

3/8/2014

Diagnostic Microbiology

320 MIC

Lecture: 4

Identification of Microbes

Agglutination and Precipitation Reactions

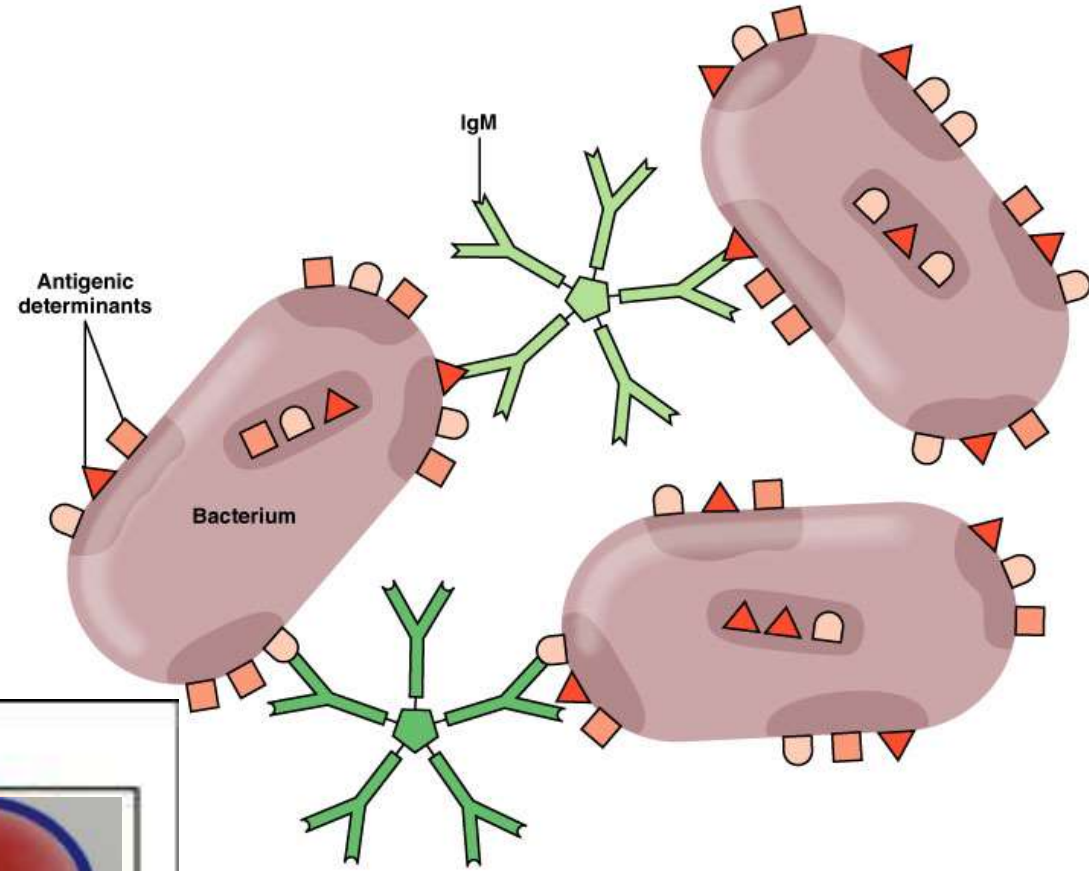
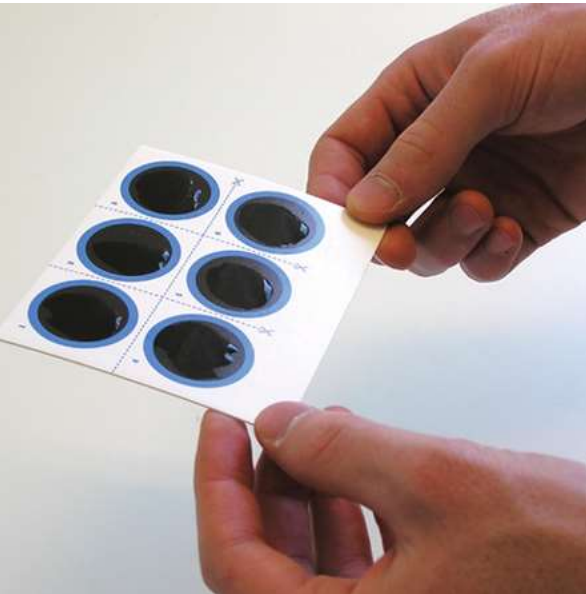
Agglutination testing:

Antibody cross links whole-cell antigens, forming complexes that settle out and form visible clumps.

