C-REACTIVE PROTEIN

Immunoturbidimetric Method

DIATEK

CLINICAL SIGNIFICANCE :

CRP (C-reactive protein) is an acute phase protein, the concentration is seen to increase as a result of the inflammatory process, most notably in response to pneumococcal (bacterial) infection, histolytic disease and a variety of disease states. Originally discovered by Tillet et al. in 1930 in patient sera with acute infection, CRP has now come to be used as a marker or general diagnostic indicator of infections and inflammation, in addition to serving as a monitor of patient response to therapy and surgery. Furthermore, regular measurements of CRP in infants can be a useful aid in the early diagnosis of infectious disease.

TEST PRINCIPLE :

This method utilizes the interaction of antigen and antibody to directly determine the CRP in speicimen. Immunturbidimetric test for the determination of CRP is based on photometric measurement of the Antibody - Antigen reaction between goat-Ab on human CRP of the sample. The amount of Ag - Ab Complex is measured as increase in absorbance at 340 nm with the help of single point calibration or with the use of multipoint calibration curve.

REAGENTS COMPOSITION :

Reagent 1 (R1) Buffer Reagent : Buffer (pH : 7.2)

Reagent 2 (R2) CRP Antibody : Buffer, Anti-human CRP antibody, Stabilizers

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

KIT CONTENTS

CODE No.	*CRPLW01*	*CRPLW02*
Pack size :	(30 ml)	(LW120 ml)
Reagent 1 (R1)	2 x 12.5 ml	5 x 20 ml
Reagent 2 (R2)	1 x 5 ml	3 x 6.7 ml

REAGENT PREPARATION

All the Reagents are ready to use and unopened reagent is stable upto expiry. Once opened the onboard stability is 30 days in Fully Automated Analyser under on board cooling system.

SAMPLES : Use fresh Serum

Materials required (But not provided) :

MULTIPOINT CALIBRATION :

Use CRP High Calibrator for preparing 6 calibrators ranging nearly 6 - 200 mg/L and use normal saline as 0 (For Semi Auto input value as 0.01 for normal saline).

CRP Control (Use of assayed QC sera is recommended to validate test result).

Use CRP Control Low and High for test validation.

Before the assay begins, bring all the reagents to room temperature.

For Semi-auto Analyser

ASSAY CONDITIONS for MULTI POINT ASSAY:

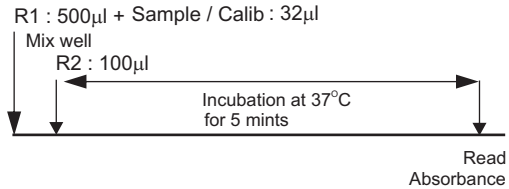
Wavelength (Primary) :..... 340 nm
Cuvette: ..... 1 cm light path
Constant temperature 37ºC
Reaction (Mode)..... End Point
Number of Standard..... 6
Standard Concentration..... As prepared
Curve..... Non linear
Linearity..... Upto highest Calib. conc.
Unit..... mg/L
Reaction Slope..... Increasing
Blanking..... Reagent.

It is recommended to use CRP High Calibrator nearly 200 mg/L

	CRP Calibrator prepartion				
	C6	C5	C4	C3	C2
Dilution	Neat	1:2	1:4	1:8	1:16
Factor	1.0	0.5	0.25	0.125	0.0625

Calibrators concentration to be calculated by multiplying the factor correspond to dilution. Normal saline to be used as calibrator C1 value 0.01mg/l

Assay Procedure summary Semi- Automated Analyser:



PROCEDURE MULTI POINT ASSAY:

Pipette into test tubes labeled as (B) Calibrators (1 to 6) and Test (T) as follows.

