Experiment no.: 10

Experiment name: determination of C-reactive protein in blood serum.

The aim of the Experiment: *Determination of C-reactive protein by a* SLIDE TEST.

Equipment and martials used in the Experiment:

- Automatic pipettes.
- − Saline solution (0.9% NaCl, only for semi-quantitation procedure).
- _ Mechanical rotator, adjustable at 100 r.p.m. Laboratory alarm clock

Property of the machine:

Normal UV-Vis spectrophotometer:

Machine usage:

16- Wavelength set up step.

17- Blank against the solvent solution using a proper cuvette.

18- Reach O.D.

Experiment procedure or protocol:

I. Qualitative Test

1. Bring the test reagents and samples to room temperature (Note 1).
2. Mix the Reagent vial gently. Aspirate dropper several times to obtain a thorough mixing.
3. Place 1 drop (50 μL) of the serum under test into one of the circles on the card. Dispense 1 drop of positive control serum and 1 drop of negative control serum into two additional circles.
4. Add 1 drop of CRP-Latex Reagent to each circle next to the sample to be tested.
5. Mix the contents of each circle with a disposable stirrer while spreading over the entire area enclosed by the ring. Use separate stirrers for each mixture.
6. Rotate the slide means of a mechanical rotator (100 r.p.m.) for a period of **2 minutes** (Note 2).
7. Observe immediately under a suitable light source for any degree of agglutination.
8. **CRP-Latex Reagent**. Suspension of polystyrene latex particles coated with specific anti-human C-reactive protein

   R1 antibodies in a buffered saline solution. Contains 0.95 g/L of sodium azide. Human serum with a CRP concentration > 15 mg/L. Contains 0.95 g/L of sodium azide.

Animal serum with a maximum concentration of human CRP of 1 mg/L. Contains 0.95 g/L of sodium azide.

Experiment data and results:

II. Semi-quantitative Test

1. Dilute sample with NaCl 9 g/L following the 2-fold dilutions procedure as follow:

Dilution	1/2	1/4	1/8	1/16	1/32
Sample (µL)	100				
ClNa 9 g/L (µL)	100	100	100	100	100
Transfer (µL)	100	100	100	100	
CRP (mg/L) non-diluted sample	12	24	48	96	192

2. Test each dilution as described in Qualitative Test.

Conclusion:

- What is the role of this experiment?
- How does its deficiency affect the health?
- What are the normal level values?
- How can you determine its quantity on the blood?
- Discuss the methodology?