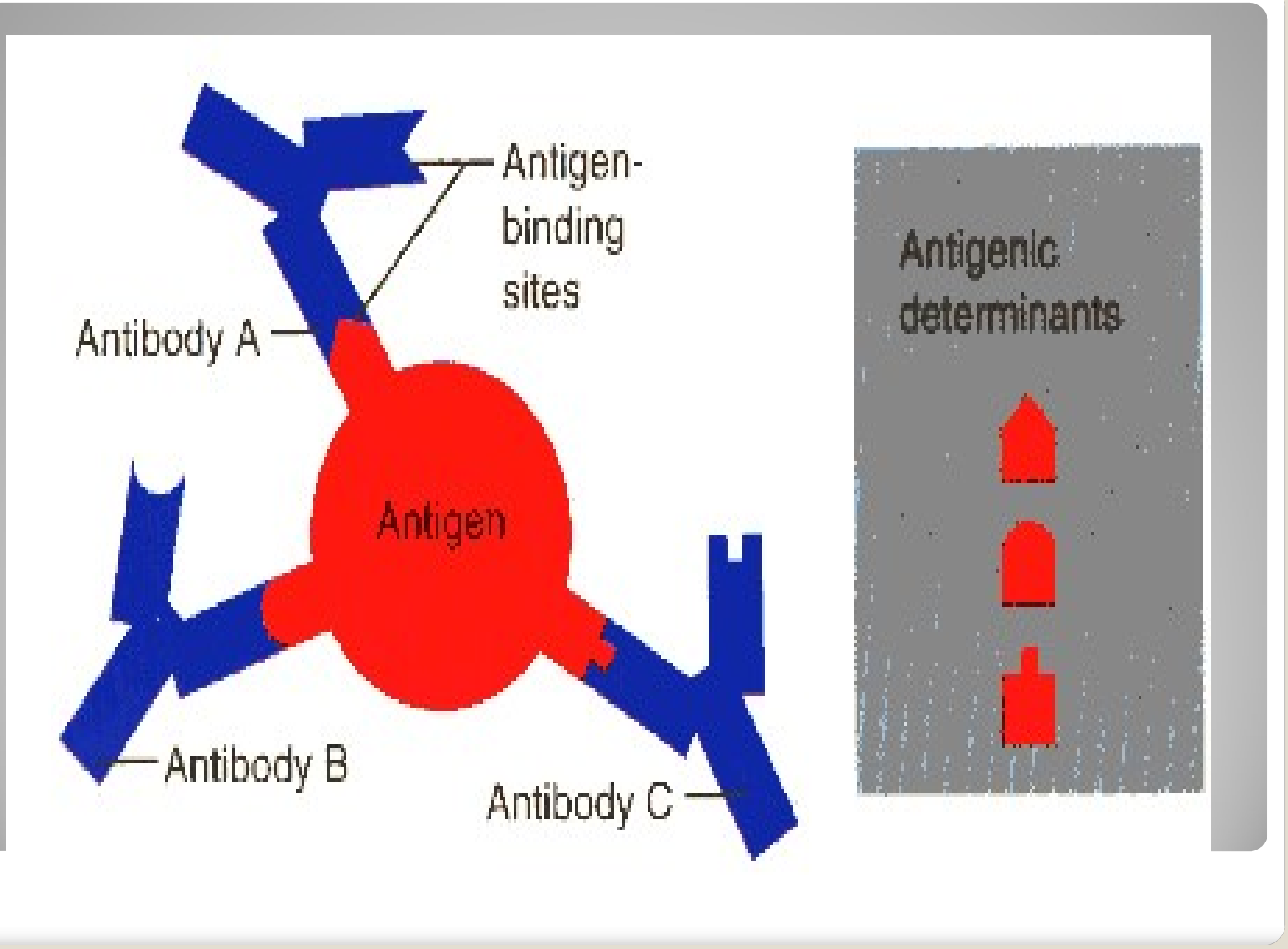
Serological Reaction

Dr.Eman Tariq Ali
(Immunity)

- **Serology**: Is the science of measuring antibody or antigen in body fluids. The immune reaction is the production of antibody (substances) that protect the body against the antigen.
- **Serologic reactions**: that are in vitro Antigen-antibody reactions provide methods for the :
- Diagnosis of disease .
- Identification and quantitation of antigens and antibodies.

