

Lec 3

C-reactive Protein (CRP) as an example for Latex agglutination test

The main biologic sign of inflammation is an increase in the erythrocyte sedimentation rate (ESR). In addition an increase in plasma concentrations of a group of proteins known as acute-phase proteins is a good indicator of local inflammatory activities and tissue damage. The acute phase proteins include C-reactive protein (CRP), inflammatory mediators (e.g. complement components c3 and c4, fibrinogen, etc.).

CRP is prominent among the acute-phase proteins because it provides fast and adequate information of the actual clinical situation; as a result CRP is a direct and quantitative measure of the acute-phase reactions.

CRP is a method of choice for screening for inflammatory and malignant diseases and monitoring therapy in inflammatory disease. Elevations of CRP occur in nearly to diseases states, including bacterial infection, viral infections, myocardial infarction specificity rules out CRP as a definitive diagnostic tool.

The CRP test has been widely used to detect infection in circumstances where microbial diagnosis is difficult. These conditions include septicemia and meningitis in neonates, infections in immunosuppressed patients, serious post operative infections etc.

CRP levels rise following the tissue injury or surgery. In uncomplicated cases the level of CRP peaks about 2 days postoperatively and gradually returns to normal levels within 7 to 10 days. CRP is synthesized more rapidly than other acute phase proteins; assays of CRP are the measurement of choice in suspected inflammatory conditions.

Antistreptolysin O test

The antistreptolysin O (ASO) test is the most widely used serological test for the detection of *Streptococcus A* sequelae. Since streptolysin is only one of several *Streptococcus A* exoenzymes, the ASO test will not detect the other antibodies to exoenzymes of *Streptococcus A*.

In the course of streptococcal infections, the extracellular products of the bacteria act as antigens to which the body responds by producing specific antibodies. Streptolysin O (SLO) is one of two hemolysins (the other being Streptolysin S) produced by virtually all strains of *Streptococcus pyogenes*. The letter "O" indicates that this toxin is oxygen labile. The SLO toxin has direct toxic effects on heart tissue. The toxic

effects of SLO can be demonstrated in-vitro, as when added to a suspension of red blood cells, hemolysis will occur in minutes. In the course of a streptococcal infection, SLO stimulates the production of specific antistreptolysin antibodies, which in-vitro, neutralize the hemolytic properties of the antigen, SLO.

A titer of antistreptolysin O (ASO) is useful in the investigation of the disease processes related to streptococcal infections, such as acute poststreptococcal glomerulonephritis and rheumatic fever.

Complement fixation test

Complement is fixed during the interaction of antigens and antibodies. The complement fixation test is sensitive test that can be used to detect and quantities of antigens and antibodies.

1. The primary reacting ingredients are antigen, antibody, and complement.

Sources of Complement:

- **Normal guinea pig serum** often is used as a primary source of complement because guinea pigs have high levels of complement with efficient lytic properties.
- **Different sources** of complement are used in different *in vitro* test (e.g., rabbit complement is used in cytotoxicity tests performed for transplantation).

2. To use the complement fixation test to determine the presence of antibody to a known antigen in a patient's serum, a test system and an indicator system are used.

- **Test system.** The serum which is heated to 56°C to inactivate native complement, has added to it measured amounts of antigen and complement (e.g., normal guinea pig serum). If antibody specific for the known antigen is present in the serum, antigen-antibody complexes will form that consume (or fix) all the complement. The initial reaction, however, cannot be seen.
- **Indicator system.** In a second step, an indicator system consisting of sheep red blood cell (SRBC) plus hemolysin, antibody specific for SRBC, is added to test for the presence of free complement.

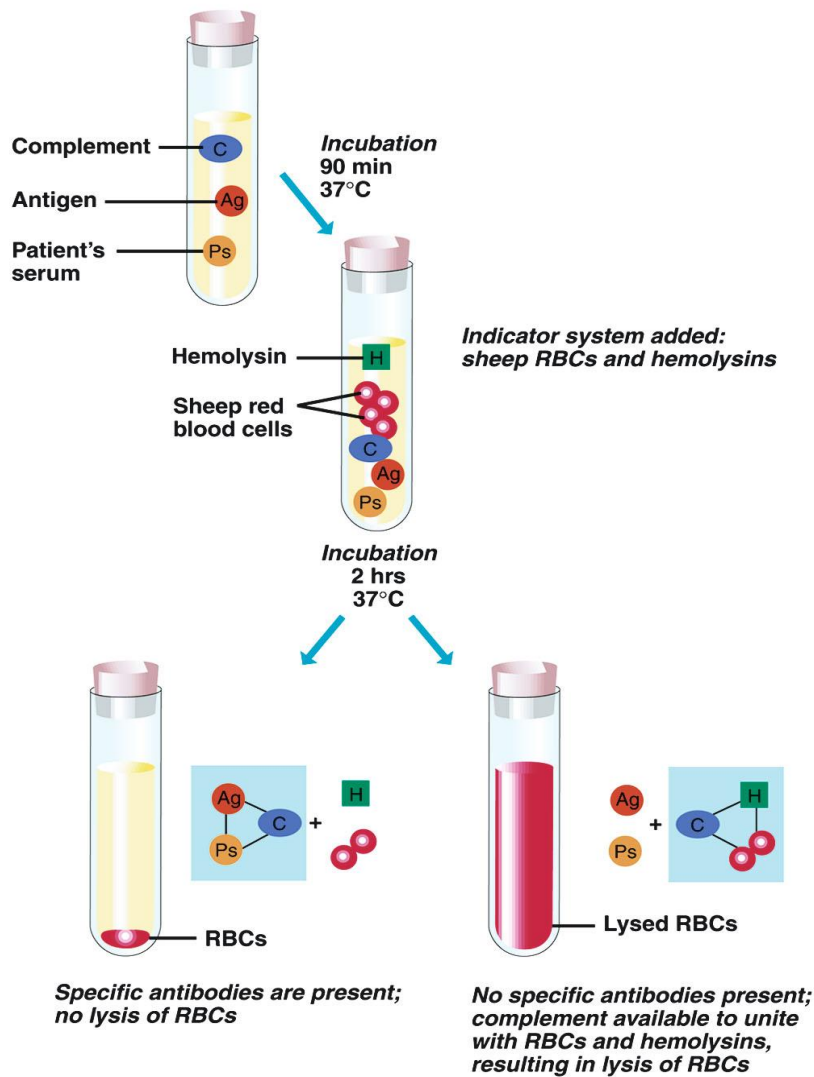
Interpretation of the test is based on the presence of hemolysis.