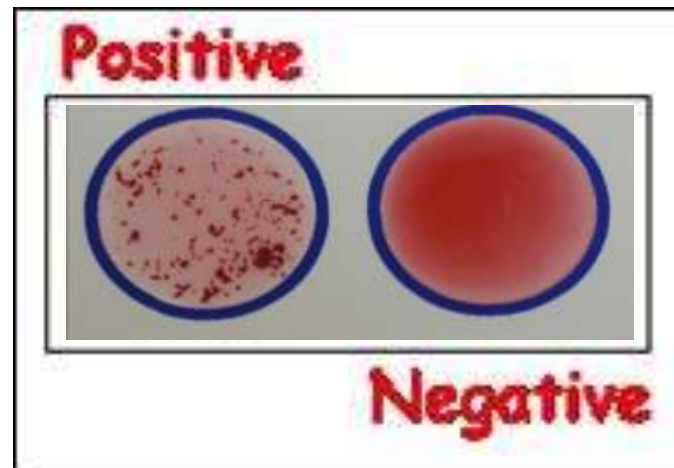
–Blood typing, some bacterial and viral diseases.

- Agglutination is the clumping of **insoluble particles**
- The interaction of particulate antigens (cells that carry antigens) with antibodies leads to agglutination reactions.
- Diseases may be diagnosed by combining the patient's serum with a known antigen.
- These reactions are easy to see and interpret with the unaided eye
- **Latex agglutination test:** Latex beads are coated with specific antibody, and agglutinated by homologous antigen. The test is used in diagnosis of *S. aureus*, *H. influenzae*, *N. meningitides*.

Agglutination and Precipitation Reactions



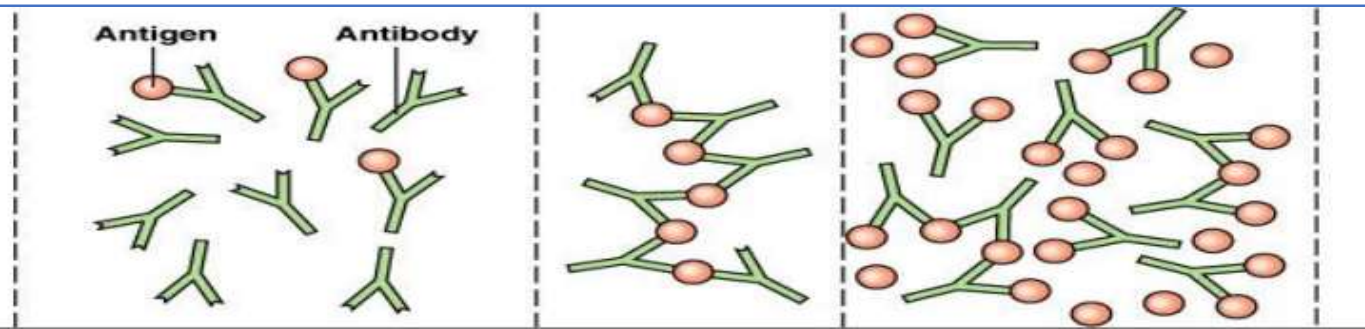
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Agglutination and Precipitation Reactions

