CRP (TURBIDIMETRY)

INTENDED USE

BIOLINE CRP reagent is for the Quantitative determination of CRP in human serum.

CLINICAL SIGNIFICANCE

C-reactive protein (CRP) is the best known among the acute-phase proteins, a group of proteins whose concentration increases in blood as a response to inflammatory disorders (acute-phase response). CRP is normally present in low concentration in blood of healthy individuals (< 5 mg/L). It is elevated up to 500 mg/L in acute inflammatory processes associated with bacterial infections, post- operative conditions or tissue damage already after 6 hours reaching a peak at 48 hours. The measurement of CRP represents a useful laboratory test for detection of acute infection as well as for monitoring inflammatory processes also in acute rheumatic and gastrointestinal diseases. CRP testing shows various advantages in comparison to the erythrocyte sedimentation rate (ESR) and the leukocyte count. In fact, it is more sensitive, the increase occurs earlier and its levels return to the reference range more rapidly after healing.

METHOD AND PRINCIPLE

Determination of CRP concentration by photometric measurement of the antigen-antibody reaction of antibodies to human CRP with CRP present in the sample.

REAGENT COMPOSITION

R1: Tris pH 7.5 100 mmol/l
R2: Tris pH 8.0 , Antihuman CRP antibodies (goat)

WARNINGS AND PRECAUTIONS

1. The reagents contain sodium azide (0.95 g/L) as preservative. Do not swallow! Avoid contact with skin and mucous membranes.

2. Reagent 2: contains animal material. Handle the product as potentially infectious according to universal precautions and good clinical laboratory practices.In very rare cases, samples of patients with gammopathy might give falsified results.

3. Please refer to the safety data sheets and take the necessary precautions for the use of laboratory reagents. For diagnostic purposes, the results should always be assessed with the patient's medical history, clinical examinations and other findings.

REAGENT PREPARATION

Reagent R1 and R2 are ready to use.

REAGENT STORAGE AND STABILITY

All the kit components are ready to use, and will remain stable until the expiration date printed on the label, when stored tightly closed at 2-8ºC and contaminations are prevented during their use. Do not freeze: frozen reagents could change the functionality of the test.
--Mix reagents gently before use.

REAGENT DETERIORATION

Presence of particles and turbidity in R1 and if control results are not accurate.

SPECIMEN COLLECTION AND STABILITY

Serum, heparin plasma or EDTA plasma

Stability:			
	15 days	at	20 – 25°C
	2 months	at	4 – 8°C
	3 years	at	–20°C

Only freeze once.

INTERFERENCES

Due to its antibodies, Bioline CRP is a specific immunoassay for human CRP. No interference was observed by ascorbic acid up to 30 mg/dL, bilirubin up to 40 mg/dL, hemoglobin up to 500 mg/dL and lipemia up to 2000 mg/dL triglycerides as well as by anticoagulants in usual concentrations. For further information on interfering substances refer to Young DS

ASSAY PROCEDURE FOR SEMIAUTO ANALYZER.

Wavelength	546 nm
Optical path	1 cm
Temperature	37°C

Sample/Calibrator	10 µl
Reagent 1	800 µl
Reagent 2	200 µl

Mix and aspirate into the analyzer, take Reading A1 after 5 sec and Reading A2 after 120 sec. Calculate the Δ Abs by subtracting A2-A1.

CALCULATIONS

$$\text{CRP (mg/l)} = \frac{\Delta \text{ A Sample}}{\Delta \text{ A Standard}} \times n$$

Where n = Calibrator Concentration (mg/l)

QUALITY CONTROL

To ensure adequate quality, control sera normal and abnormal control should be used. These controls must be performed & validated before the patient samples are assayed. The control frequency must be at least once a day, after each calibration and should be adapted to Quality Control procedures of each laboratory and the regulatory requirements. Results should be within the defined ranges. If values fall outside of the defined ranges, each laboratory should take corrective measures. Quality control material should be used in accordance with local guidelines

EXPECTED VALUES

Adults	< 6 mg/L
Newborn up to 3 weeks	< 4.1 mg/L
Infants and children	< 2.8 mg/L

Each laboratory should check if the reference ranges are transferable to its own patient population and determine own reference ranges if necessary.

PERFORMANCE CHARACTERISTICS

Measuring Range

The measuring range is from 2 mg/L upto 150 mg/L. When values exceed these ranges, samples should be diluted with NaCl solution (9 g/L) and the result multiplied by dilution factor.

Prozone Limit

No prozone effect was observed up to a CRP concentration of 2000 mg/L.

Sensitivity/Limit of Detection

The lower limit of detection is 2 mg/L.

Precision (n = 20)

Intra-assay precision	Mean [mg/L]	SD [mg/L]	CV [%]
Sample 1	6.6	0.3	4.7
Sample 2	20.4	0.6	3.0
Sample 3	88.5	3.1	3.5

Inter-assay precision	Mean [mg/L]	SD [mg/L]	CV [%]
Sample 1	7.2	0.4	5.7
Sample 2	22.2	0.4	1.8
Sample 3	97.8	2.4	2.5

Method Comparison: A comparison of Bioline CRP (y) to a commercially available test (x) using 65 samples gave following results: y = 0.99 x +0.00 mg/L; r = 0.997

LIMITATIONS OF THE PROCEDURE

- False positive results may be obtained in conditions such as, rheumatoid arthritis, scarlet fever, tonsilitis, several streptococcal infections and healthy carriers.
- Early infections and children from 6 months to 5 years may cause false negative results.
- A single CRP determination does not produce much information about the actual state of the disease. Titrations at biweekly intervals during 4 or 6 weeks are advisable to follow the disease evolution.
- Clinical diagnosis should not be made on findings of a single test result, but should integrate both clinical and laboratory data.

GENERAL TECHNICAL PARAMETERS.

Mode	**2-POINT KINETICS**
Wavelength (Filter)	**546 nm**
Reaction Direction	**Increasing**
Sample Vol.	**10 µl**
Reagent Vol.	**1000 µl**
Delay Time / Lag Time	**5 Sec**
Measuring Time	**120 Sec**
Reagent Blank Abs.(Max)	**NMT 1.500**
Calibration Method	**Linear**
Linearity	**150 mg/L**
Decimal Places	**1**
Temp.	**37ºC**
Unit	**mg/L**
Ref. Low (Male / Female)	**0 mg/L**
Ref. High (Male / Female)	**6 mg/L**

REFERENCES

1. Lars-Olof Hanson et al. Current Opinion in Infectious diseases 1997; 10: 196-201.
2. 2. M.M. Pepys. The Lancet 1981; March 21: 653 – 656.
3. Chetana Vaishnavi. Immunology and Infectious Diseases 1996; 6: 139 – 144.
4. 4. Yoshitsugy Hokama et al. Journal of Clinical Laboratory Status 1987; 1: 15 – 27.