Immunological techniques

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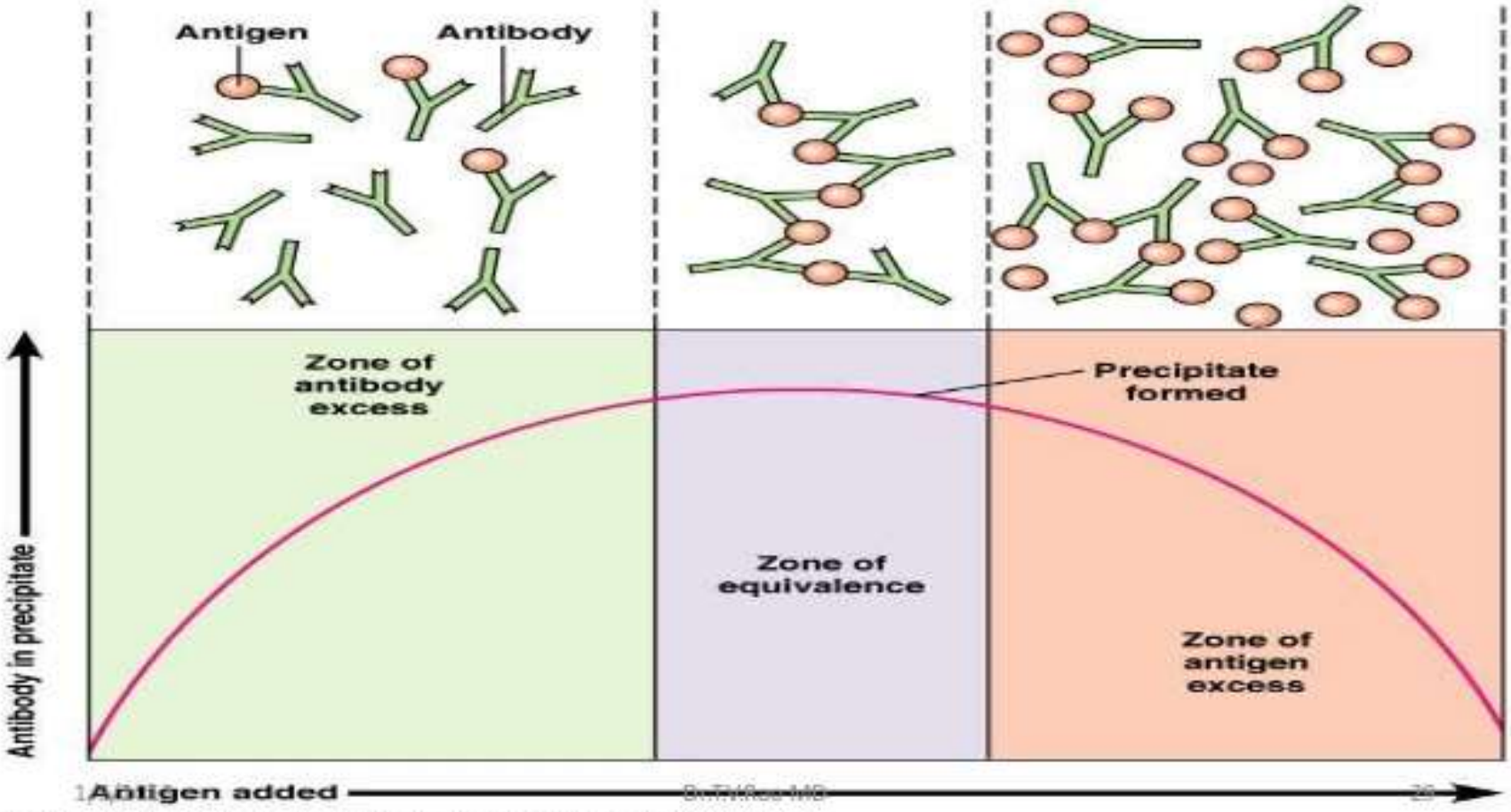
Precipitation Curve



Serology

• Serology is the study of blood serum and other bodily fluids for the identification of antibodies. Serological tests are performed on blood serum, and body fluids such as semen and saliva.