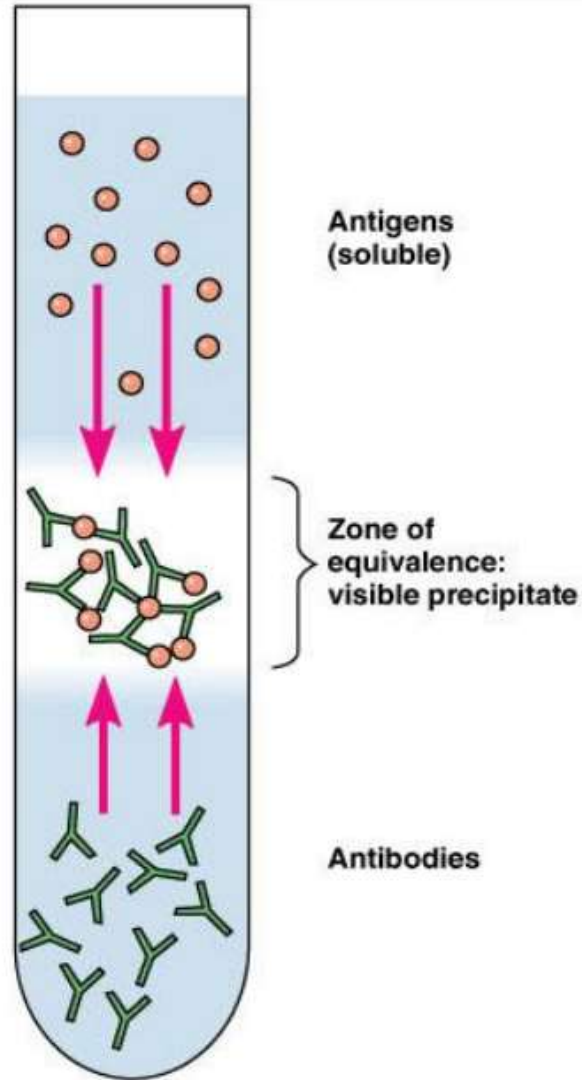
Precipitation Tests: soluble antigen is made insoluble by an antibody- Most precipitation reactions are carried out in agar gels media

- precipitation involves the aggregation of **soluble molecule**
- One of the easiest of serological tests- Based on the idea that antigens and antibody mixed in the proper proportion form large macromolecular complexes called precipitates
- These tests require both **specificity and sensitivity** of the antibodies. **Sensitivity** is the ability to recognize and bind to the antigen, **specificity** is the characteristic of binding only to one antigen and no others
- The interaction of soluble antigens with IgG or IgM antibodies leads to precipitation reactions.
- Precipitation reactions depend on the formation of lattices and occur best when antigen and antibody are present in optimal proportions. Excesses of either component decrease lattice formation and subsequent precipitation

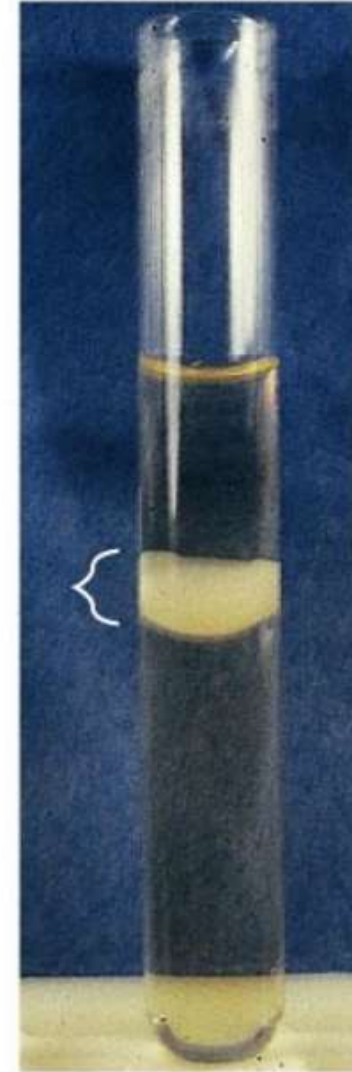


Agglutination and Precipitation Reactions

- The precipitin ring test is performed in a small tube.

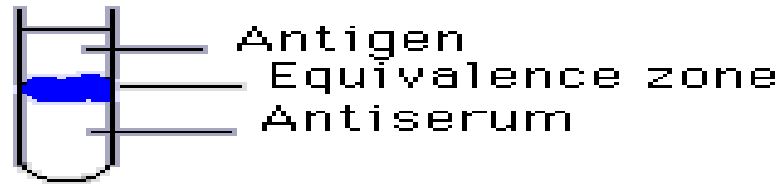


(a)

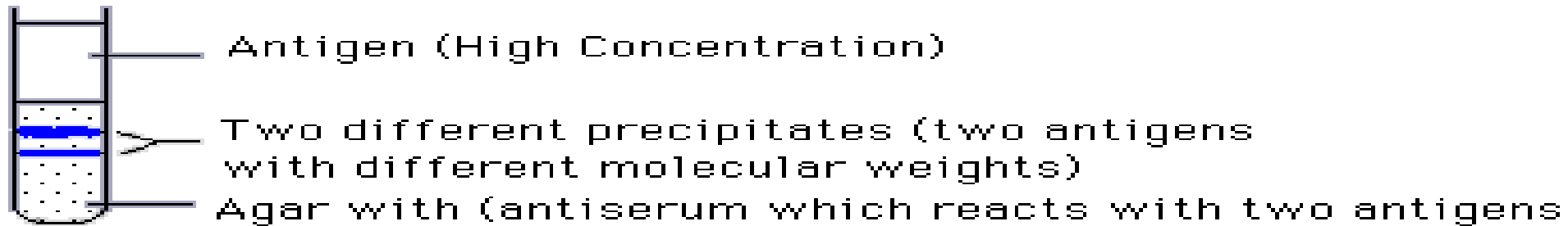


(b)

