Application Sheet

Laboratory Name

Test Name: C-Reactive Protein Gen.3

System information

For **cobas c** 311/501 analyzers:
CRPL3: ACN 210

Intended use

Immunoturbidimetric assay for the in vitro quantitative determination of CRP in human serum and plasma on Roche/Hitachi **cobas c** systems.

Summary[1,2,3,4,5,6,7,8]

C-reactive protein is the classic acute phase protein in inflammatory reactions. It is synthesized by the liver and consists of five identical polypeptide chains that form a five-membered ring having a molecular weight of 105000 daltons. CRP is the most sensitive of the acute phase reactants and its concentration increases rapidly during inflammatory processes. Complexed CRP activates the classical complement pathway. The CRP response frequently precedes clinical symptoms, including fever. In normal healthy individuals CRP is a trace protein with a range up to 5 mg/L. After onset of an acute phase response the serum CRP concentration rises rapidly and extensively. The increase begins within 6 to 12 hours and the peak value is reached within 24 to 48 hours. Levels above 100 mg/L are associated with severe stimuli such as major trauma and severe infection (sepsis). CRP response may be less pronounced in patients suffering from liver disease. CRP assays are used to detect systemic inflammatory processes; to assess treatment of bacterial infections with antibiotics; to detect intrauterine infections with concomitant premature amniorrhexis; to differentiate between active and inactive forms of disease with concurrent infection, e.g. in patients suffering from SLE or Colitis ulcerosa; to therapeutically monitor rheumatic disease and assess anti-inflammatory therapy; to determine the presence of post-operative complications at an early stage, such as infected wounds, thrombosis and pneumonia, and to distinguish between infection and bone marrow rejection. Postoperative monitoring of CRP levels of patients can aid in the recognition of unexpected complications (persisting high or increasing levels). Measuring changes in the concentration of CRP provides useful diagnostic information about how acute and how serious a disease is. It also allows judgements about the disease genesis. Persistence of a high serum CRP concentration is usually a grave prognostic sign which generally indicates the presence of an uncontrolled infection.

Test principle[9,10]

Particle enhanced immunoturbidimetric assay.
Human CRP agglutinates with latex particles coated with monoclonal anti-CRP antibodies. The aggregates are determined turbidimetrically.

Reagents - working solutions

R1 TRIS[a)] buffer with bovine serum albumin; preservatives

R2 Latex particles coated with anti-CRP (mouse) in glycine buffer; immunoglobulins (mouse); preservative

a) TRIS = Tris(hydroxymethyl)-aminomethane

R1 is in position B and R2 is in position C.

Precautions and warnings

For in vitro diagnostic use.
Exercise the normal precautions required for handling all laboratory reagents.
Disposal of all waste material should be in accordance with local guidelines.
Safety data sheet available for professional user on request.
For USA: For prescription use only.

Reagent handling

Ready for use
Mix **cobas c** pack well before placing on the analyzer.
Carefully invert reagent container several times prior to use to ensure that the reagent components are mixed.

Storage and stability

CRPL3	
Shelf life at 2-8 °C	See expiration date on **cobas c** pack label.
On-board in use and refrigerated on the analyzer:	12 weeks
Diluent NaCl 9 %	
Shelf life at 2-8 °C:	See expiration date on **cobas c** pack label.
On-board in use and refrigerated on the analyzer:	12 weeks

Specimen collection and preparation

For specimen collection and preparation only use suitable tubes or collection containers.
Only the specimens listed below were tested and found acceptable.
Serum.
Plasma: Li-heparin, K_2-EDTA, K_3-EDTA plasma
The sample types listed were tested with a selection of sample collection tubes that were commercially available at the time of testing, i.e. not all available tubes of all manufacturers were tested. Sample collection systems from various manufacturers may contain differing materials which could affect the test results in some cases. When processing samples in primary tubes (sample collection systems), follow the instructions of the tube manufacturer.
Centrifuge samples containing precipitates before performing the assay.