- **Simple serological techniques are called simple, because**:
- these procedures involving direct demonstration and observation of reactions,
- they do not require the participation of accessory factors such as; indicator system, or specialized equipment.
- Some examples of these tests are the agglutination reactions, and precipitation reactions

- **Antigens**
- A molecule which can be specifically bound by an antibody (typically a protein or carbohydrate recognized as "foreign").
- **Antigen** is substance which when introduced parentally into the body stimulates the production of an antibody with which it reacts specifically and in an observable (fever, rash).
- The antigens may classified according to their immunogenicity & immunoreactivity into:
- **I-Complete antigen** : substances with both immunogenicity and immunoreactivity By convention , we call complete antigen as antigen.
- **II- Incomplete antigen (hapten):** substances only with immunoreactivity Hapten +carrier complete antigen (immunogen) Hapten: Only possess immunoreactivity Carrier: Make Hapten obtain the immunogenicity B

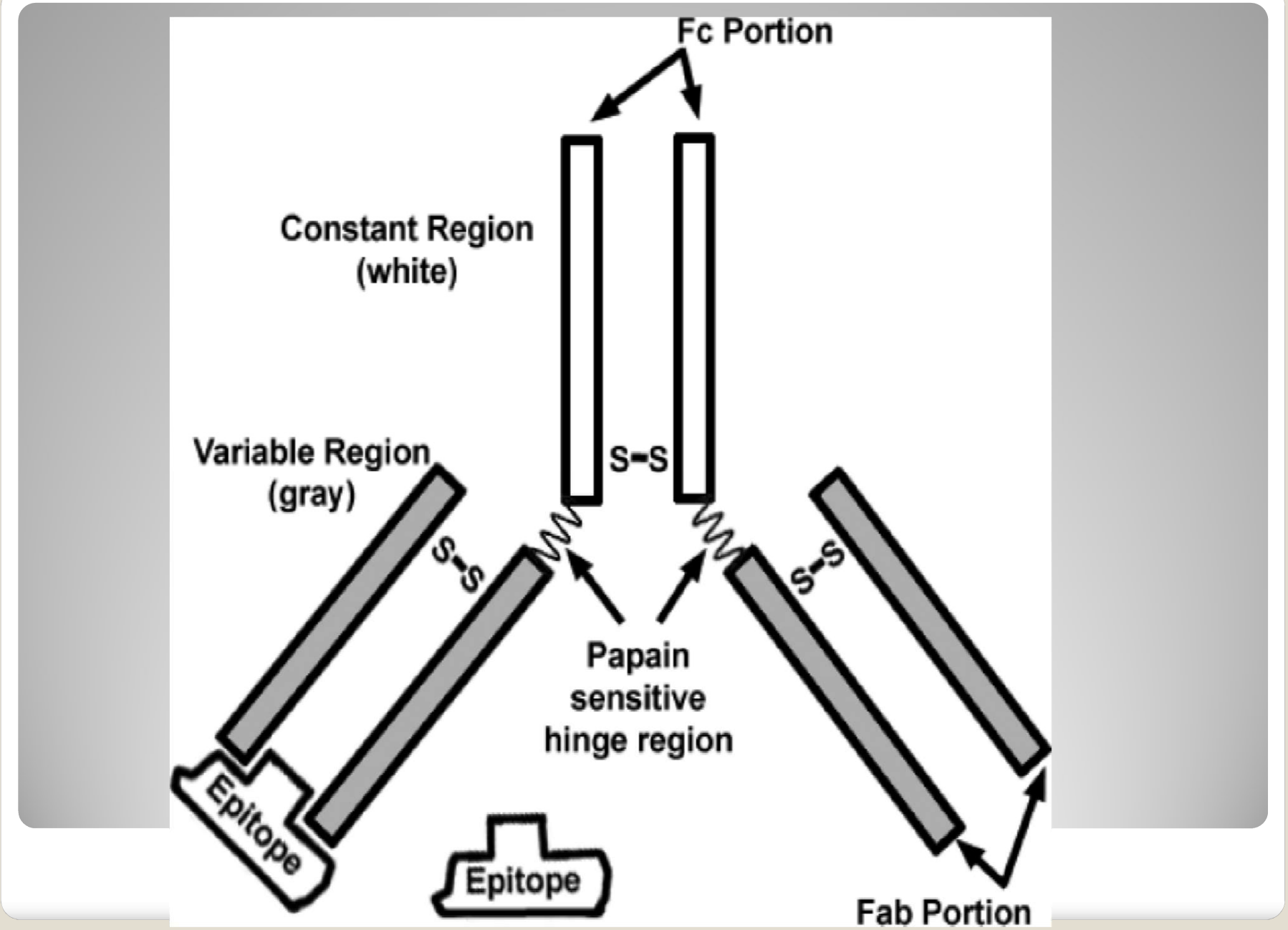

Antigen-
binding
sites
Antibody A
Antigen
Antibody B
Antibody C
Antigenic
determinants

- # Antibodies

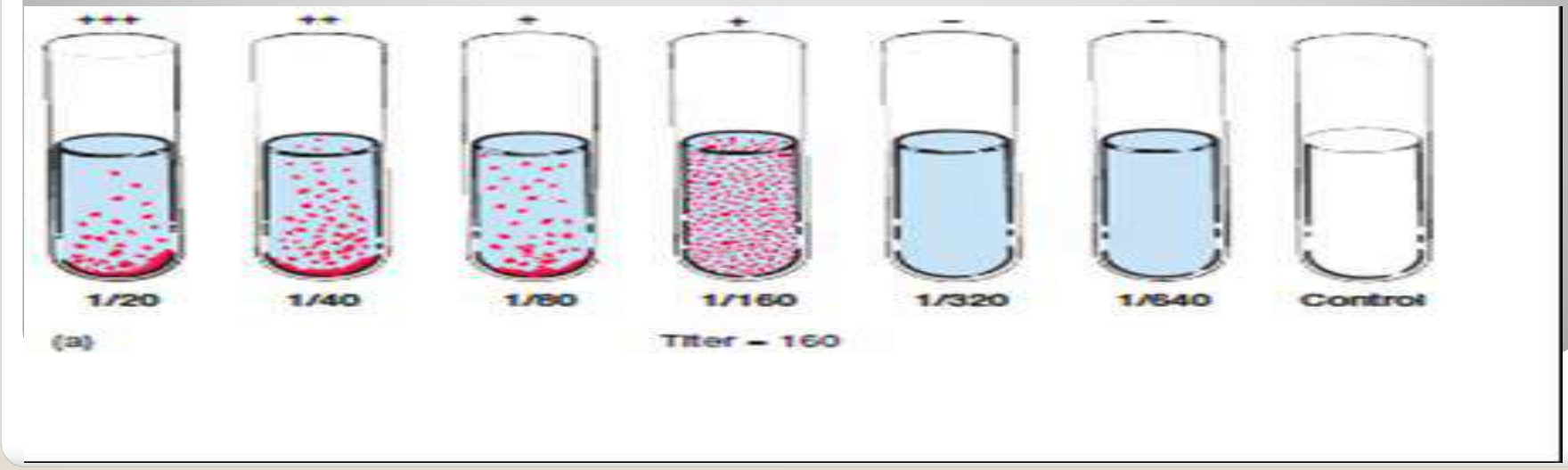
- **Antibody Molecules :**Also known as an **immunoglobulin** (Ig), is a large, Y-shape protein produced by plasma cells that is used by the immune system to identify and neutralize pathogens such as bacteria and viruses

