Immunologic Systems

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Principle

- Diagnostic microbiology makes great use of the specificity of the binding between antigen and antibody.

Immunological Methods

- **Precipitation**
- **Agglutination**
- **Neutralization**
- **Complement Fixation**
- **Labeling Methods**

1. **radioimmunoassay,** or **RIA**
2. **enzyme immunoassay,** or **EIA**

- **Western Blot**

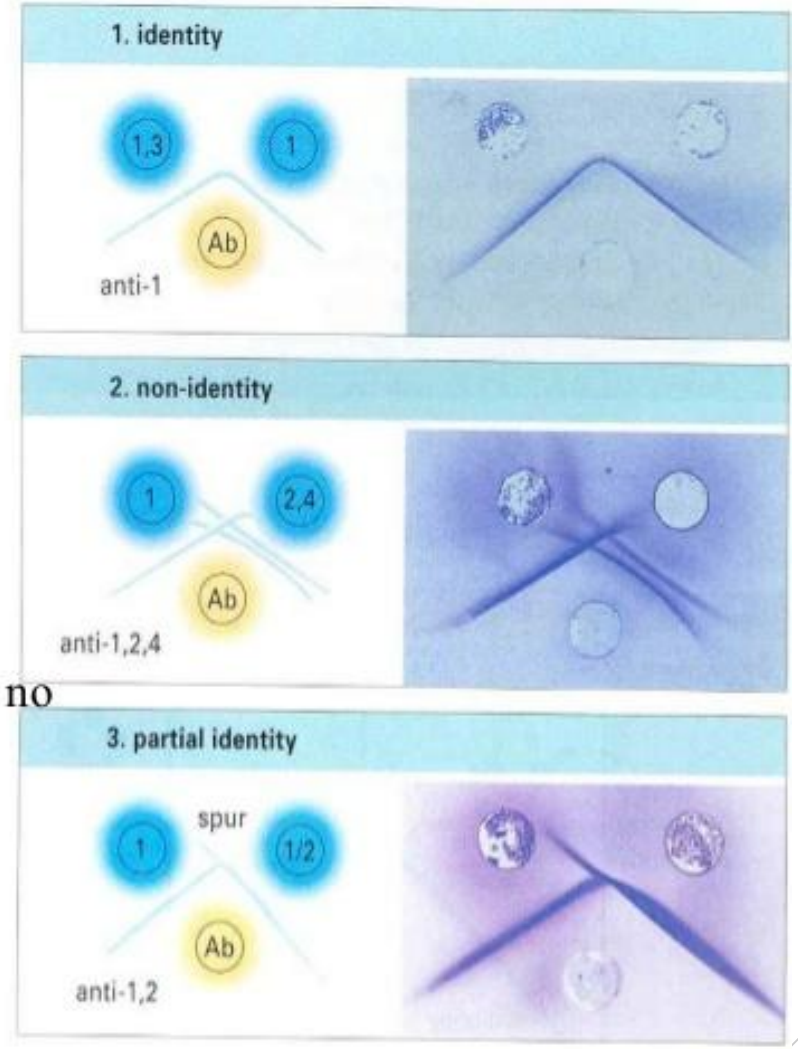
Precipitation

- When antigen and antibody combine in the proper proportions, a visible precipitate is formed.
- Optimum antigen–antibody ratios can be produced by allowing one to diffuse into the other, most commonly through an agar matrix **(immunodiffusion)**.
- **Counterimmunoelectrophoresis (CIE)** is immunodiffusion carried out in an electrophoretic field.