• In practice, the term usually refers to the diagnostic identification of antibodies in the serum or the detection of antigens of infectious agents in serum.

• Such antibodies are typically formed in response to an infection (against a given microorganism), against other foreign proteins (in response, for example, to a mismatched blood transfusion), or to one’s own proteins (in instances of autoimmune disease).

❖ Precipitation Reactions

• Precipitation reactions are serological assays for the detection of immunoglobulin levels from the serum of a patient with infection.
• Precipitation reactions are based on the interaction of antibodies and antigens.
• They are based on two soluble reactants that come together to make one insoluble product, the precipitate.
• These reactions depend on the formation of lattices (cross-links) when antigen and antibody exist in optimal proportions.

• Excess of either component reduces lattice formation and subsequent precipitation.
• Precipitation reactions differ from agglutination reactions in the size and solubility of the antigen and sensitivity.
• Antigens are soluble molecules and larger in size in precipitation reactions.
• There are several precipitation methods applied in clinical laboratory for the diagnosis of disease.
• These can be performed in semisolid media such as agar or agarose, or non-gel support media such as cellulose acetate.

• Assay for both qualitative and quantitative detection were available using gel based assays.
• The principle of the precipitation reaction that allows for the quantitative measurement is called equivalence.
• Precipitation reaction will be maximal at that point where antigen and antibody are in equal numbers.
• The equivalence point is where maximal cross-linking will occur and the antigen and antibody macromolecule will be the least soluble.

• The most commonly used serologic precipitation reactions are the Ouchterlony test (based on double immunodiffusion and named after the Swedish physician who invented it), and the Mancini method (based on single radial immunodiffusion).

• **Precipitin**: Any antibody which reacts with an antigen to form a precipitate.