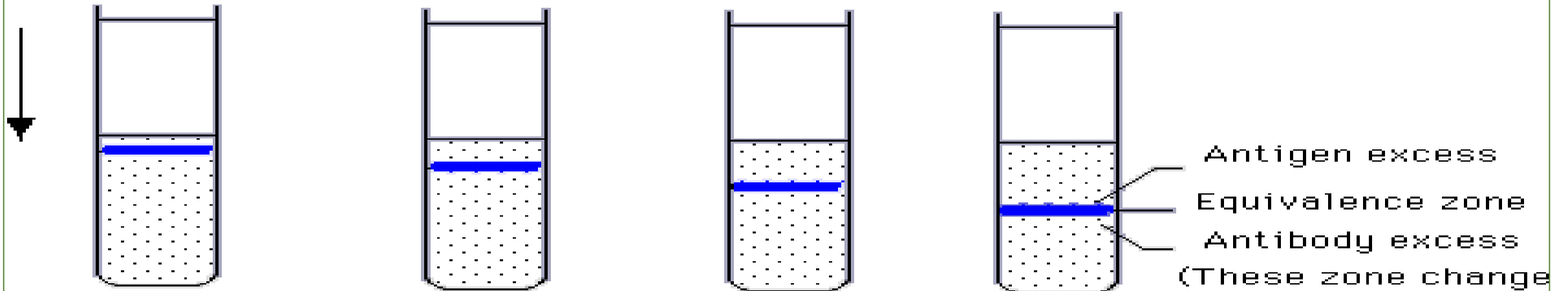
Ring precipitation test



Single dimensions single diffusion



Pseudomigration of the precipitate with time

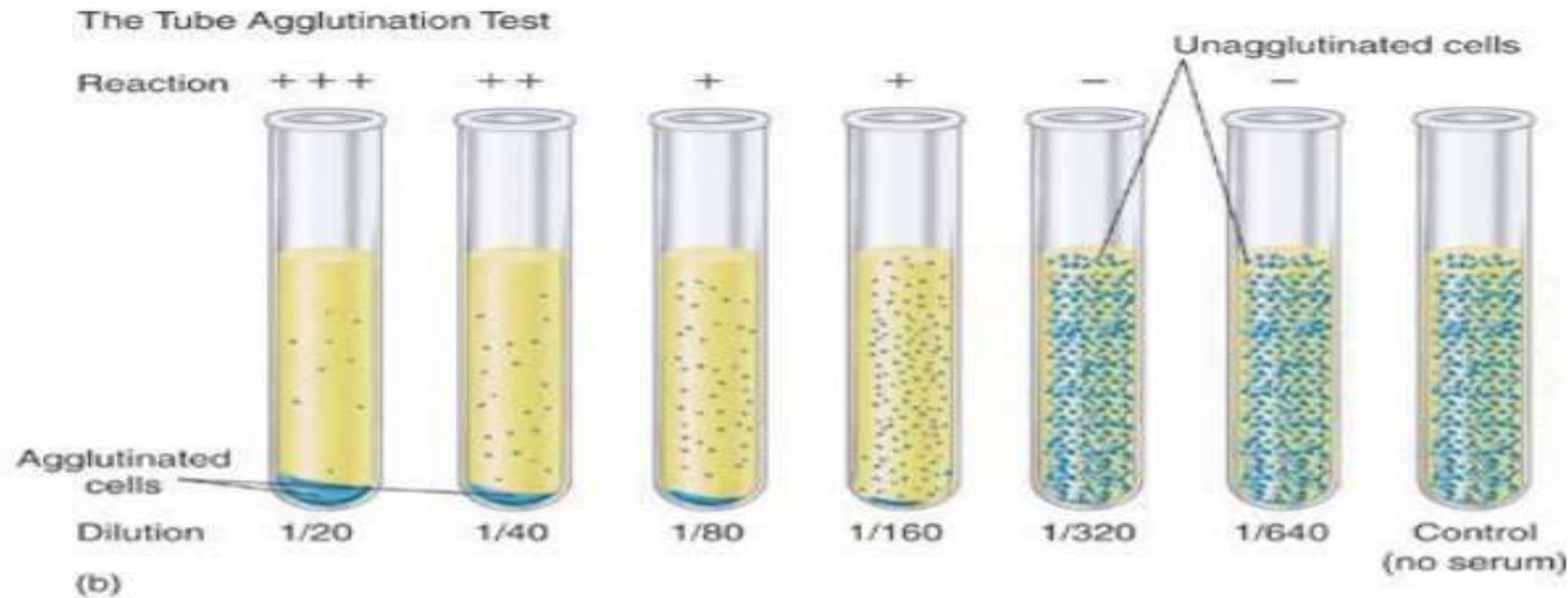
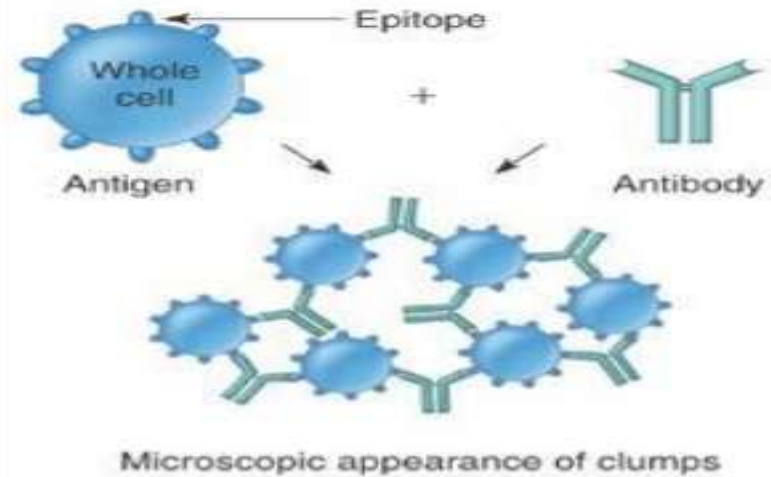


