied by the dilution factor.
Sample carryover may occur when a high CRP sample >160mg/L is run directly before a sample with low CRP. On the AU2700/5400 contamination parameters are available on request. On the AU400/400^e/480, and AU600/640/640^e/680, measures should be taken to repeat samples.
< 2.0 mg/L that follow CRP results > 160mg/L.
Prozone settings are required for the AU2700/5400.

2. The Highly Sensitive (Neonatal / Cardiac) application is intended for use in the monitoring of neonates. Basal levels of CRP in neonates are very low. This assay protocol was specifically designed for optimal performance at these levels.

3. When using the Highly Sensitive Application on the Beckman Coulter AU400/400^e/480/600/640/640^e/680 systems, patients with CRP concentrations above 400mg/L may demonstrate an inappropriately low CRP value, which could be within the linear measuring range. Patients with inflammatory and/or infectious conditions should have their CRP measured using the Normal Application, particularly when used for patient monitoring.

4. Increases in the C-Reactive Protein values are not specific and should not be interpreted without a complete clinical history. When used for cardiovascular risk assessment, these measurements should be compared to previous C-Reactive Protein values.

5. Samples with very high CRP concentrations (> 750 mg/dL) can generate false low results without appropriate "Z" flags due to excess antigen in the sample.

6. In very rare cases Gammopathy, especially monoclonal IgM (Waldenström's macroglobulinemia), may cause unreliable results.

Expected Values

C-Reactive Protein is a non-specific indicator for a wide range of disease processes. Reference intervals may be affected by different factors.

Expected values <10 mg/L[3]
Recommended Cardiac risk assessment categories:[5]
Low < 1 mg/L
Average 1.0 to 3.0 mg/L
High > 3.0 mg/L

Newborns with no evidence of infection have CRP concentrations < 1 mg/L.[7]
Because of the variation depending on age, sex, diet, and geographical location, each laboratory should determine its own expected values for the different patient groups as dictated by good laboratory practice.

Specific Performance Characteristics

The following data was obtained using the CRP Latex reagent on Beckman Coulter AU analyzers according to established procedures. Results obtained in individual laboratories may differ.

Precision[15]

Estimates of precision, based on CLSI recommendations,[14] are consistent with typical performance. The within run precision is ≤ 5% CV or SD ≤ 0.20 mg/L (Normal Application), SD ≤ 0.02 mg/L (Highly Sensitive Cardiac / Neonatal Application) and the total precision is ≤ 10% CV or SD ≤ 0.25 mg/L (Normal Application), SD ≤ 0.02 mg/L (Highly Sensitive Cardiac / Neonatal Application). Assays of serum pools and control sera were performed and the data reduced following the CLSI guidelines above.

N= 80	Within run		Total	
Mean, mg/L	SD	CV%	SD	CV%
Normal Application				
6.56	0.07	1.09	0.12	1.85
64.79	0.78	1.20	2.16	3.34
137.71	0.96	0.70	2.26	1.64
Highly Sensitive (Cardiac / Neonatal) Application				
0.21	0.01	4.08	0.01	4.87
1.05	0.03	2.96	0.03	2.50
2.99	0.02	0.62	0.03	0.92
10.00	0.09	0.95	0.15	1.53
59.38	0.54	0.91	1.00	1.68
146.95	1.12	0.76	2.34	1.59

Functional Sensitivity

Precision results (25-fold determination) for a level below the bottom of the measuring range. This is the lowest CRP level the assay can measure accurately with a CV of < 20% for each application. Values less than these should not be reported.

Mean Concentration (mg/L)		SD	CV%
Normal Application			
AU400/400^e	0.15	0.03	17.0
AU640/640^e	0.15	0.02	16.1
AU2700/5400	0.32	0.03	8.0
Highly Sensitive (Cardiac / Neonatal) Application			
AU400/400^e	0.05	0.01	13.1
AU640/640^e	0.06	0.01	9.3
AU2700/5400	0.09	0.02	19.1

CRP Latex

Method Comparison[15]

Patient samples were used to compare this CRP Latex Reagent. The table below demonstrates representative performance on AU analyzers.

Normal application	
Y Method	AU640/640[e]
X Method	Method 2
Slope	1.024
Intercept	0.594
Correlation Coeff. (r)	0.998
No. of Samples (n)	113
Range (mg/L)	0.78 – 167.38
Highly Sensitive (Cardiac / Neonatal) Application	
Y Method	AU640/640[e]
X Method	Method 2
Slope	0.993
Intercept	-0.825
Correlation Coeff. (r)	0.997
No. of Samples (n)	118
Range (mg/L)	0.20 – 155.86

Lower Limit of Detection

The LLD is calculated as mean recovery +3 SD of a 20-fold determination of an analyte-free sample.
Normal application: ≤ 0.15 mg/L
Highly Sensitive (Cardiac / Neonatal) application: ≤ 0.07 mg/L

References

1. Morrow, A.D., Ridker, P.M. C-reactive protein, inflammation, and coronary risk Med. Clin. of North Am. 2000; 84: 149161.
2. Ridker, P.M. Novel risk factors and markers for coronary disease. Adv. Int. Med.2000; 45: 391-419.
3. Raifai N, Ridker PM. Population Distributions of C-reactive Protein in Aparently Healthy Men and Women in the United States: Implication for Clinical Interpretation. Clin Chem 2003;49:666-669.
4. Ridker, P.M., Cannon, C. P., Morrow, D., Rifai, N., Rose, L. M., McCabe, C. H., Pfeffer, M. A., Braunwald, E., C-Reactive Protein Levels and Outcomes after Statin Therapy. N. Eng. J. Med. 2005, 352, 20-28.
5. Pearson TA 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. http://www.circulationaha.org.
6. NACB "Biomarkers of Acute Coronary Syndrome & Heart Failure" (draft guidelines) R.H. Christianson, et al AACC Press, 2004.
7. Wasunna A., Whitelaw A., Gallimore R., Hawkins P.N., Pepys M.B. C-reactive protein and bacterial infection in preterm infants. Eur.J. Pediatr. 1990; 149: 424-7.
8. Benitz, W.E., Han, M.Y., Madan, A. Ramachandra, P. Serial serum C-reactive protein levels in the diagnosis of neonatal infection. Neonatals1998; 102: 4.
9. WHO/DIL/LAB/99.1 Rev.2 World Health Organization. Use of Anticoagulants in Diagnostic Laboratory Investigations 2002.
10. CLSI/NCCLS, Interference Testing in Clinical Chemistry, EP7-A, 2002.
11. In-house data available upon request.
12. Ashwood, E.R., Burtis, C.A., Tietz Textbook of Clinical Chemistry, 2nd edition, W.B. Saunders, 1994.
13. Young, D.S., Effects of Drugs on Clinical Laboratory Tests, 5th Edition, AACC Press, 2000.
14. CLSI/NCCLS Evaluation of Precision Performance of Quantitative Measurement Methods, EP5-A2, 2004.
15. Data is on file for specific AU analyzers.

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