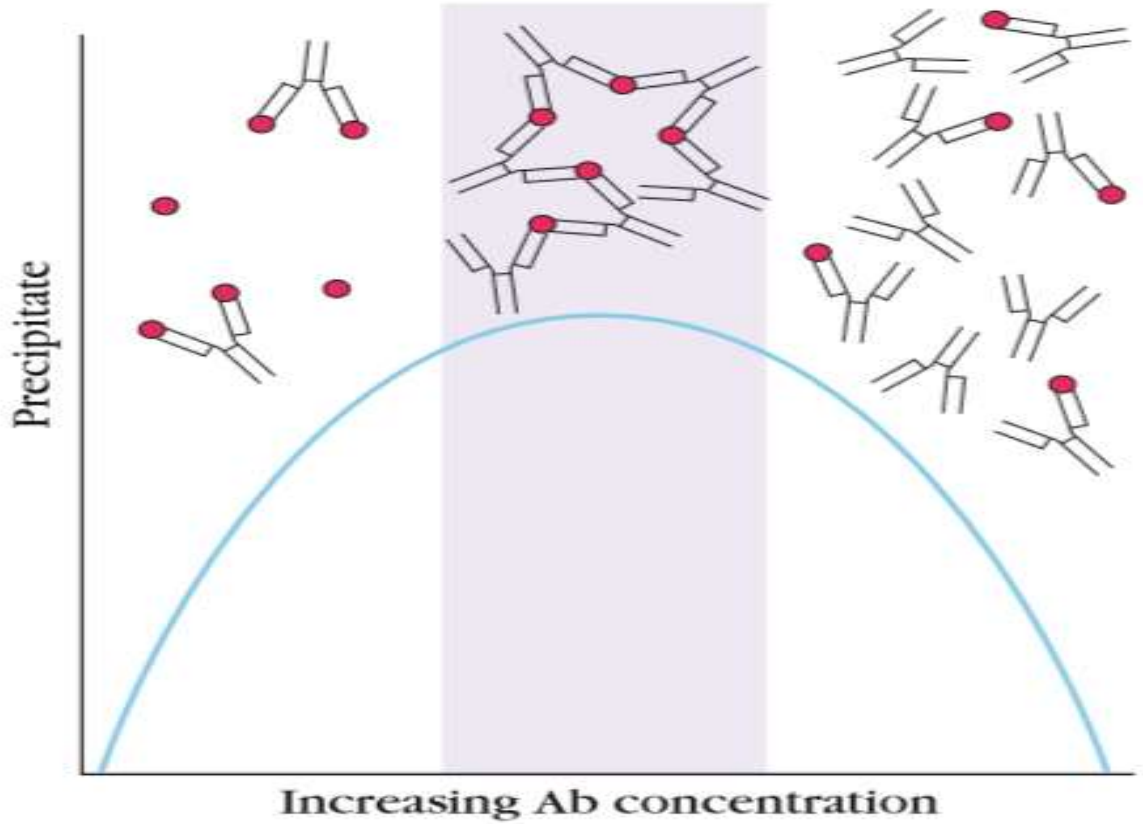
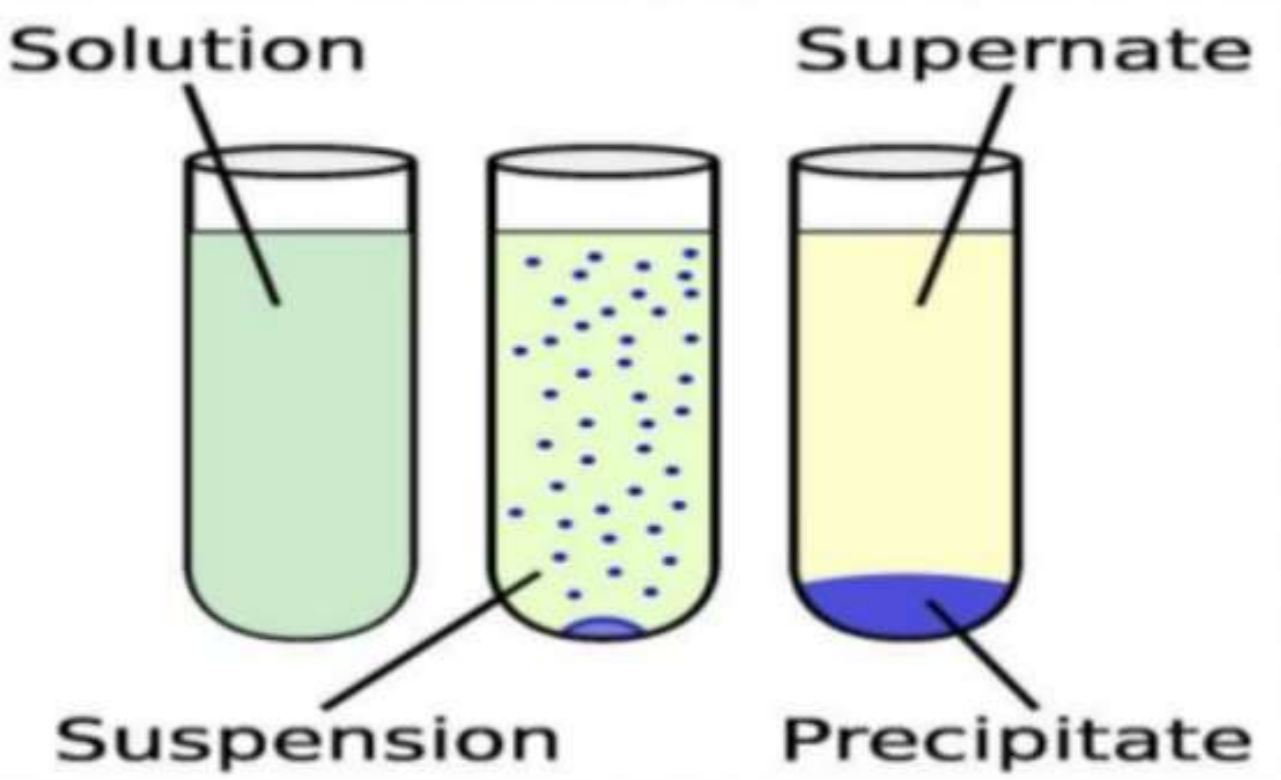