The precipitate is forming and dissolving as the antigen migrates.

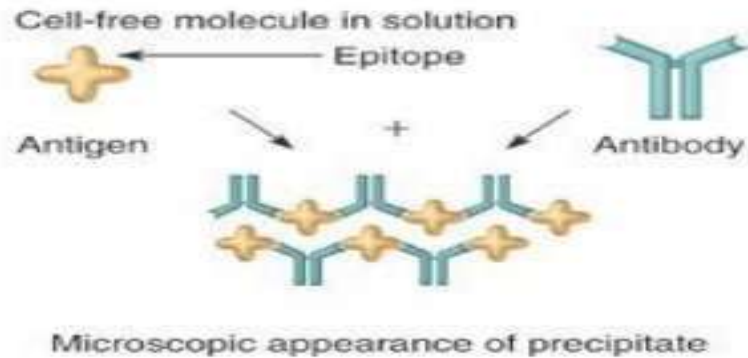
Agglutination and precipitation reactions

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Agglutination*



Precipitation*

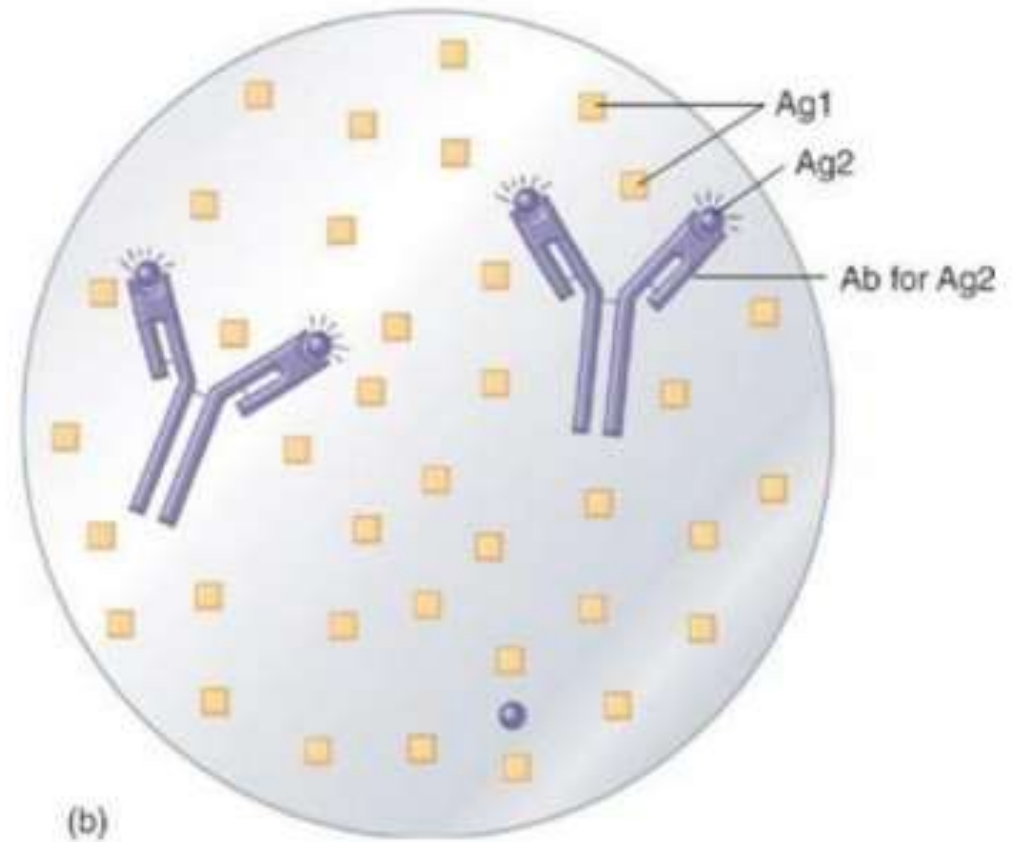
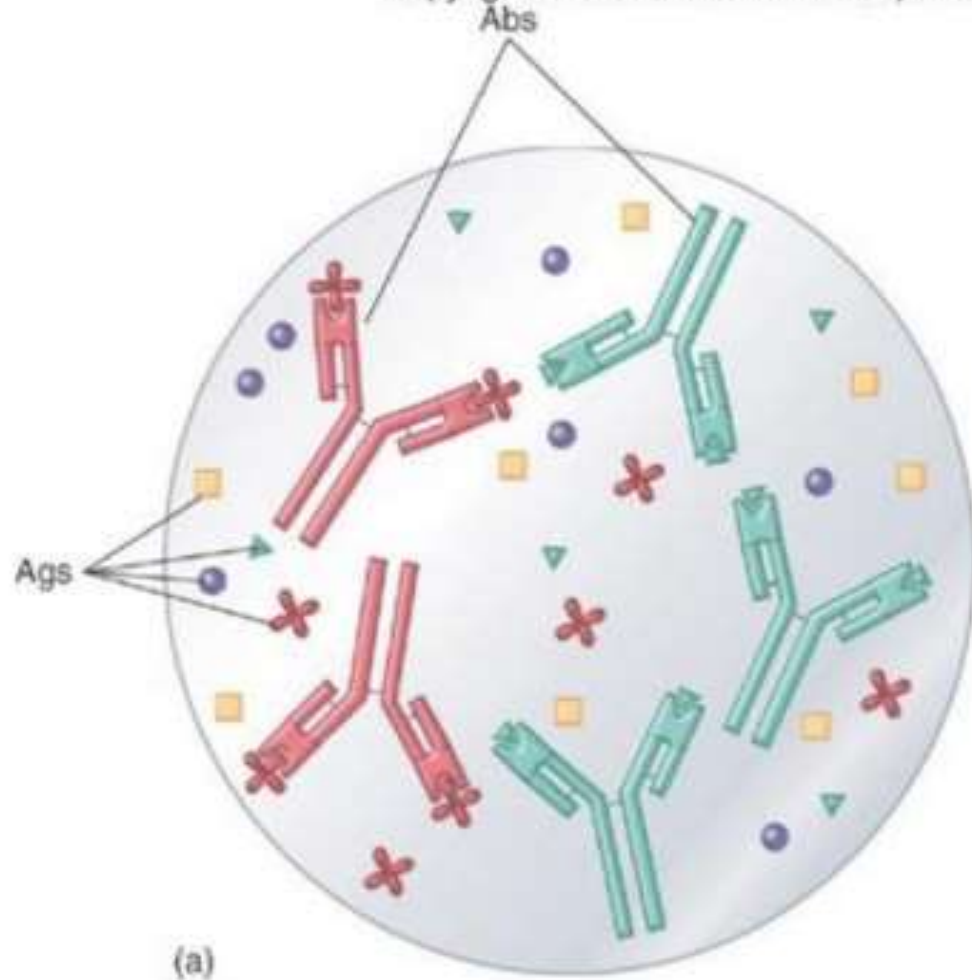


(a)

*Although IgG is shown as the Ab, IgM is also involved in these reactions.

