C REACTIVE PROTEIN (CRP)

In vitro diagnostic reagents for the quantitative determination of C Reactive Protein (CRP) in serum by means of particle-enhanced turbidimetric immunoassay.

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: 7.0, 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.

Calibration Curve and Controls

Analytical Range up to 90 mg/L.

Calibrator 1	100 µl of CRP Calibrator*
Calibrator 2	100 µl of Calibrator 1 + 100 µl of Saline Solution
Calibrator 3	100 µl of Calibrator 2 + 100 µl of Saline Solution
Calibrator 4	100 µl of Calibrator 3 + 100 µl of Saline Solution
Calibrator 5	100 µl of Saline Solution
(*) See values on the label or on the insert. Multiply by the appropriate factor.	

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.

Stability

Reagents in the original vial is 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 1 month 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 colourless. Any turbidity may be sign of deterioration and reagent should be discarded.

WR is stable for up to one month at 4°C. **It is recommended that each Laboratory prepares a fresh Working Reagent based on its workload.**

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.

Materials required but not provided

Spectrophotometric analyser. Controls.

Specimens

Fresh or deep frozen serum. CRP remain 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 haemolysed or contaminated samples.

Heavily lipaemic sera and turbid frozen serum samples must be cleared with a delipidating agent. Delipidation 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		550 nm	
Temperature		37°C	
Cuvette		1cm light path	
Measurement against distilled water blank.			
Bring the reagents at 37°C and pipette:			
	Calibrator	Sample	Blank
Calibrator	3 µl	—	—
Sample	—	3 µl	—
Distilled Water	—	—	3 µl
Work. Reagent	500 µl	500 µl	500 µl
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 the sample absorbance (A2-A1) in the calibration curve.

If is an one point calibration:

$$\frac{(A2-A1)_{sample} - (A2-A1)_{blank}}{(A2-A1)_{calibrator} - (A2-A1)_{blank}} \times CalibratorConcentration$$

Linearity

The range interval for the multipoint calibration method is from 0 to 90 mg/L.

With this method you can use the one point calibration procedure using a calibrator without dilutions, because it 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 appropriated factor.

Reference Values

Values < 6 - 8 mg/L are within the normal range. Each laboratory should establish an expected range for the geographical area in which it is located.

Sensitivity

Calculating the mean plus 3SD of twenty replicates of zero standard 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 bilirrubin, 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.

Literature

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