Immunodiffusion

Diffusion patterns

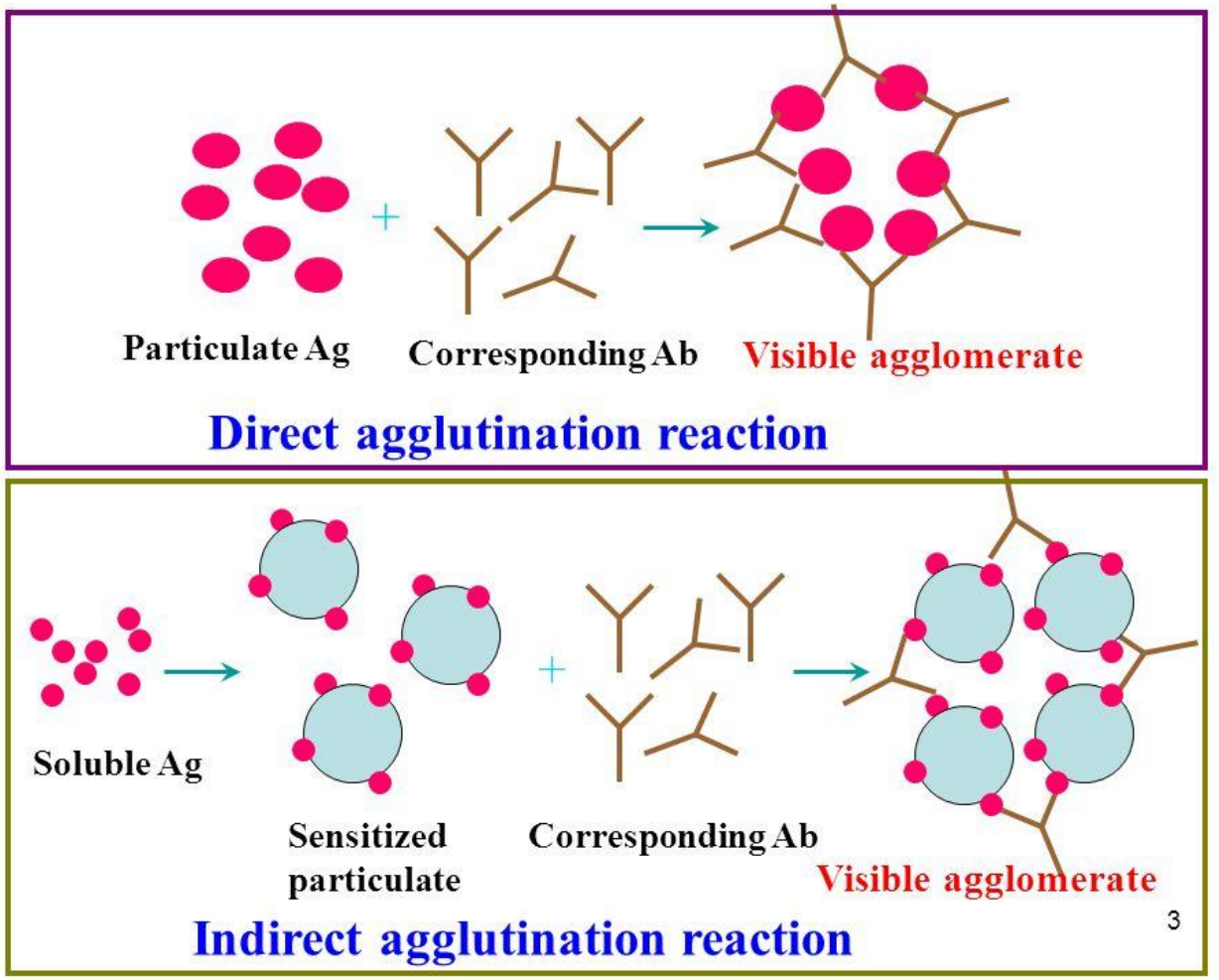
- Fusion of lines at their junction to form an arc
  - Serologic identity / presence of common epitope
- Crossed lines
  - Demonstrates 2 separate reactions
  - Compared antigens shared no common epitopes
- Fusion of 2 lines with spur
  - Partial identity



Agglutination

- The amount of antigen or antibody necessary to produce a visible immunologic reaction can be reduced if either is on the surface of a relatively large particle.
- The process is termed slide agglutination, hemagglutination, or latex agglutination depending on the nature of the sensitized particle.