	B	C1	C2	C3	C4	C5	C6	T
Reagent R1	500µl	500µl	500µl	500µl	500µl	500µl	500µl	
Deionised water	32µl							
Normal Saline		32µl						
CRP Calibs.			32µl	32µl	32µl	32µl	32µl	
Specimen								32µl
Mix well								
Reagent R2	100µl	100µl	100µl	100µl	100µl	100µl	100µl	100µl
Incubate for 5 mint at 37ºC								
Mix and Read **Absorbance** for each tube against Blank (B)								

1. Pipette into test tube Reagent1(R1) 500 µl and Add (Normal Saline / Calibrators / Samples) 32 µl as described above.
2. Mix well and Reagent 2 (R2) 100 µl
3. Incubate for 5 Min. at 37°C.
4. Read **Absorbance** of Calibrators (C1 to C6) and Test (T) at bicromatic mode Primary wavelength 340 nm and Secondary wave legth 680 nm against Blank (B).
5. Plot a non linear Point to point curve and calculate result from curve.

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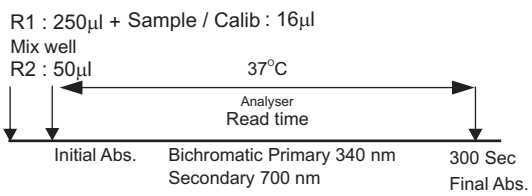

PROCEDURE MULTI POINT ASSAY FULLY AUTOMATED:

	B	C1	C2	C3	C4	C5	C6	T
Reagent R1	250µl	250µl	250µl	250µl	250µl	250µl	250µl	
Normal Saline		16µl						
CRP Calibs.			16µl	16µl	16µl	16µl	16µl	
Specimen								16µl
Reagent R2	50µl	50µl	50µl	50µl	50µl	50µl	50µl	50µl
Read **Initial Absorbance A1** for each tube against Blank (B)								
Incubate for 5 mint at 37ºC								
Mix and Read **Final Absorbance (A2)** of Calibrators (C1 to C6) and Tes (T) at Bichromatic mode with Primary wavelength 340 nm and Secondary wavelength 700 nm (670 - 700 nm) against Blank (B).								

1. Calculate the change of Absorbance ∆ **Abs (A2 - A1)** of each Calibrator and Test Sample.
2. Plot a Calibration Curve (Pont to Point) with **Calibrator Concentration** against corresponding ∆ **Abs.** from lower to higher sequentially.
3. Calculate the concentrations of Test samples / controls based on the calibration curve.
4. Do not attempt to extrapolate above or below the range of Calibrators
5. **Programmes for specific autoanalysers are available on request.**

PRECAUTIONS :

1. Storage conditions as mentioned on the kit to be adhered.

2. Use clean glassware and microtips while pipetting Reagents

3. Avoid contamination of the reagent during the assay process.

4. No interference for Intralipid (1000 mg/dL), Bilirubin (40 mg/dL), and Hemoglobin (500mg/dl)

5. For accuracy of results, the assay procedure, reagent preparation and storage has to be meticulously followed.

6. No prozone Phenomenon observed till CRP concentration of 400 mg/L

7. As with all the diagnostic procedures, the physician should evaluate data obtained by the use of this kit in light of other clinical information.

TEST SPECIFICATIONS:

Precision: n=20

Intra-assay	Low	Medium	High
Mean	24.51	49.04	73.49
Std. Dev.	0.25	0.48	0.68
%CV	1.00	0.98	0.92

Precision: n=5

Inter-assay	Low	Medium	High
Mean	25.36	50.16	76.13
Std. Dev.	0.44	1.11	1.36
%CV	1.73	2.21	1.79

TEST COMPARISON

This method (Y) was compared with another commercially avai (X) and following linear regression equation obtained Y = 1.0028X + 0.124 with a correlation coefficient of 0.9995 when 70 patient sample were anlysed..

REFERENCE RANGE :

Adult : upto 6 mg/L

The above reference range is guideline and all the laboratories must establish their own normal reference range. Final diagnosis should be made with correlation of clinical factors.

Measuring range :

1 - 200 mg/L (Multi point Calibration)

BIBLIOGRAPHY

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