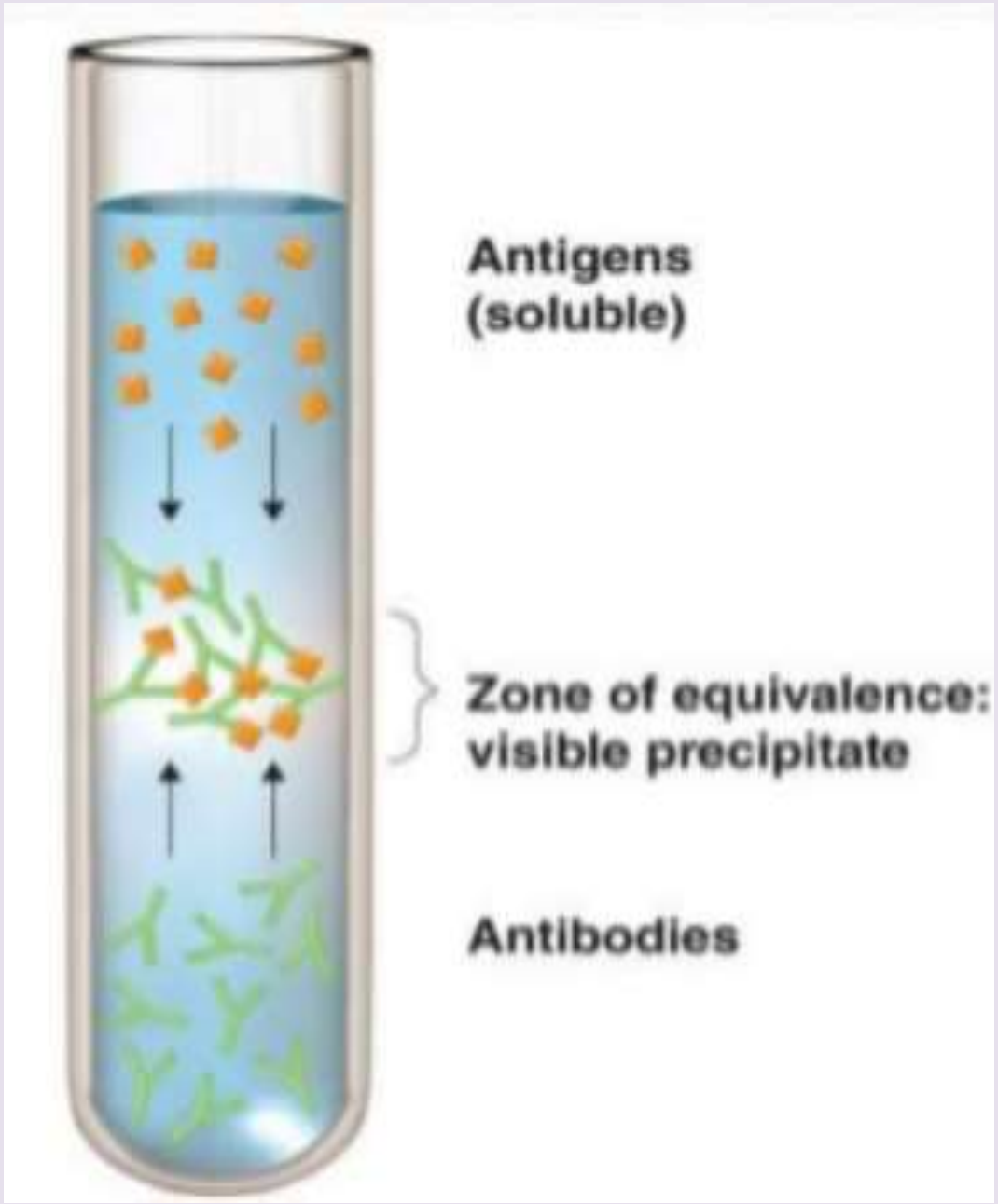