Agglutination



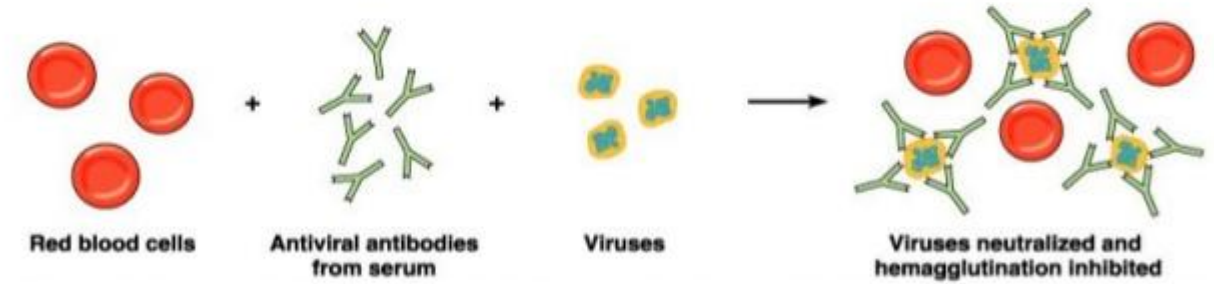
Neutralization

- Neutralization takes some observable function of the agent, such as cytopathic effect of viruses or the action of a bacterial toxin, and neutralizes it.

Neutralization

Neutralization Reactions

- Eliminate the harmful effect of a virus or exotoxin



(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.

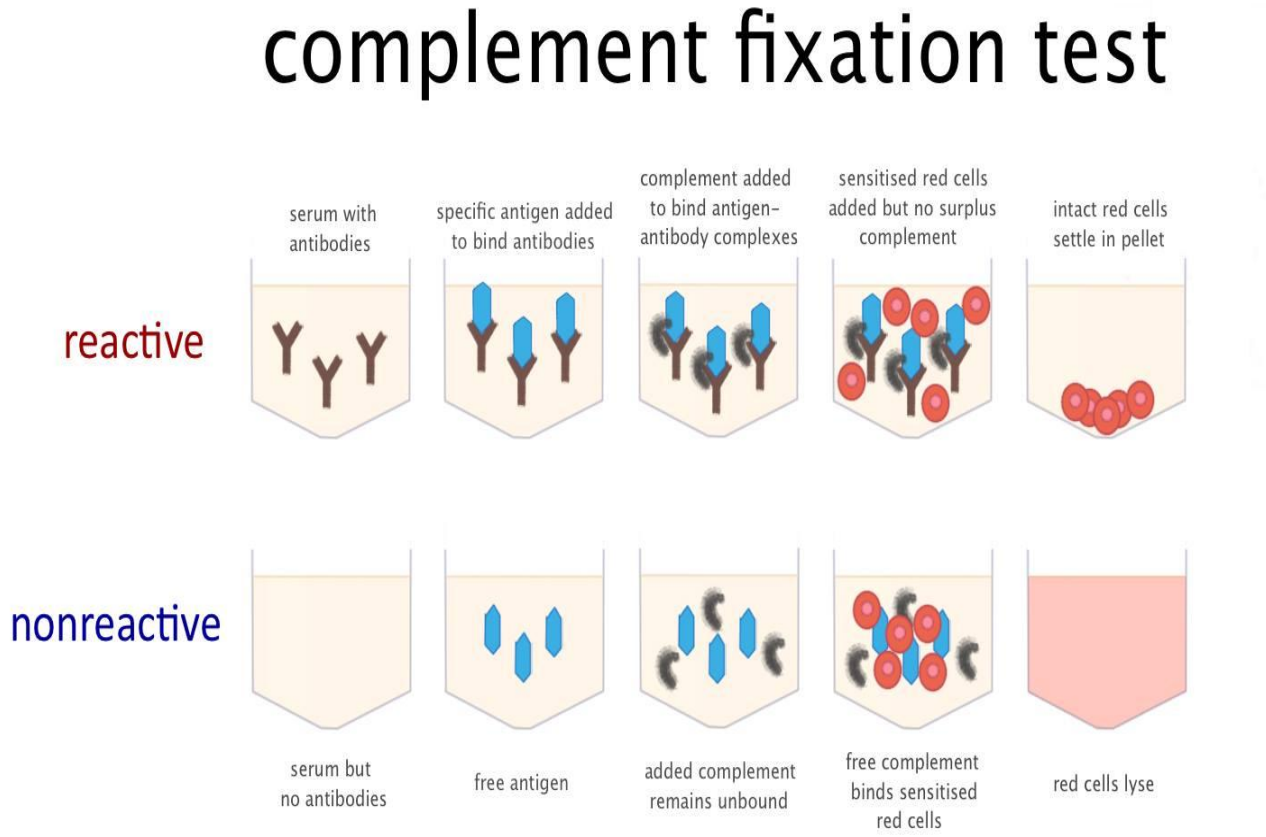
Figure 18.8b

Complement Fixation

- Complement fixation assays depend on two properties of complement.

1. The first is fixation (inactivation) of complement on formation of antigen–antibody complexes.
2. The second is the ability of bound complement to cause hemolysis of sheep (RBCs coated with anti-sheep RBC antibody ([sensitized RBCs]).

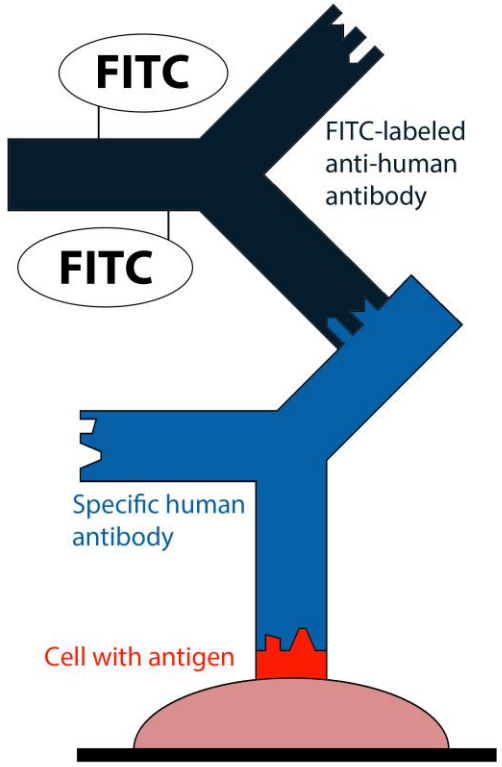
Complement Fixation

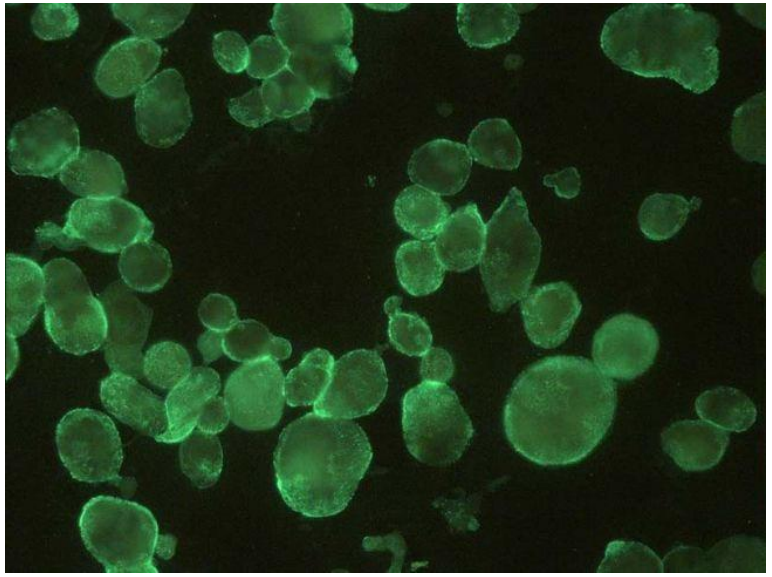


Labeling Methods

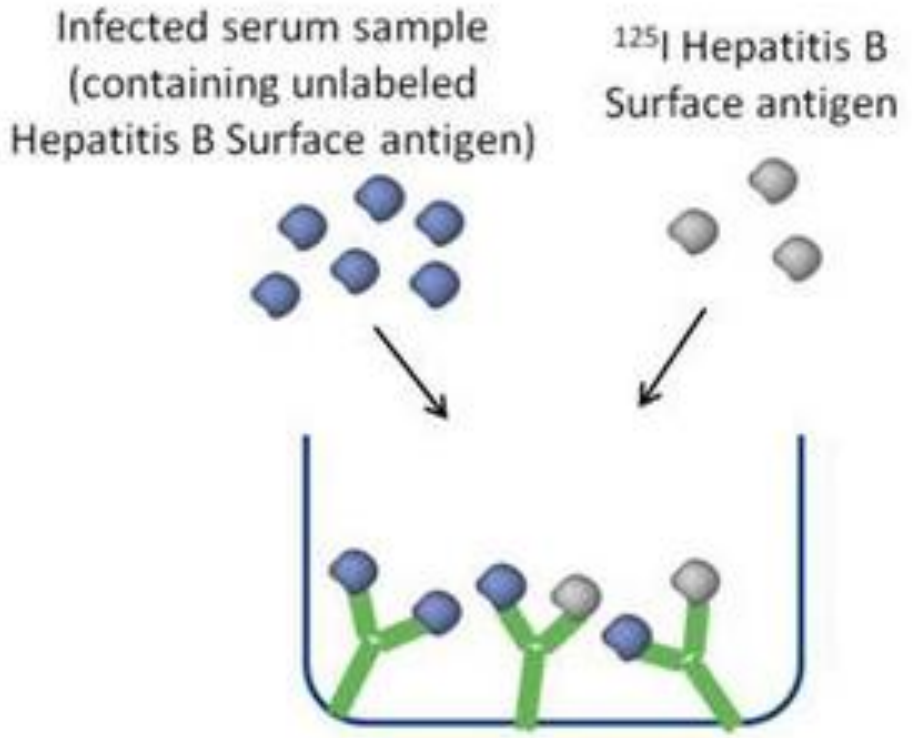
- Detection of antigen–antibody binding may be enhanced by attaching a label to one (usually the antibody) and detecting the label after removal of unbound reagents. The label may be a fluorescent dye (immunofluorescence), a radioisotope **(radioimmunoassay,** or **RIA),** or an enzyme **(enzyme