CRP

Turbilatex Method

Intended Use:

CRP is in vitro Medical Device Reagents/Kits for the detection and screening of C-Reactive Protein levels in various conditions including autoimmune disorder in human specimen. It is intended for *in vitro* diagnostics use by trained healthcare professionals in IVD laboratory only and not near patients.

Clinical Significance

C-Reactive Protein (CRP) is a normal alpha globulin, which increases in inflammatory processes. The name CRP is derived from the fact that this protein has the capacity to precipitate the somatic C-carbohydrate of Pneumococcus. Elevated CRP levels are usually observed in a variety of infections and inflammatory conditions where there is tissue destruction.

The CRP level measurement is useful in differential diagnostic of neonatal septicemia and meningitis. CRP levels are always elevated after myocardial infarction and surgery. The CRP test can also help in determining post-surgical complications.

Principle

CRP-Turbilatex is a quantitative turbidimetric test for the measurement of C- reactive protein (CRP) in human serum or plasma.

Latex particles coated with specific anti- human CRP are agglutinated when mixed with samples containing CRP. The agglutination causes an absorbance change, dependent upon the CRP contents of the patient sample that can be quantified by comparison from a calibrator of known CRP concentration.

Reagent Composition

R1 Diluent	Tris buffer 20 mmol/L, pH 8.2.
R2 Latex	Latex particles coated with goat anti-human CRP ,pH 7.3
CRP Calibrator	Human serum CRP.Concentration is stated on vial label. and it is traceable to the Certified Reference Material ERM-DA472/IFCC

Precaution

Components from human origin have been tested and found to be negative for the presence of HBsAg, HCV, and antibody to HIV (1/2). However handle cautiously as potentially infectious. This reagent contains sodium azide 0.95 g/L. Avoid any contact with skin or mucous.

Calibration

Use CRP Calibrator ONLY.

The sensitivity of the assay and the target value of the calibrator have been standardized against the Reference Material ERM-DA 472(IFCC).

Preparation

CRP Calibrator: Ready to use.

Working Reagent:

Swirl the latex vial before use. Mix Latex and Diluent in a 1:4 ratio (i.e 2 ml R2 + 8 ml R1) prior to use.

Working Reagent is stable during 20 days at 2-8^{0}C

Stability and Storage

All the components of the kit are stable until the expiration date on the label when stored tightly closed at 2-8°C and contaminations are prevented during their use. Do not use reagents over the expiration date.

Reagent deterioration: Presence of particles and turbidity.

Additional Equipment

- Thermostatic bath at 37°C.
- Spectrophotometer or photometer thermostatable at 37°C with a 540 nm filter.

Samples

Fresh serum. Stable 7 days at 2-8°C or 3 months at -20°C.

The samples with presence of fibrin should be centrifuged before testing. Do not use highly hemolized or lipemic samples.

Assay Parameters

Mode	Two Point / Fixed Time
Reaction	Ascending
Wavelength	540 nm (530-550)
Blank With	Distilled Water
Sample Volume	5 µl
Reagent Volume	400 µl : 100 µl
Delay Time	10 (Sec)
Read Time	120 (Sec)
Calibrator Conc.	See Vial Label
Linearity Limit	Up to 100 mg/L
Unit	mg/L

Procedure

1. Bring the reagents and the photometer (cuvette holder) to 37°C.
2. Assay conditions:
   Wavelength: 540 nm (530-550)
   Temperature: 37°C
   Cuvette light path: 1 cm
3. Adjust the instrument to zero with distilled water.
4. Pipette into a cuvette

Addition Sequence	Test
R1 Diluent	400 µl
R2 Latex	100 µl
Calibrator or sample	5 µl

5. Mix and read the absorbance immediately Abs (A1) and after 2 minutes Abs (A2) of the sample addition.

Calculation

$$\text{CRP (mg/L)} = \frac{\text{Abs (A2-A1) sample}}{\text{Abs (A2-A1) calibrator}} \times \text{Calibrator conc.}$$

Quality Control

Control Sera are recommended to monitor the performance of manual and automated assay procedures.
Each laboratory should establish its own Quality Control scheme and corrective actions if controls do not meet the acceptable tolerances.

Reference Values

Normal values: up to 6mg/L.
Each laboratory should establish its own reference range.

Performance Characteristics

1. Linearity limit: Up to 100 mg/L, under the described assay conditions.

   Samples with higher concentrations should be diluted 1/5 in NaCl 9 g/L and retested again. The linearity limit depends on the sample / reagent ratio, as well as the analyzer used. It will be higher by decreasing the sample volume, although the sensitivity of the test will be proportionally decreased.
2. Detection limit: Values less than 1 mg/L give non-reproducible results.
3. Prozone effect: No prozone effect was detected upto 250 mg/L.
4. Sensitivity: 2.9 mA.mg/L.
5. Precision:

	Mean (mg/L)	CV (%)
Intra - assay N = 10	8.0	6.7
	19.7	3.7
	71.8	2.6
Inter – assay N = 10	8.7	5.3
	40.6	5.6
	60.8	4.3

Accuracy: Results obtained with this reagent did not show systematic differences when compared with commercial reagents of similar characteristics. Details of comparison are available on request

Interferences: Bilirubin (40 mg/dl), hemoglobin (12 g/L) lipemia (10g/L) and Rheumatoid factors (800 IU/ml), do not interfere. Other substances may interfere.

Notes

1. This method may be used with different instruments. Any application to an instrument should be validated to demonstrate that results meet the performance characteristics of the method. It is recommended to validate periodically the instrument. Contact to the distributor for any question on the application method.
2. The linearity limit depends on the sample/reagent ratio, as well as the analyzer used. It will be higher by decreasing the sample volume, although the sensitivity of the test will be proportionally decreased.
3. Clinical diagnosis should not be made on findings of a single test result, but should integrate both clinical and laboratory data.
4. For automatic instruments, avoid the presence of bubbles in the reagents that may interfere with the assay results

References

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Packaging

R1. Diluent: 1 x 40ml

R2. Latex : 1 x 10ml

CRP-CAL: 1 x 1ml

For *in vitro* Diagnostic Use Only