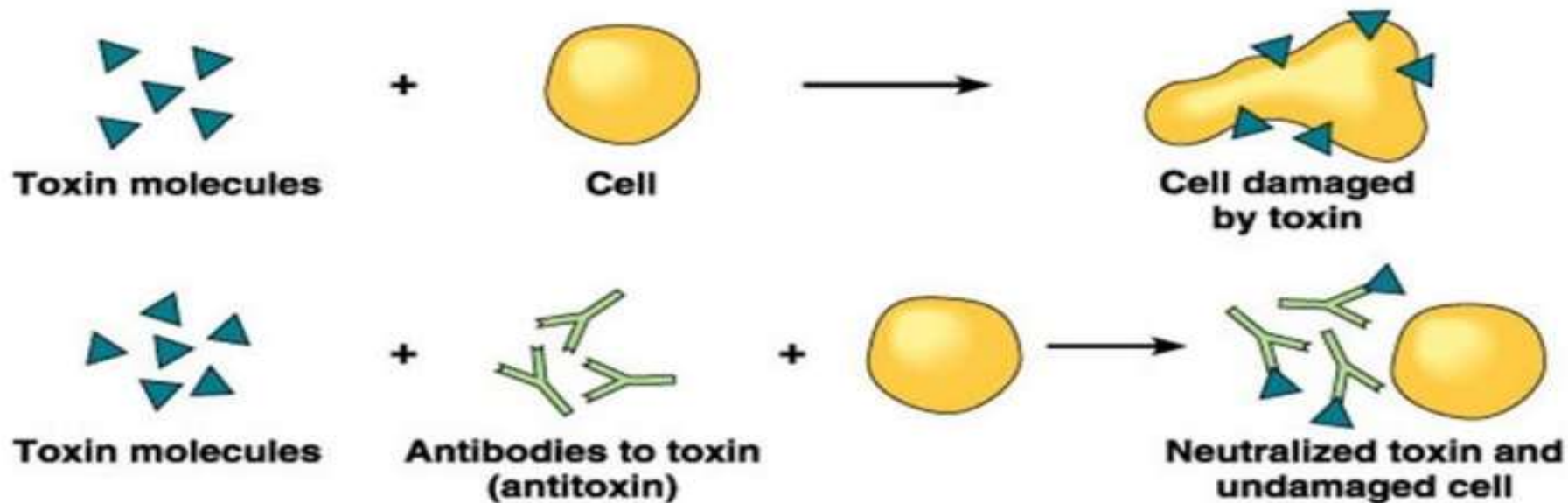
Specificity and sensitivity in immune testing

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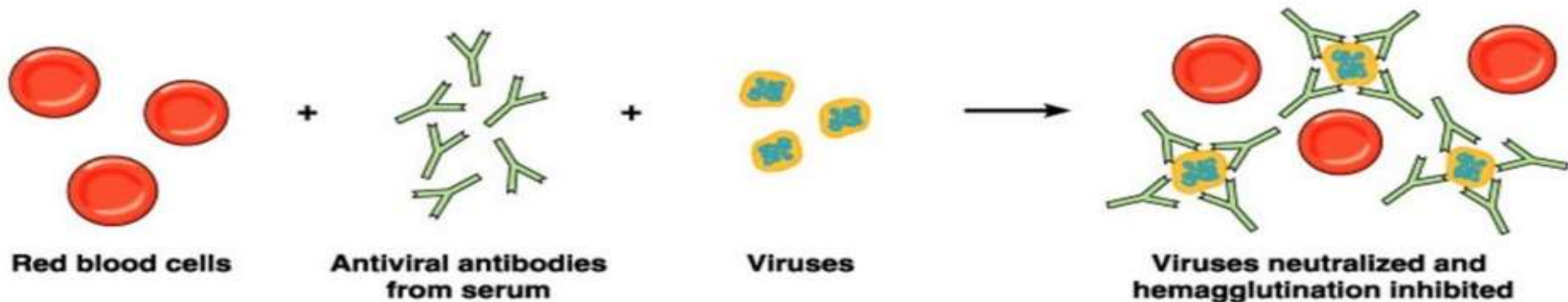


