Quantitative determination of C-reactive protein (CRP) in Serum by means of particle-enhanced turbidimetric immunoassay.

Only for in vitro use in clinical laboratory (IVD).

Clinical Significance
C-reactive protein (CRP) is one of the acute phase proteins being synthesised by hepatocytes. The serum concentration of CRP increases during acute stages of diverse diseases associated with inflammation and tissue injury. Elevated CRP has been demonstrated in nearly all bacterial and fungal infections. In addition, it has been shown to be increased in other diseases as neoplasia and rheumatic diseases as well as in major surgery. The diagnosis usefulness of CRP is based on the velocity and on the magnitude of its increase. Serum concentrations are raised within hours of disease onset and the increase can be as much 2000-fold. A rapid fall of CRP levels indicates recovery.

Principle
This CRP test is based upon the reactions between C reactive protein (CRP) and latex-covalently bound antibodies against human CRP. CRP values are determined photometrically.

Reagents
Each CRP kit contains:
A. Buffer - 45 mL of TRIS buffer, pH: 8.2, and 0.09 % sodium azide as preservative.
B. Latex reagent - 5 mL of Polystyrene particles (0.5%) coated with goat antibodies anti-human-CRP serum in a glycine buffer (0.1 M, pH: 8.2), containing NaCl (0.15 M) and bovine serum albumin (0.5%). Preservative: Sodium azide 0.075%.
C. Calibrator - 1 mL Human - based reference fluid. Preservative: sodium azide, 0.075 %. All raw materials of human origin used in the manufacture of this product showed no reactivity when tested for HBsAg, anti-HIV-1/2 and HCV with commercially available test methods. However, this product should be handled as though capable of transmitting infectious diseases.

Reagent Preparation
Working Reagent is prepared with 1 part of Latex Reagent and 9 parts of Buffer Reagent. Prepare a fresh WR based on its workload. Shake gently the reagents before pipetting.

Stability
Reagents in the original vial are stable to the expiration date on the vial label when capped and stored at +2 - +8°C. Immediately following the completion of an assay run, the reagent vial should be capped until next use in order to maximize curve stability. Once opened the reagent can be used within 6 months if stored tightly closed at +2 - +8°C after use. Do not freeze reagents. The CRP latex reagent should have a white, turbid appearance free of granular particulate. Visible agglutination or precipitation may be a sign of deterioration, and the reagent should be discarded. The CRP buffer reagent should be clear and colorless. Any turbidity may be sign of deterioration and reagent should be discarded.

Sample --- (A2 - A1)
Calibrator Concentration

For quality control use a suitable control material. The control intervals and limits must be adapted to the individual laboratory requirements. Values obtained should fall within established limits. Each laboratory should establish corrective measures to be taken if values fall outside the limits. Control must be assayed and evaluated as for patient samples.

Precautions
For in vitro diagnostic use only. Do not pipette by mouth. Reagents containing sodium azide must be handled with precaution. Sodium azide can form explosive azides with lead and copper plumbing. Since absence of infectious agents cannot be proven, all specimens and reagents obtained from human blood should always be handled with precaution using established good laboratory practices. Disposal of all waste material should be in accordance with local guidelines. As with other diagnostic tests, results should be interpreted considering all other test results and the clinical situation of the patient.

Specimens
Fresh or deep frozen serum. CRP remains stable for 8 days at +2 to +8°C. If the test should be performed later, it is recommended to freeze the serum. Avoid successive freezing and thawing. Discard hemolysed or contaminated samples. Heavily lipemic sera and turbid frozen serum samples must be cleared with a delipidating agent. Dilapidation of samples do not affect the results of CRP in serum samples. The cleared patient serum sample must be used on the same day, as turbidity may reoccur.

Procedure
Wavelength: 540 nm (530 – 550nm)
Temperature: 37°C
Cuvette: 1cm light path
Measure against distilled water blank.

Mix and measure absorbance immediately (A1) incubate 2 min (37°C), after incubation read absorbance (A2).

Calculation
Plot the calibration curve and the sample concentration is obtained by interpolation

Reference Values
Adults : Upto 6 mg/L
New born up to 3 weeks: <4.1 mg/L
Infants and Children : <2.8 mg/L
Each laboratory should establish an expected range for the geographical area in which it is located.

Reagent Performance
Linearity
The range interval for the multipoint calibration method is from 0 to 100 mg/L. With this method you can use the one point calibration procedure using a calibrator with dilutions. One point calibration is linear at least up to 80 mg/L. When values exceed the range the samples should be diluted with saline solution and the result should be multiplied by the appropriate factor.

Sensitivity
Calculating the mean plus 3SD of twenty replicates of zero standards resulted in a lower limit of detection less than 2 mg/L.

Specificity
The assay is specific for CRP determination. There is no significant interference by bilirubin, haemoglobin, or rheumatoid factor or Intralipid (up to 0.5 %). Other substances can interfere. For a comprehensive review of interfering substances, refer to the publication by Young.

Prozone Effect
The system did not show prozone phenomenon at least up to 430 mg/L.
Assay Precision
Intra-assay coefficients of variation (CV) for three samples (CRP values ranging from 8 to 33 mg/L) were between 2.8 and 4.2 %. Inter-assay CVs were between 3.9 and 4.6 %.

Method Comparison
25 samples were correlated with a nephelometric commercial procedure. When comparing the results by lineal regression the result was: \( y = 1.08 x - 3.4 \) and \( r=0.9916 \)

Analytical characteristics have been obtained in a single experiment in a conventional spectrophotometer. Therefore, the data expressed in the present document should be interpreted as a guide example.

Test Parameters

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Specification</th>
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<tbody>
<tr>
<td>Mode</td>
<td>Fixed Time Kinetic</td>
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<tr>
<td>Wavelength (nm)</td>
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<td>Sample Volume (µl)</td>
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<td>Working Reagent Volume (µl)</td>
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<td>Measuring Time (A2) (Sec.)</td>
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<td>Units</td>
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<td>Linearity Limit</td>
<td>Upto 100 (Multipoint calibration)</td>
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<tr>
<td></td>
<td>Upto 80 (One point calibration)</td>
</tr>
</tbody>
</table>

Literatures
6. Passing H, Bablok W. A new biometrical procedure for testing the equality of measurements from two analytical methods.

Manufactured in Spain for

A Division of

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