- If all the complement has been fixed, none will be free to lyse the SRBCs, which constitutes a positive complement fixation test.
- If no antibody is present in the patient's serum, then the complement is not fixed and is free to interact in the indicator system and lyse the SRBCs, which constitutes a negative complement fixation test.

3. Properly conducted complement fixation tests require the incorporation of appropriate controls to ensure that the results are not adversely affected by the presence of anticomplementary ingredients. The antigen or the serum itself may have anti-complementary properties (e.g., denatured or aggregated immunoglobulin, heparin, chelating agents, microbial contaminants), may fix all the complement in the system, or may remove calcium or magnesium ions (both of which are essential for complement-mediated lysis).

The following tests are done by Complement Fixation:

- Adenovirus
- Fungal Panel (*Blastomyces*, *Coccidioides*, & *Histoplasma*)
- Influenza A & B
- Parainfluenza 1, 2, & 3
- Poliovirus 1, 2, & 3
- Respiratory Syncytial Virus (RSV)



Coombs Test

A **Coombs test** (also known as **antiglobulin test** or **AGT**) is used in immunohematology and immunology. There are two Coombs tests:

- I. **Direct Coombs test (DCT) or direct antiglobulin test (DAT)**
- II. **Indirect Coombs test or indirect antiglobulin test or (IAT).**

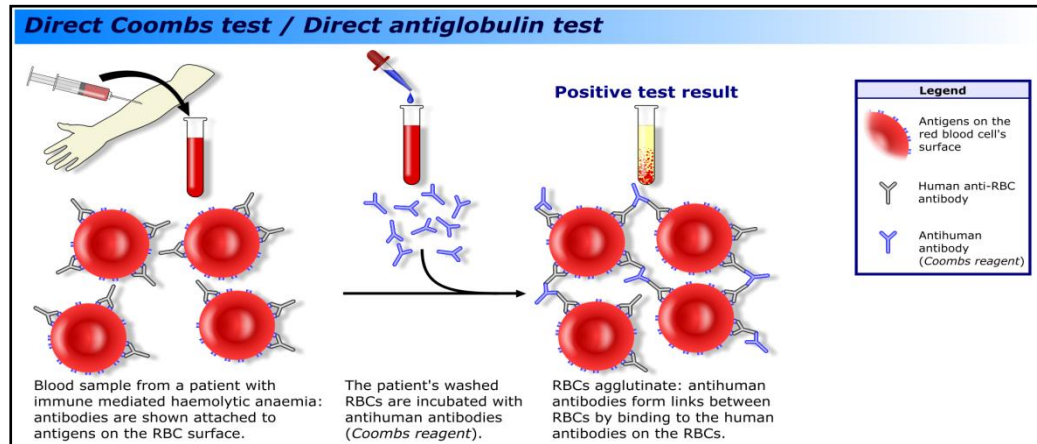
The direct antiglobulin test is used to demonstrate the **sensitization** of RBCs *in vivo* with IgG antibodies and/or complement components (C3b, C3d, C4). This test is useful in the investigation of (or the Application of this test):

Hemolytic transfusion reactions

Hemolytic disease of the fetus and newborn

Autoimmune hemolytic anemia

In **direct Coombs test**: a **blood sample** is taken and the RBCs are washed (removing the patient's own plasma) and then incubated with antihuman globulin (also known as "Coombs reagent"). If this produces agglutination of RBCs, the direct Coombs test is positive, a visual indication that antibodies (and/or complement proteins) are bound to the surface of red blood cells.



The **indirect Coombs test** detects antibodies against RBCs that are present **unbound** in the patient's serum. This test is performed to detect presence of Rh-antibodies or other antibodies in patients serum in case of the following:

- To check whether an Rh-negative women (married to Rh-positive husband) has developed Anti Rh-antibodies.
- Transfusion of Rh positive blood**
- Pregnancy, if infant is Rh positive (if father is Rh-positive)**
- Abortion of Rh-positive fetus.**

In IAT: **serum** is extracted from the blood sample taken from the patient. Then, the serum is incubated with RBCs of known antigenicity; that is, RBCs with known reference values from other patient blood samples. If agglutination occurs, the indirect Coombs test is positive.

Indirect Coombs test / Indirect antiglobulin test