Stability:[11]	11 days at 15-25 °C
	2 months at 2-8 °C
	3 years at (-15)-(-25) °C

Materials provided

See “Reagents – working solutions” section for reagents.

Materials required (but not provided)

- See “Order information” section
- General laboratory equipment

In addition, other suitable control material can be used.

Assay

For optimum performance of the assay follow the directions given in this document for the analyzer concerned. Refer to the appropriate operator’s manual for analyzer-specific assay instructions.
The performance of applications not validated by Roche is not warranted and must be defined by the user.

Application for serum and plasma

cobas c 501 **test definition**

Assay type	2-Point End	
Reaction time / Assay points	10 / 13-29	
Wavelength (sub/main)	800/570 nm	
Reaction direction	Increase	
Units	mg/L (nmol/L, mg/dL)	
Reagent pipetting		Diluent (H_2O)
R1	150 µL	
R2	48 µL	24 µL

Sample volumes	*Sample*	*Sample dilution*	
		Sample	*Diluent (NaCl)*
Normal	2 µL	–	–
Decreased	4 µL	25 µL	75 µL
Increased	2 µL	–	–

Calibration

Calibrators	S1: H_2O		
	S2: C.f.a.s. Proteins		
	Multiply the lot-specific C.f.a.s. Proteins calibrator values by the factors below to determine the standard concentrations for the 6-point calibration curve:		
	S2: 0.10000		S5: 2.0000
	S3: 0.3325 (c 501/502)	0.3500 (c 311)	S6: 4.0000
	S4: 1.0000		
Calibration mode	6-point spline		
Calibration frequency	Full calibration		

- after reagent lot change
- as required following quality control procedures

Traceability: This method has been standardized against an internal method traceable to CRM 470 (RPPHS - Reference Preparation for Proteins in Human Serum).[12]

Quality control

At least once daily run solutions at two levels of a quality control material with known concentrations.

Refer to Brown Clinic Quality Control Requirements, Rules and Reviews Policy

Refer to Brown Clinic Quatliy Control Specialty and Subspecialty Policy

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Calculation

Roche/Hitachi **cobas c** systems automatically calculate the analyte concentration of each sample.

Conversion factors:	mg/L x 9.52 = nmol/L	mg/dL x 95.2 = nmol/L
	mg/L x 0.1 = mg/dL	mg/dL x 10 = mg/L
	mg/dL x 0.01 = g/L	g/L x 100 = mg/dL

Limitations - interference

Criterion: Recovery within ± 10 % of initial values at a CRP concentration of 5.0 mg/L (47.6 nmol/L).
Icterus:[13] No significant interference up to an I index of 60 for conjugated and unconjugated bilirubin (approximate conjugated and unconjugated bilirubin concentration: 60 mg/dL or 1026 µmol/L).
Hemolysis:[13] No significant interference up to an H index of 1000 (approximate hemoglobin concentration: 622 µmol/L or 1000 mg/dL).
Lipemia (Intralipid):[13] No significant interference up to an L index of 1000. There is poor correlation between the L index (corresponds to turbidity) and triglycerides concentration.
Rheumatoid factors up to 1200 IU/mL do not interfere.
High dose hook-effect: No false result occurs up to a CRP concentration of 1200 mg/L (11424 nmol/L).
Drugs: No interference was found at therapeutic concentrations using common drug panels.[14,15]
Therapeutic drugs: Significantly decreased CRP values may be obtained from samples taken from patients who have been treated with carboxypenicillines.
In very rare cases, gammopathy, in particular type IgM (Waldenström's macroglobulinemia), may cause unreliable results.[16]
Although measures were taken to minimize interference caused by human anti-mouse antibodies, erroneous findings may be obtained from samples taken from patients who have been treated with monoclonal mouse antibodies or have received them for diagnostic purposes.
For diagnostic purposes, the results should always be assessed in conjunction with the patient's medical history, clinical examination and other findings.