- •In blood serum of immunised annimals there are specific proteins – immunoglobulins, that bind antigens causing their production (behring, Kitaso, 19 th century)
- •Serum – liquid that remains at the top of the tube after centrifugation of coagulated blood
- •Plasma – liquid that remains at the top of tube after centrifugation of not coagulated blood

- The ability of an antibody to communicate with the other components of the immune system is mediated via its Fc region (located at the base of the "Y"), which contains a conserved glycosylation site involved in these interactions.
- The production of antibodies is the main function of the humoral immune system An immune response generates antibodies or proteins called immunoglobulin (Igs).
- Antibodies are further classified into multiple isotype or classes .
- In immunocytochemistry, the IgG isotype is preferred because its generation and binding is more consistent.
- IgM antibodies can be used if no other isotype is available.
- The IgG molecules can be broken down into four subclasses, IgG1, IgG2, IgG3, and IgG4


Fc Portion
Constant Region
(white)
Variable Region
(gray)
S-S
S-S
S-S
Papain
sensitive
hinge region
Epitope
Epitope
Fab Portion

- **Titer**
- is a way of expressing concentration
- •Titer testing employs serial dilution to obtain approximate quantitative information from an analytical procedure
- •The titer corresponds to the highest dilution factor that still yields a **positive reading**



- **Factors affecting (antigen antibody reaction)**
- Many factors influence antigen-antibody reactions. They can be conveniently classified in two groups according to whether they act on the equilibrium constant or not:

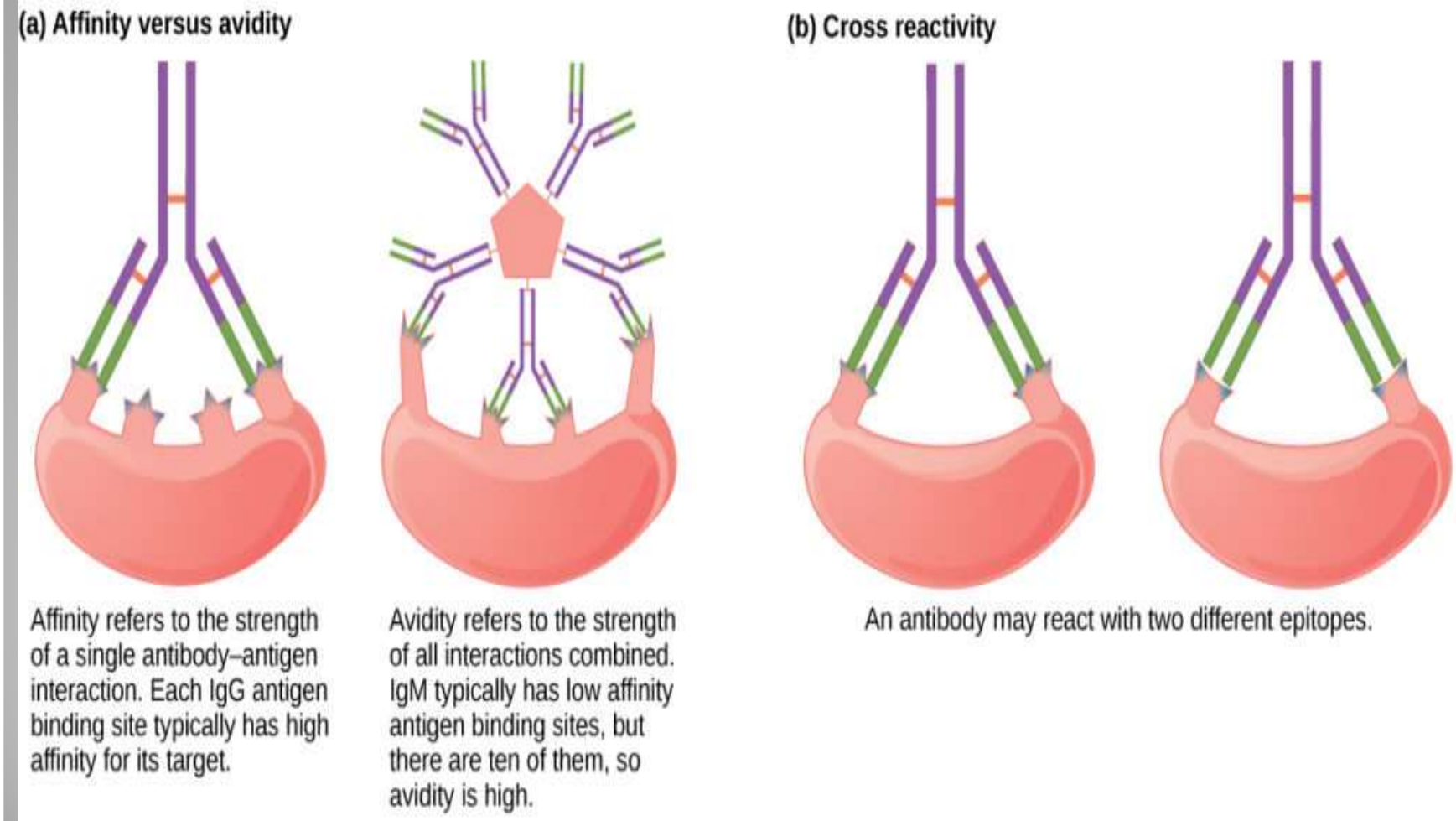
✓ Factors acting on the equilibrium constant.

✓ Other factors on the antigen antibody reaction.

Table IV - Factors affecting the antigen-antibody reaction

Factors acting on the equilibrium constant
- Temperature
- pH
- Ionic strength
- Enzyme treatment of red cells
Other factors
- Concentrations of antigen and antibody
- Zygosity (number of antigen sites per cell)
- Duration of incubation

- **Other factors on the antigen antibody reaction**



- **Sensitivity = a / a+c**
- = a (true positive) / a+c (true positive + false negative)
- = Probability of being test positive when disease present.
- **specificity = d / b+d**
- = d (true negative) / b+d (true negative + false positive)
- = Probability of being test negative when disease absent

	Disease present	Disease absent
Test positive	a (TP)	b (FP)
Test negative	c (FN)	d (TN)
	Sensitivity: a/ (a+c)	Specificity: d/ (b+d)

TP: True positive, FP: False positive, FN: False negative, TN: True negative

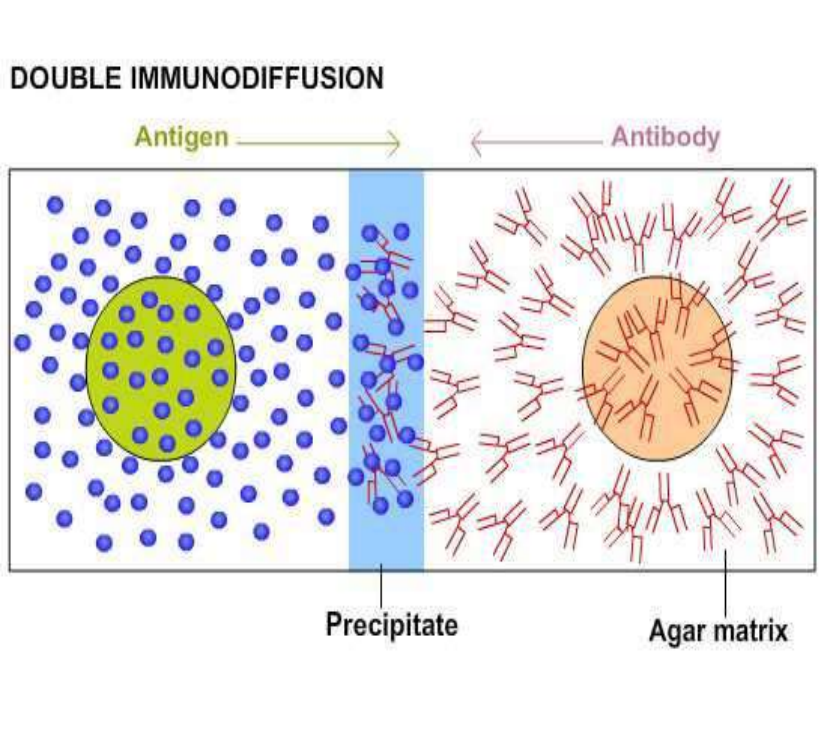
- **I-primary binding tests**
- Primary binding tests are tests that directly measure the binding of antigen and antibody (i.e.; directly measure or visualize the immune complex).
- They are the most sensitive techniques in terms of the amount of detectable antigen or antibody.
  - Immunofluorescence tests
  - Enzyme linked immunosorbent assay(ELISA) .
  - Radioimmunoassay (RIA)
  - Radioimmunodifusion (RID