FIGURE 20-2 Immunoprecipitation in solution. When bi- or multivalent antibodies are mixed in solution with antigen, the antibodies can form cross-linkages with two or more antigen molecules, leading to the formation of a cross-linked precipitate (middle panel of graph). Precipitate formation requires that neither antigen (left panel in graph) nor antibody (right panel in graph) molecules are in excess. In either of these two cases, primarily monovalent binding takes place, as shown in the diagram. *[http://nfs.unipv.it/nfs/minf/*



Precipitation reaction

Difference in the visual appearance of an aggregate and a precipitate.


Antigens
(soluble)
Zone of equivalence:
visible precipitate
Antibodies

❖ Agglutination reactions:

• The cross-linking that occurs between di- or multivalent antibodies and multivalent, bacterial, or other cellular antigens can result in visible clumping of the complexes formed between cells bearing the antigens and the antibody molecules.

• This clumping reaction is called agglutination, and antibodies that produce such reactions are called agglutinins.

• Agglutination reactions are identical in principle to precipitation reactions; the only difference is that the cross-linked product is visible to the naked eye because of the larger size of the antigens.

▪ Hemagglutination reactions can be used to detect any antigen conjugated to the surface of Red blood cells:

• When antibodies bind antigens on the surface of red blood cells (RBCs), the resultant clumping reaction is referred to as hemagglutination.

• In the example shown in figure, control buffer was added to well 10 of the microtiter tray.

• Antibodies to sheep red blood cells (SRBCs) were added to well one of this tray, and then this antiserum was serially diluted into wells 2 through 9, such that the concentration of antibodies to the SRBCs in well 2 was half that in well 1, and so on.

• The same number of SRBCs was then added to each well.

• In well 10, in the absence of any agglutinating antibody, the SRBCs settle into a tight button in the bottom of the well.
• This tight button represents a negative result in a hemagglutination assay.
• In well 1, the high concentration of anti-SRBC antibodies induced cross-linking of the SRBCs, so that they form a large clump and do not fall down to the bottom of the well.
• The diffuse shading of RBCs seen in well 1 represents a positive interaction between the antibodies and the SRBC surface antigen.

• The concentration of anti-SRBC antibodies in wells 2 and 3 remains high enough to allow hemagglutination, but once the antibodies have been diluted eightfold (well 4), there are too few antibodies to generate cross-links and the SRBCs can again settle into the bottom of the well.

• The responses in wells 1, 2, and 3 therefore represent a positive hemagglutination reaction.

• Hemagglutination reactions are routinely performed to type RBCs.

• With tens of millions of blood-typing determinations run each year, this is one of the world's most frequently used immunoassays.

• In typing for the human ABO antigens, human RBCs are mixed with antisera to the A or B blood group antigens.

• If the antigen is present on the cells, they agglutinate, forming a visible clump on the slide.

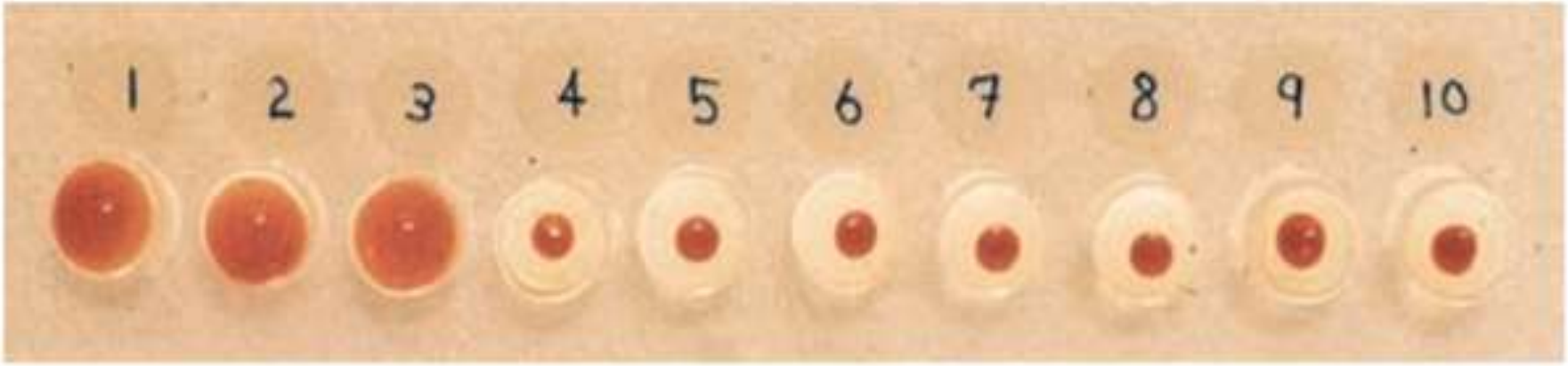


FIGURE 20-4 Demonstration of hemagglutination using antibodies against sheep red blood cells (SRBCs). The control tube (10) contains only SRBCs, which settle into a solid "button." The experimental tubes 1 to 9 contain a constant number of SRBCs plus serial twofold dilutions of anti-SRBC serum. The spread pattern in the experimental series indicates positive hemagglutination through tube 3. *[Louisiana State University Medical Center/MIP. Courtesy of Harriet C. W. Thompson.]*

▪ Bacterial agglutination can be used to detect antibodies to bacteria:

• A bacterial infection often elicits the production of antibacterial antibodies specific for surface antigens on the bacterial cells; such antibodies can be detected by bacterial agglutination reactions.

• The principle of bacterial agglutination is identical to that for hemagglutination, but in this case the visible pellet is made up of bacteria, cross-linked by antibacterial antibodies.

• Agglutination reactions can also provide quantitative information about the concentration of antibacterial antibodies in a patient's serum.

• The patients' sera are serially diluted, as described above.

• The last well in which agglutination is visible tells us the agglutinin titer of the patient, defined as the reciprocal of the greatest serum dilution that elicits a positive agglutination reaction.

• The agglutinin titer of an antiserum can be used to diagnose a bacterial infection.

• Patients with typhoid fever, for example, show a significant rise in the agglutination titer to Salmonella typhi.

• Agglutination reactions also provide a way to type bacteria.

• For instance, different species of the bacterium Salmonella can be distinguished by agglutination reactions with a panel of typing antisera.

❖ Complement fixation test (CFT)

• Complement fixation test is used to detect and quantify antibody in serum that does not form visible precipitate or agglutinate when reacted with antigen until complement is used.