ACTION REQUIRED
Special Wash Programming: The use of special wash steps is mandatory when certain test combinations are run together on Roche/Hitachi **cobas c** systems. The latest version of the carry-over evasion list can be found with the NaOHD-SMS-SmpCln1+2-SCCS Method Sheets. For further instructions refer to the operator's manual. **cobas c** 502 analyzer: All special wash programming necessary for avoiding carry-over is available via the **cobas** link, manual input is not required.
Where required, special wash/carry-over evasion programming must be implemented prior to reporting results with this test.

Limits and ranges

Measuring range

0.3-350 mg/L (2.9-3333 nmol/L)

Determine samples having higher concentrations via the rerun function. Dilution of samples via the rerun function is a 1:2 dilution. Results from samples diluted using the rerun function are automatically multiplied by a factor of 2.

Lower limits of measurement

Limit of blank and Limit of Detection

Limit of Blank = 0.2 mg/L (1.9 nmol/L)
Limit of Detection = 0.3 mg/L (2.9 nmol/L)
The Limit of Blank and Limit of Detection were determined in accordance with the CLSI (Clinical and Laboratory Standards Institute) EP17-A requirements.
The Limit of Blank is the 95th percentile value from n ≥ 60 measurements of analyte-free samples over several independent series. The Limit of Blank corresponds to the concentration below which analyte-free samples are found with a probability of 95 %.
The Limit of Detection is determined based on the Limit of Blank and the standard deviation of low concentration samples.
The Limit of Detection corresponds to the lowest analyte concentration which can be detected (value above the Limit of Blank with a probability of 95 %).

Limit of Quantitation (Functional sensitivity)

0.6 mg/L (5.7 nmol/L).
The Limit of Quantitation was determined using the result of functional sensitivity testing. The Limit of Quantitation (functional sensitivity) is the lowest CRP concentration that can be reproducibly measured with an interassay coefficient of variation of < 20 %. It has been determined using low C-reactive protein samples.

Expected values

Consensus reference interval for adults:[17] < 5 mg/L (< 47.6 nmol/L)
Each laboratory should investigate the transferability of the expected values to its own patient population and if necessary determine its own reference ranges.

Specific performance data

For Known Interfering Substances section refer to package insert.
For Known Non-Interfering Substance refer to package insert.
For Additional Technical Information refer to package insert.

References

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Alternative method

Refer to Brown Clinic Back-up Testing Policy

Source document

Reagent Name: CRPL3
Method Sheet Version: V9.0 English

Order information

REF	CONTENT		Analyzer(s) on which **cobas c** pack(s) can be used
04956842 190	C-Reactive Protein Gen.3 (250 tests)	System-ID 07 6993 2	Roche/Hitachi **cobas c** 311, **cobas c** 501/502
11355279 216	Calibrator f.a.s. Proteins (5 x 1 mL)	Code 656	
11355279 160	Calibrator f.a.s. Proteins (5 x 1 mL, for USA)	Code 656	
10557897 122	Precinorm Protein (3 x 1 mL)	Code 302	
10557897 160	Precinorm Protein (3 x 1 mL, for USA)	Code 302	
11333127 122	Precipath Protein (3 x 1 mL)	Code 303	
11333127 160	Precipath Protein (3 x 1 mL, for USA)	Code 303	
04489357 190	Diluent NaCl 9 % (50 mL)	System-ID 07 6869 3	

Effective date

Effective date for this procedure:

Author

Source documentation compiled by Roche Diagnostics

Revised by: Heather J Hall, MBA, MT(ASCP), CG(ASCP)[cm] Date: 4/9/2018

Approved by: Aaron Shives MD (Signature on file Date: 4/11/2018

REVIEW – REVISION SUMMARY DOCUMENTATION

Date: By: Revision Summary: