MEDTEST DX
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C-Reactive Protein
High Sensitivity CRP (HS)
Wide Range Reagent Set

Intended Use
For the quantitative determination of C-reactive protein in serum or plasma by latex particle enhanced immunoturbidimetric assay using the Mindray BS-200 analyzer. For *in vitro* diagnostic use only.

Introduction
C-reactive protein (CRP) is an acute phase protein that is involved in the activation of complement, acceleration of phagocytosis, and detoxification of substances released from damaged tissue. As such, CRP is considered to be one of the most sensitive indicators of inflammation. In response to an inflammatory stimulus, a rise in CRP may be detected within 6 hours. CRP is a sensitive, though considered to be a non-specific indicator of acute phase reactants.[1, 2,3]

Measurement of C-reactive protein is most frequently used for the evaluation of injury to body tissues or, for the detection of an inflammatory event somewhere in the body. CRP levels in serum are typically elevated in patients with arthritis or liver disease such as hepatitis A, hepatitis B, or biliary cirrhosis, and after severe infections such as septic shock.

The CRP-HS is intended for the quantitative determination of human CRP by latex particle enhanced immunoturbidimetric assay (ITA). ITA methods for quantitative determination of antibody and antigen immunoprecipitation complexes have been described.[4,5,6,7]

Principle of the Test
Latex particles coated with antibody specific to human CRP aggregate in the presence of CRP from the sample forming immune complexes. The immune complexes cause an increase in light scattering which is proportional to the concentration of CRP in the serum. The light scattering is measured by reading turbidity (absorbance) at 570 nm. The CRP concentration is determined from a calibration curve developed from CRP standards of known concentration.

Reagents
R-1: Buffer Reagent
Glycine buffer: 170 mM
R-2: Latex Suspension
Latex particles coated with rabbit anti-human CRP antibodies: 0.20% (w/v)

Reagent Preparation
Reagents are ready to use and do not require reconstitution. Mix gently before using.

Reagent Storage and Stability
1. All reagents should be stored at 2-8°C and protected from light.
2. Unopened reagents can be used until the expiration date on the package and bottle labels.
3. Once the reagent vial has been opened, store tightly capped at 2-8°C and use within 1 month.

Precautions
1. For *in vtro* diagnostic use only.
2. Not to be used internally in humans or animals. Normal precautions for handling laboratory reagents should be followed.
3. Do not mix or use reagents from one test kit with those from a different lot number.
4. Do not use reagents past their expiration date stated on each reagent container label.
5. Do not pipette by mouth. Avoid ingestion and contact with skin.
6. Reagents in this kit contain <0.1% (w/v) sodium azide as a preservative. Sodium azide may form explosive compounds in metal drain lines. When disposing of reagents through plumbing fixtures, flush with copious amounts of water.
7. All specimens, controls and calibrators should be handled as potentially infectious, using safe laboratory procedures (NCCLS M29-T2).[8]

Specimen Collection and Storage
1. Freshly drawn serum is preferred and should be used within the day of collection. Samples may also be stored refrigerated (2-8°C) for one week or at -30°C for up to 1 year. Use undiluted samples for this assay.
2. Lithium heparin or EDTA plasma samples may also be used.
3. Use plastic tubes for storing the sample, do not use glass.
4. Collect specimens per NCCLS document H4-A3.[9]

Interference
1. All interference studies were performed according to the procedures recommended in NCCLS guideline No. EP7-P for interference testing in clinical chemistry.[10]
2. Hemoglobin to 500 mg/dl, Lipid (Triglycerides) to 3000 mg/dl, Bilirubin to 30 mg/dl and RF to 560 IU/ml were found not to interfere with this assay.
3. Dust particles or other particulate matter in the reaction solution may result in extraneous light-scattering, which may affect the accuracy of this test.
4. See Young, et al for other interfering substances.[11]

Materials Supplied
1. Reagent 1 (R-1) Buffer Reagent
2. Reagent 2 (R-2) Latex Suspension

Materials Required But Not Supplied
1. Multi-point calibrators: CRP Multi-Calibrator Set, catalog number C7568-STD. Approx. values: 2.5, 10.0, 20.0, 80.0, 160.0 mg/L.
2. CRP control Set, catalog number C7568-CTL.
3. Mindray BS-200 Analyzer
4. Mindray BS-200 Operation manual
5. Isotonic saline
6. Pipettes capable of accurately dispensing the required volumes