immunoassay,** or **EIA)**. The presence or quantitation of antigen–antibody binding is measured by fluorescence, radioactivity, or the chemical reaction catalyzed by the enzyme.

Immunofluorescence

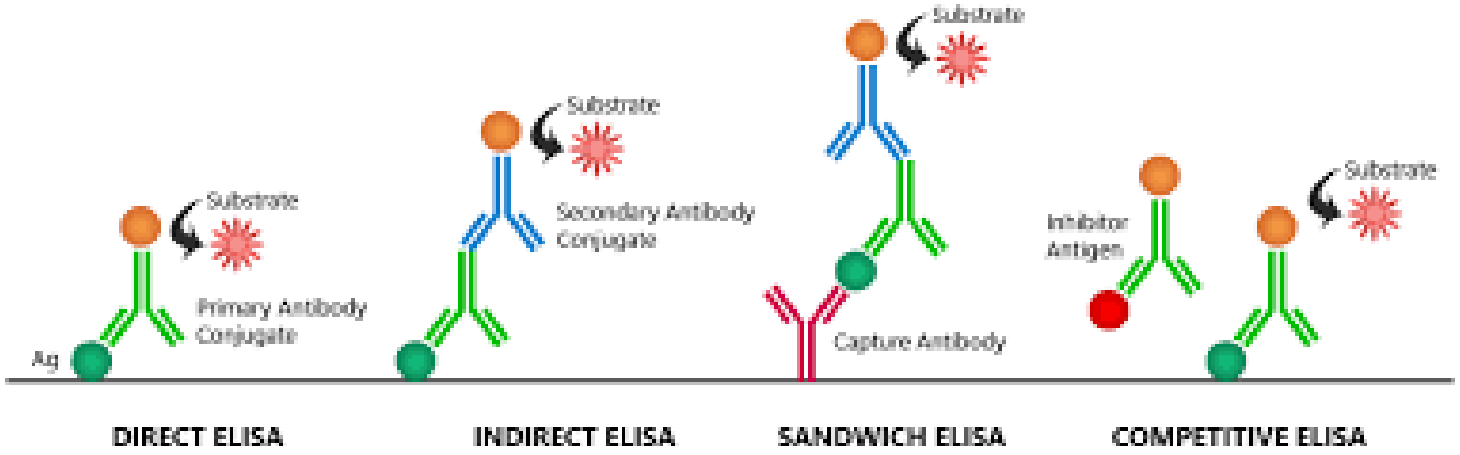




Radioimmunoassay



Enzyme Immunoassay



Western Blot

- Its greatest use has been in the diagnosis of HIV infections, in which virions are electrophoresed in a polyacrylamide gel to separate the protein and glycoprotein components and then transferred onto nitrocellulose. This is then incubated with patient serum, and antibody to the different viral components is detected by using an antihuman globulin IgG antibody conjugated with an enzyme label.

Western Blot