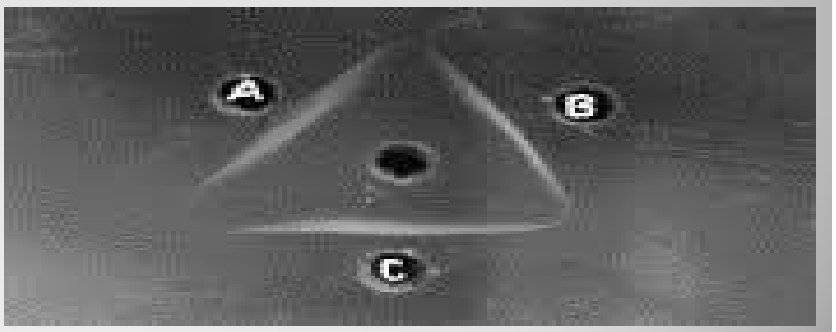
- **II-Secondary binding tests**
- Secondary binding tests are tests that detect and measure the consequences (secondary effect) of antigen-antibody interaction.
- These consequences include:
  - Precipitation of soluble antigens
  - Clumping (agglutination) of particulate antigens
  - Complement fixation test
  - They are usually less sensitive than primary binding tests, but may be easier to perform

Serological reactions

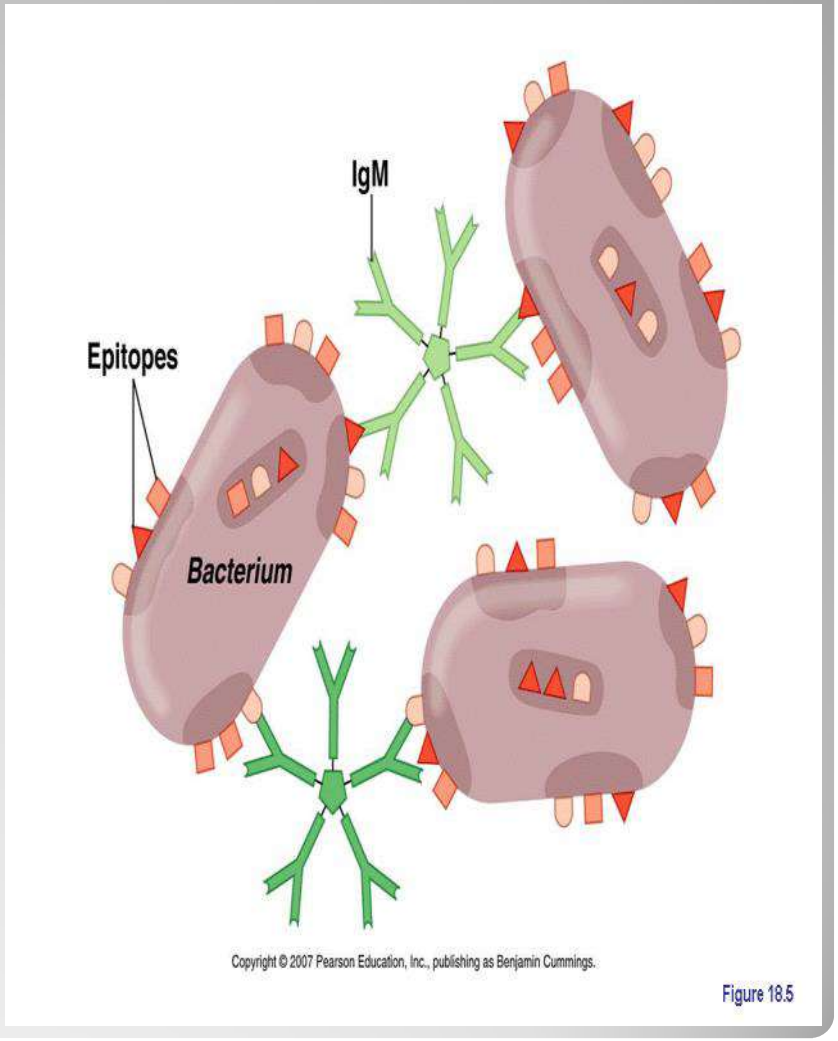
- •Agglutination – antigen + dilution of serum = visible agglutination
- •Latex agglutination – antibody bound on latex particles + Ag = big agglutination
- •Precipitation – Ag+Ab = ring in the touch zone
- •Immunodiffusion – diffusion of Ag and Ab in agar. In the meeting point – line of precipitation
- •Hemagglutination – passive agglutination – ag is bound on the surface of RBC
- •CF – complement fixation- Ag +Ab +C´+ Ery + antieryab –lysis