Mindray BS-200 Test Parameters

Test :	CRP	R1 :	180
No.:	015	R2 :	180
Full Name:	CRP	Sample Volume:	7
Standard No.:		R1 Blank:	
Reac.Type:	Endpoint	Mixed Rgt. Blank:	
Pri. Wave:	578nm	Linearity Range:	0.1 – 160.0
Sec. Wave:		Linearity Limit:	
Direction:	Increase	Substrate Limit:	
Reac. Time:	0 / 18	Factor:	
		Compensate: Slope 1.0	Intercept: 0
Incuba. Time:	3	□ Prozone check	
Unit:	mg/L	q1: q2: q3: q4:	
Precision:	0.1	PC:	Abs:

Calibration Parameters

Rule:	Spline	Calibrator 1:	Deionized Water
Sensitivity:		Calibrator 2:	CRP Std 1
Replicates:	1	Calibrator 3:	CRP Std 2
Interval (day):		Calibrator 4:	CRP Std 3
Difference Limit:		Calibrator 5:	CRP Std 4

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Calibration Parameters (continued)

SD:		Calibrator 6:	CRP Std 5
Blank Response:			
Error Limit:			
Coefficient:	0		

Calibration Curve
It is recommended that a multi-point calibration curve be developed using a CRP Multi-standard Set. It is recommended that the user determine calibration frequency as this will depend on the instrument and type/number of other assays being run. Initially, calibration should be performed each day.

Quality Control
It is recommended that commercially available control serum with known concentrations of CRP be included in all assay runs. Levels in the range of 2.5 mg/L and 55.0 mg/L are recommended. Quality control requirements should be performed in conformance with local, state, and/or Federal regulations or accreditation requirements.

Calculations
CRP levels are determined by the automated analyzer using the prepared calibration curve.

Limitations of the Procedure
1. The CRP-HS has a measurable range from 0.1 to 160.0 mg/L using the manufacturer's CRP Multi-Calibrator Set and the correct instrument parameters.
2. Reagents should not be used after the expiration date indicated on the kit label. Do not mix reagents with different lot numbers.
3. If the CRP concentration is greater than highest calibrator value, dilute one part sample with four parts isotonic saline and re-assay. Multiply results by 5 to compensate for the dilution.

Performance
The following performance data was obtained using a Hitachi 717 analyzer and standard protocol.

Sensitivity
When saline is used as a sample, the range of absorbance change per minute is –0.0050 to 0.0050, while a standard CRP solution containing 10.00 mg/L is 0.0650 to 0.1000 after subtracting the saline blank.

Specificity
When serum containing a known level of CRP (2.5 mg/L) is measured, the assay value obtained is within ± 10%.

Precision
Samples tested were commercial human CRP control serum. Studies were performed following a modification of NCCLS document EP5-T2.[12]

Within Day

Sample I	Sample II
N=20	N=20
Mean=2.31 mg/L	Mean=44.92 mg/L
SD=0.04	SD=0.24
CV=1.90%	CV=0.50%

Day to Day*

Sample I	Sample II	Sample III
N=21	N=21	N=21
Mean=0.47 mg/L	Mean=2.18 mg/L	Mean=9.76 mg/L
SD=0.03	SD=0.07	SD=0.12
CV=6.97%	CV=3.34%	CV=1.23%

*NOTE: Day to Day data does not reflect Mindray BS-200 performance.

Assay Range
0.1 – 160.0 mg/L

Lower Limit of Detection
0.1 mg/L

Functional Sensitivity
To at least 0.15 mg/L

Correlation
y = 1.036x - 0.44
r = 0.996 (n = 38, range = 0.2 – 17.9 mg/L)
x = CRP (HS) Similar analyzer.
y = CRP(HS) Mindray BS-200 analyzer

Expected Values
Expected value for CRP in healthy individuals is below 3.0 mg/L.[13] It is recommended that each laboratory establish its own expected range.

References

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8. NCCLS document, "Protection of Laboratory Workers from Infectious Disease Transmitted by Blood, Body Fluids, and Tissue", 2nd Ed. (1991).
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10. NCCLS document, "National Evaluation Protocols for Interference Testing", Evaluation Protocol Number 7, Vol. 4, No. 8, (June 1984).
11. Young, D.S., et al, *Clin Chem* 21:1D, 1975.
12. NCCLS document, "Evaluation of Precision Performance of Clinical Chemistry Devices", 2nd Ed. (1992)
13. Liuzzo, G., et al, N Eng J Med, 331:417-424, 1994.
14. U.S. Patent nos. 6,248,597; 6,828, 158.

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Use by (YYYY-MM) | LOT Lot and batch code | REF Catalog number | Manufacturer | IVD *In vitro* diagnostic medical device
Temperature limitation | Consult instructions for use | CE mark | EC REP Authorized representative in the European Community