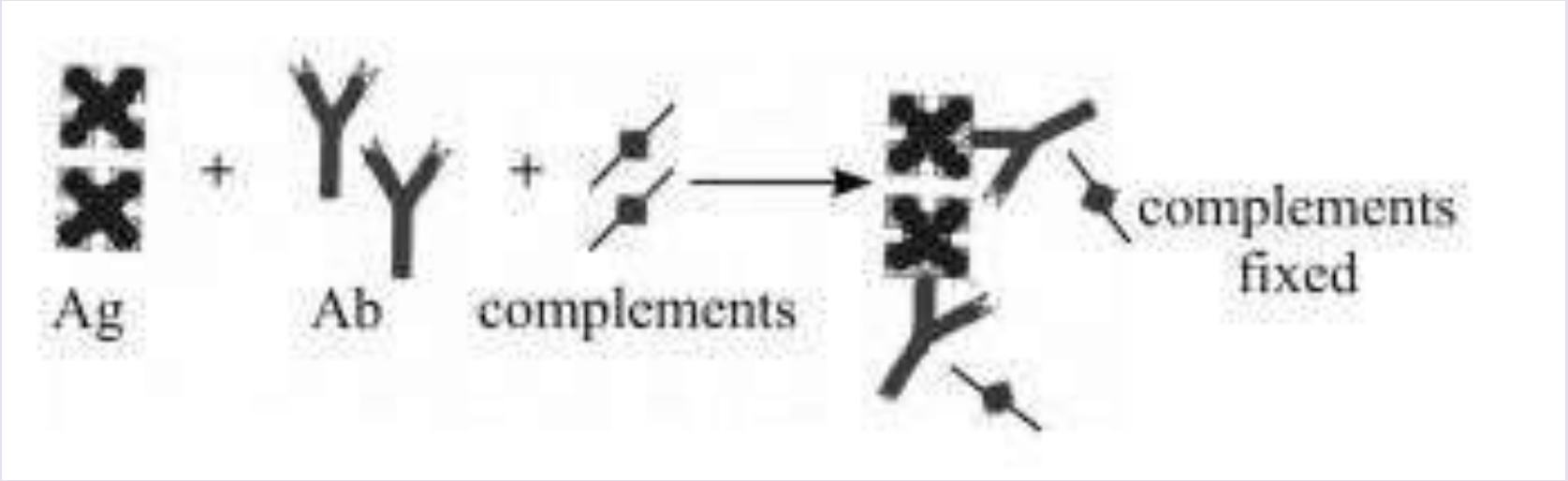
• Complement is a heat labile globular protein present in normal serum.

• Whole complement system is composed of 9 protein components i.e. C1, C2, C3….C9.

• Complement can only bind Ag-Ab complexes. When complement takes part in antigen-antibody reactions it is bound or fixed to the Ag-Ab complexes.

• When these complexes are on bacteria, RBCs or other cells, the complement brings about the lysis of these cells. Complement cannot bind free antibody.

• Antigen-antibody complex fixes the complement.



- But the fixation of complement with Ag-Ab complex do not have any visible effect like agglutination and precipitation.
- So it is necessary to use indicators system.

• The indicator system consists of sheep RBC coated with anti-sheep RBC antibody (Amboreceptor).
• The test serum is inactivated by heating at 56°C for 30 minutes to destroy the complement activity of test serum and to remove anti-complementary effect of some non-specific inhibitors in the serum.

▪ Requirements for CFT:

Test system:

• Antigen: cardiolipin/ Viral Ag/ washed Sheep RBCs
• Hemolysin: inactivated patient's serum

- Complement (Guinea pig serum)

- Procedure of Complement Fixation Test:

