BIOGENIX INC. PVT. LTD.

CRP Latex Particle Enhanced Turbidimetric Immunoassay

Intended Use

This reagent is intended for in vitro quantitative determination of C-reactive protein (CRP) in human serum.

Clinical Significance

C-Reactive Protein (CRP) is an acute phase protein produced by the liver in response to inflammation, infection and tissue injury. Increased CRP concentrations occur much earlier than other acute phase reactants and this rapid response to trauma or infection is the distinguishing feature of CRP. In addition, CRP levels return to normal quickly at the end of an acute episode making CRP useful for both the detection of acute episodes as well as in treatment monitoring.

Principle

Latex particles coated with specific rabbit 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 calibrators of known CRP concentrations.

Kit components

1	R1(Diluent): Tris buffer
2	R2(Latex Reagent): Latex particles coated with specific rabbit anti-human CRP
3	Calibrator (Conc. as mentioned on vial label)

Storage & Stability

- **R1 & R2 & Calibrator**

The sealed reagents are stable for 24 months from manufacture date, when stored at 2-8ºC.

- **Working reagent**

Stable during 30 days at 2-8ºC. Shake gently the vial before use. Products must not be stored at room temperature for longer than 30 hours during use.

Precautions

1. If the reagents became turbid or the absorbance of blank reagent is higher than 1.0000, it means that the reagent is valid and you should discard it.

2. Do not freeze; frozen Latex or Diluent could change the functionality of the test.

3. Components from human origin have been tested and found to be negative for the presence of HBsAg, HCV, and antibody to HIV (1/2). It is recommended to handle with caution.

Calibration

Use BIPL CRP Calibrators, which are ready to use.

The calibration in automated analyzer is stable for 2 weeks, after which a new curve must be generated. Re-calibrate when control results are out of specified tolerances, when using different lot of reagent and when the instrument is adjusted.

Preparation

Working reagent: Swirl the latex vial gently before use.

Prepare the necessary volume as follow:

8 ml R1+ 2 ml R2

CRP Calibrator (Ready to use)

Materials required but not provided

Thermostatic bath at 37ºC.

Spectrophotometer or photometer thermostatable at 37ºC with a 540 ± 20 nm filter.

Sample

Fresh Serum (Do not use lipaemic or haemolysed sample). Stable for 7 days at 2-8℃. Samples with presence of fibrin should be centrifuged before testing.

Procedure

- **System Parameters**

Mode	: Two point/fixed time
Reaction	: Ascending
Wavelength	: 540±20 nm
Blank	: Distilled water
Sample Volume	: 5 µL
Reagent Volume	: 1000 µL (800 µL R1 + 200 µL R2)
Delay Time	: 10 (Sec)

Read Time	:	120 (Sec)
Calibrator	:	Stated on the vial
Normal range	:	Upto 6 mg/L
Linearity limit	:	150 mg/L
Unit	:	mg/L

- **Test procedures**

1. Bring the working reagent and the photometer (cuvette holder) to 37ºC.
2. Assay conditions:

Wavelength: . . . . . .540 nm (520-560)

Temperature: 37ºC

Cuvette light path: . . . . . .1 cm

3. Adjust the instrument to zero with distilled water.
4. Pipette into a cuvette:

Working Reagent (µl) (4R1:1R2)	1000
Calibrators or sample (µl)	5

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

Calculation

One point Calibration:

$$\frac{\text{(A2-A1) sample}}{\text{(A2-A1) calibrator}} \text{ X calibrator concentration = CRP (mg/L)}$$

Quality Control

It is recommended to use Quality Controls to verify the performance of the assay.

Each Laboratory has to establish its own internal quality control scheme and procedures for corrective action if controls do not recover within the acceptable tolerance.

Reference Values

It is recommended that each laboratory should establish its own reference values.

The following value may be used as guideline.

Serum: Normal values up to 6 mg/L.

Results obtained for patient samples are to be correlated with clinical findings of patient for interpretation and diagnosis.

Performance

1. Linearity limit: ***Up to 150 mg/L,*** under the described assay conditions. If the concentration is greater than linearity (150 mg/L), dilute the sample with normal saline and repeat the assay. Multiply the result with dilution factor.
2. Detection limit: Values less than ***2 mg/L*** give non-reproducible results.
3. Pro-zone effect: No pro-zone effect was detected upon 1000 mg/L.
4. Sensitivity: Δ4.2 mA. mg/L.
5. Precision:

	Intra Run		**Inter Run**	
Control	Low	High	Low	High
n	20	20	20	20
Mean(mg/L)	5.2	60.1	5.3	60.5
SD	0.14	0.9	0.16	1.0
CV (10%)	2.7%	1.5%	3.0%	1.7%

6. Accuracy: Results obtained using this reagent (y) were compared to those obtained using a commercial reagent (x) with similar characteristics. 50 samples of different concentrations of CRP were assayed. The correlation coefficient (r) was 0.99 and the regression equation was y = 0.968x + 1.197.

The results of the performance characteristics depend on the analyzer used.

Interference

Bilirrubin (20 mg/dL), lipemia (10g/L) and rheumatoid factors (300 IU/mL) do not interfere. Hemoglobin (≥ 5 g/L), interferes. Other substances may interfere.

Notes:

1. The reagent system is for in vitro use only.
2. The volume of reagents and sample can be adjusted according to different instruments. While the ratio of the reagent ⁄ sample shall be the same.

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