C-reactive protein (CRP)and (CRP)-latex test

Serological Tests

SEROLOGY

Serology is the scientific study of blood sera and their effects. It is concerned with *in-vitro* Ag-Ab reaction and with the laboratory study of the activities of the components of serum that contribute to immunity. Serology as a science began in 1901. Karl Landsteiner

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Serum reaction: Antigen (**Ag**) antibody (**Ab**) reaction (binding) in the serum, its useful for detection and measurement either Ab or Ag in the serum, which is very important in the clinical conditions & diagnosis of diseases.

Antigen and Antibody reactions can be identified by different tests. These include:

Precipitation Test: It is a type of reactions which done between **soluble** Ag & its specific Ab.

a- Immunodiffusion

b-Immunelectrophoresis

Agglutination Test: Agglutination occurs due to the cross-linking of antibodies with particulate antigens. Agglutination is the clumping of insoluble particles, whereas precipitation involves the aggregation of soluble molecules.

Types of Agglutination

Direct agglutination: This reaction done between **insoluble** Ag & its specific Ab.

Passive (indirect) agglutination: When the test Ag is soluble, sometime may be attached to a carrier so that the carrier will be agglutinated in the presence of a specific Ab, producing the reaction which is easy to see.

Reverse passive agglutination: soluble antibody coated on carrier particles binds to antigen and results in agglutination. Example: detecting cholera toxin

Example of carrier; latex particle, charcoal, and gelatin.

Example of indirect Agglutination:

Latex Agglutination •

- a) Ab or Ag molecules are bound to the surface of latex carrier particles.
- b) If Ag or Ab is present in the sample •, cross linking will occur & visible aggregates.
- Example: C-reactive protein (CRP), Anti- streptolysin O (ASO), Rheumatoid Arthritis (RA), etc.

Mechanisms of Agglutination: Occur in • two stages

- Sensitization, physical attachment of Ag (agglutinogen) & Ab (agglutinin).
- > Network formation.

3-Neutralization Test:

Viral neutralization •

4-Complement-fixation Test: •

5-Labeled Antibody Test: •

A.Fluorescent antibody tests •

B.Enzyme linked immunosorbent assay(ELISA) •

C.Western blot test •

C-reactive protein (CRP) and (CRP)-latex test:

C-reactive protein (CRP) is a protein • found in the blood which is produced by the liver and by fat cells in response to inflammation. It is one of the acute phase proteins, CRP originally thought to be an antibody to the C-polysaccharide of Pneumococci. CRP is classified as an acute phase reactant, which means that its levels will rise in response to inflammation, other common acute phase reactants include the erythrocyte sedimentation rate (ESR).

CRP increase rapidly in serum within (4-6) hours of the onset of inflammation (infection or other trauma), reach a peak within 48 hours and decline rapidly with resolution of inflammation trauma.

Elevated levels are found in conditions such as bacterial infection, rheumatic fever, viral infection, malignant diseases, tuberculosis, surgery, tissue damage and necrosis, and after a heart attack.

CRP acts somewhat like an antibody, as it is capable of opsonization (coating of foreign particle), agglutination, precipitation, and activation of complement system, bind to phosphocholine which is a common constitute of microbial membrane. In addition CRP binds to receptor on macrophage and neutrophil, which is promote phagocytosis.

Because the levels rise and then decline so rapidly, CRP is a more reliable and sensitive indicator of acute inflammation than the ESR, which may be influenced by physiological changes not associated with an inflammation process, also CRP level may fall to normal if it has been treated successfully, but ESR may still be abnormal for a while longer.

Although CRP is a nonspecific indicator of disease or trauma, monitoring of its levels can be useful clinically to follow a disease process and observe the response to treatment of inflammation and infection.

Currently, increased level of CRP is a significant risk factor for myocardial infraction in men and women. Thus, monitoring CRP may be an important preventative measure in determining the potential risk of heart attack and stroke, although only automated, high-sensitivity tests for CRP are useful for this purpose. Highsensitivity CRP testing has a lower level of detection of 0.01 mg/dL, allowing for measurement of much smaller increase than the traditional latex agglutination screening test.

Principle:

C-reactive protein test is done to detect acute inflammations and to estimate the qualitative and semi quantitative CRP in human serum samples. Normal range is less than **6mg/l**.

The reagent contains latex particles coated with anti-human c-reactive protein antibodies, agglutination occur in the presence of CRP in the patient serum.

Procedures:

Qualitative:

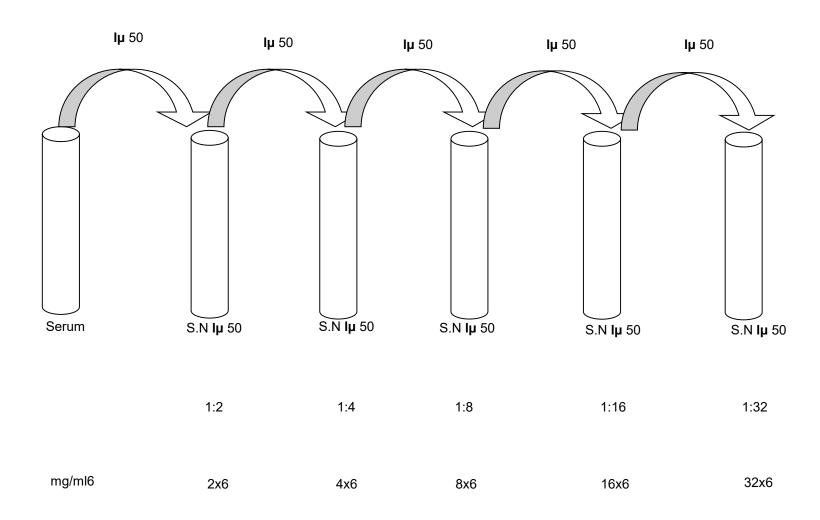
- I-Allow each component to reach room temperature.
- 2-Gently shake the latex reagent to disperse the particles.
- 3- Add one drop of serum on to black circle test slide, using the disposable pipettes provided.
- 4- Add one drop of CRP latex reagent next to the drop of serum.
- 5- Mix both drops with a stirrer and spread over the entire area of the test circle.
- 6- Gently tilt or rotate the slide backwards and forwards for 2 minutes. Interpret results immediately after 2 minutes. Extended incubation may lead to false results. Positive and negative controls should be included at regular intervals.

Results

- +ve result: presence of agglutination indicates a level of CRP in the sample ≥ 6 mg/l.
- **-ve result**: no agglutination indicates a level of CRP in the sample < 6mg/l.

Semi quantitative:

This method can be performed in the same way as the qualitative test, using dilution of the serum in normal saline, phosphate buffered saline or glycine saline as below:



Result:

The serum titer is examined as the reciprocal of the **highest dilution** showing macroscopic agglutination, e.g. If this occurs in dilution 3, the titer is 48 mg/l.

Notes:

The CRP-latex has been standardized to detect serum CRP levels at or above **6mg/l**, which is considered the lowest concentration of clinical significance.

The normal range for CRP is <6mg/l however levels within this range are associated with cardiac risk. Levels between 10-20mg/l are associated with viral infections while levels from 10-40mg/l are seen in myocardial infarction and upper respiratory tract infections. Levels between 30-200mg/l are often found post-surgery as well as in bacterial infections and rheumatoid arthritis.