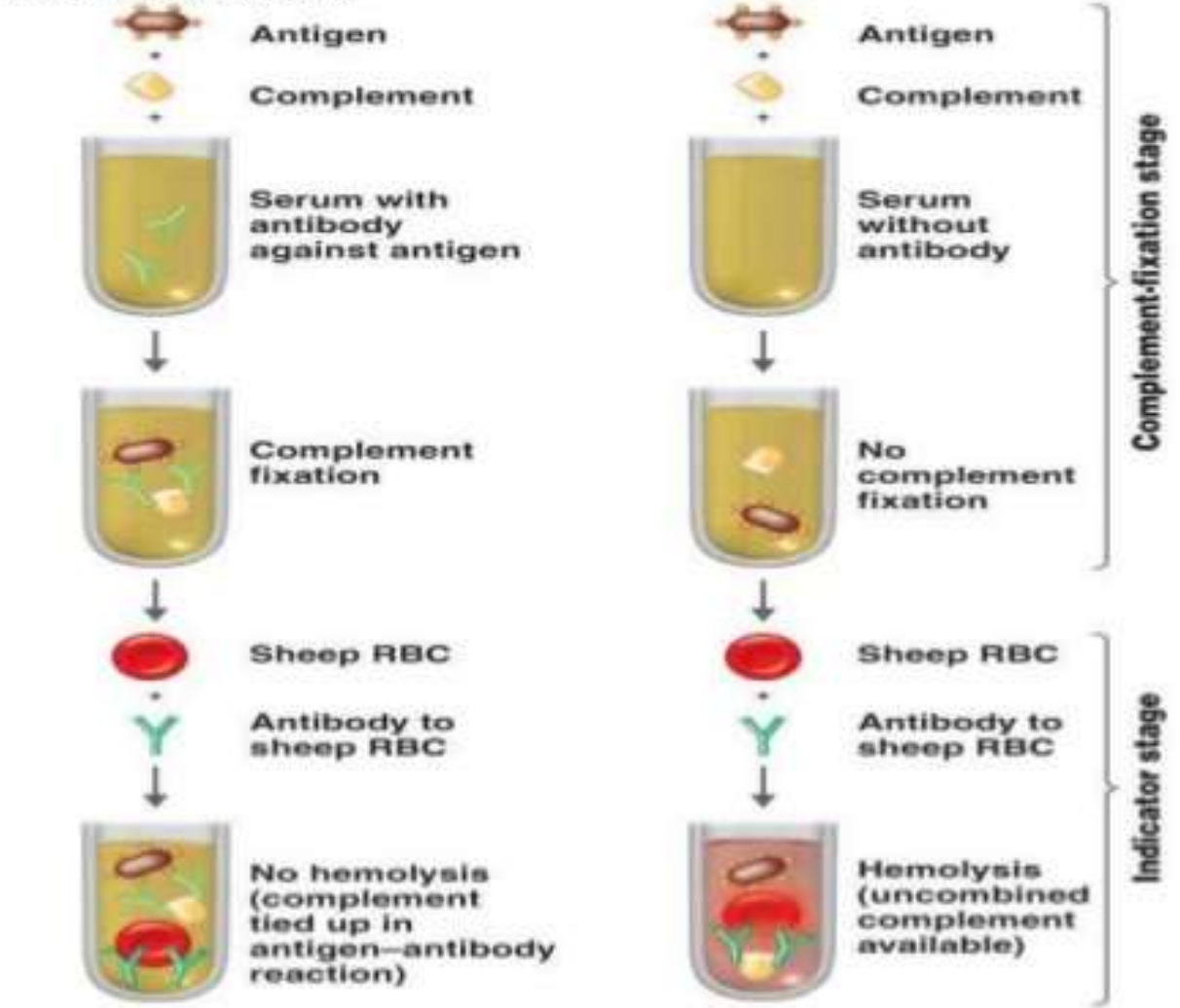
Complement Fixation Test (CFT) consists of two stage:

1) First step (Complement fixation stage):

a known antigen and inactivated patient's serum are incubated with a standardized, limited amount of complement.

Note: patient's serum is heated at 56°C for 30 minutes to inactivate endogenous complement which may disturb the test calibration.

Complement Fixation



(a) Positive test. All available complement is fixed by the antigen–antibody reaction; no hemolysis occurs, so the test is positive for the presence of antibodies.

(b) Negative test. No antigen–antibody reaction occurs. The complement remains, and the red blood cells are lysed in the indicator stage, so the test is negative.

1. If the serum contains specific complement activating antibody, the complement will be activated or fixed by the antigen-antibody complex.

2. However, if there is no antibody in the patient's serum, there will be no formation of antigen-antibody complex, thus complement will not be fixed but will remain free (In the indicator stage this complement will react with RBC coated with antibody to sheep RBC).

2. Second step (Indicator Stage): The second step detects whether complement has been utilized in the first step or not. This is done by adding the indicator system.

- If the complement is fixed in the first step owing to the presence of antibody there will be no complement left to fix to the indicator system. There won't be any lysis of RBCs.
- However, if there is no specific antibody in the patient's serum, there will be no antigen-antibody complex, therefore, complement will be present free or unfixed in the mixture.

This unfixed complement will now react with the antibody- coated sheep RBCs to bring about their lysis.

- Results and Interpretation:

- No lysis of sheep red blood cells (positive CFT) indicates the presence of antibody in the test serum,

- while lysis of sheep red blood cells (Negative CFT) indicates the absence of antibody in the serum