Neutralization Reaction

- In neutralization reactions, the harmful effects of a **bacterial exotoxin** or **virus** are eliminated by a specific antibody.
- An **antitoxin** is an antibody produced in response to a **bacterial exotoxin** or a **toxoid** that neutralizes the exotoxin.
- In a virus neutralization test, the presence of antibodies against a virus can be detected by the antibodies' ability to prevent cytopathic effects of viruses in cell cultures.
- Antibodies against certain viruses can be detected by their ability to interfere with viral hemagglutination in viral hemagglutination inhibition tests



(a) The effects of a toxin on a susceptible cell and neutralization of the toxin by antitoxin.



(b) Viral hemagglutination test to detect antibodies to a virus. These viruses will normally cause hemagglutination when mixed with red blood cells. If antibodies to the virus are present, as shown here, they neutralize and inhibit hemagglutination.

Complement Fixation Test

Complement-fixation reactions are serological tests based on the depletion of a fixed amount of complement in the presence of an antigen- antibody reaction

simplest test used to detect a patient serum antibody

. If the antibody is present in the patient's serum it binds to the antigen, and the complement reagent is completely consumed in the reaction.

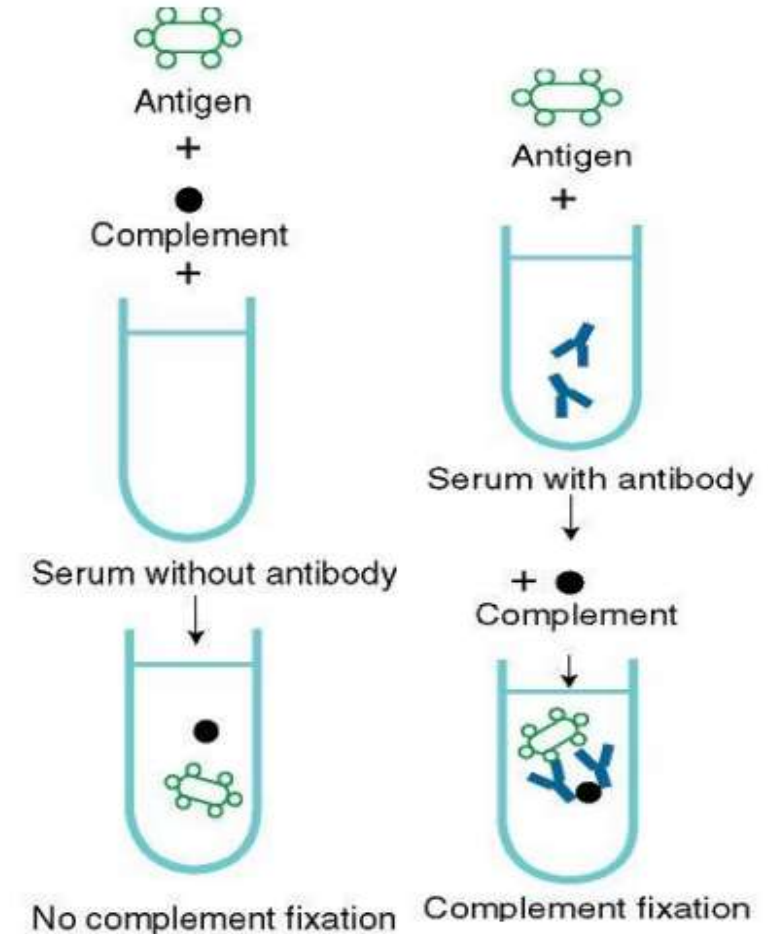
- If the antibody specific for the antigen in the assay is present in the patient's serum, then complement is completely consumed in the reaction.

Test uses four components

- Antigen, antibody, complement, and sensitized sheep RBCs.

Steps of test

1. Test antigen reacts with test antibody
2. Contents of tube from (1.) are mixed with sheep RBCs



Complement Fixation Test

Good for detecting **very small amounts of antibody**, when the amount of antibody is too low to cause a precipitation or agglutination reaction.

- It is still used to diagnose some **bacterial, viral, fungal, and rickettsial diseases**.
- There are two steps, the complement fixation step and the indicator step.
- Complement used up in first stage, no hemolysis.
- Unfixed complement, hemolysis.