- **Immunodiffusion**
- procedures are precipitation reactions carried out in an agar gel medium.
- •Antibody and antigen are loaded in different wells and diffuse through the medium.
- •antigen-antibody - visible band appears in the gel.





- **Agglutination**
- 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



- **Types of agglutination reaction Direct agglutination.**

- Corpuscular antigen-agglutinogen
- Antibody - agglutinin
- To test patient's sera (contain antibody) against large antigen.

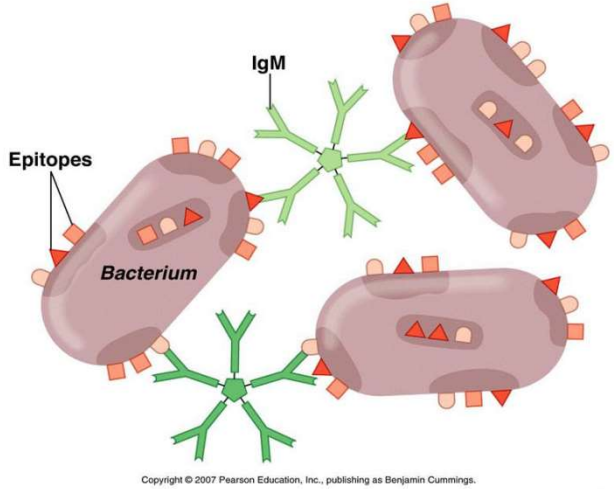
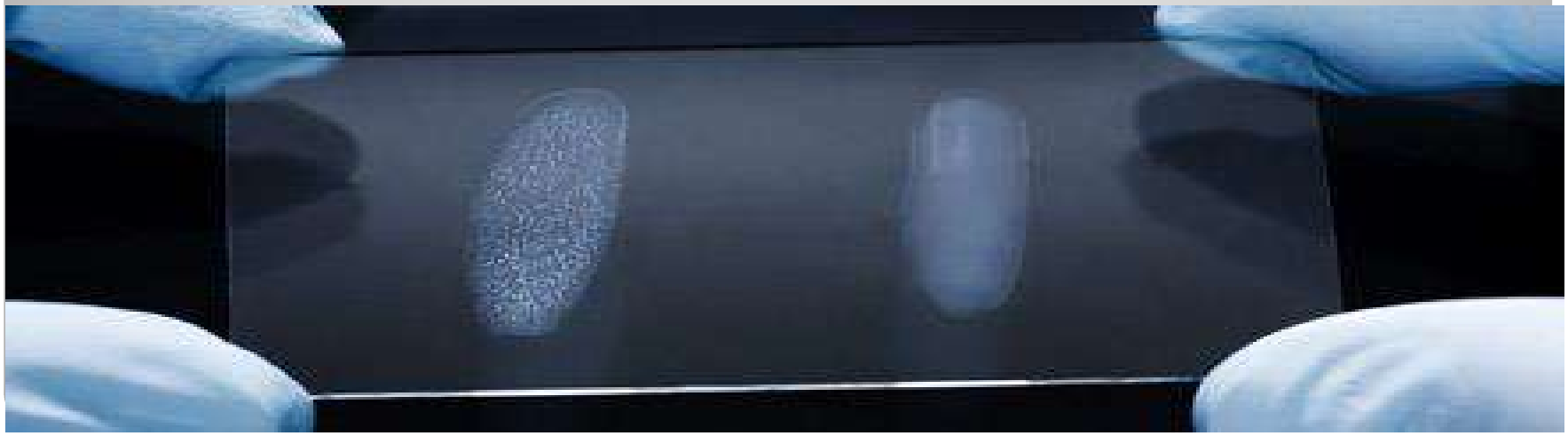
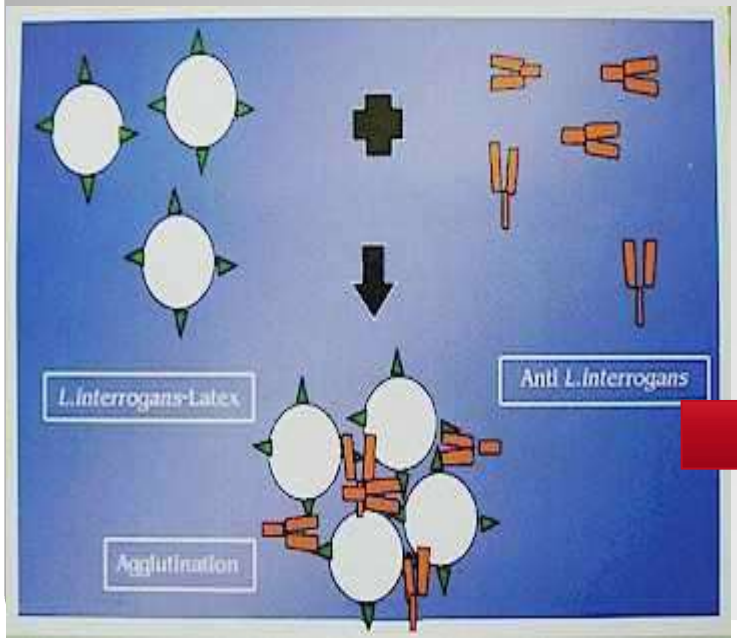


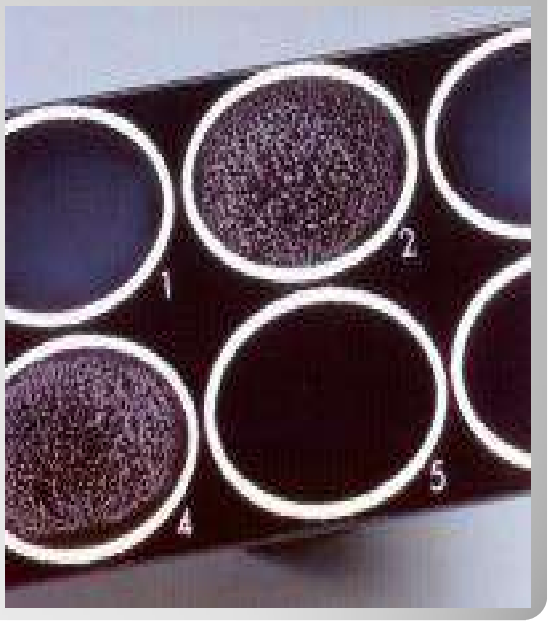
Figure 18.5

- Slide agglutination
- a rapid screening test in which antibody and antigen are mixed on a glass slide and observed for agglutination

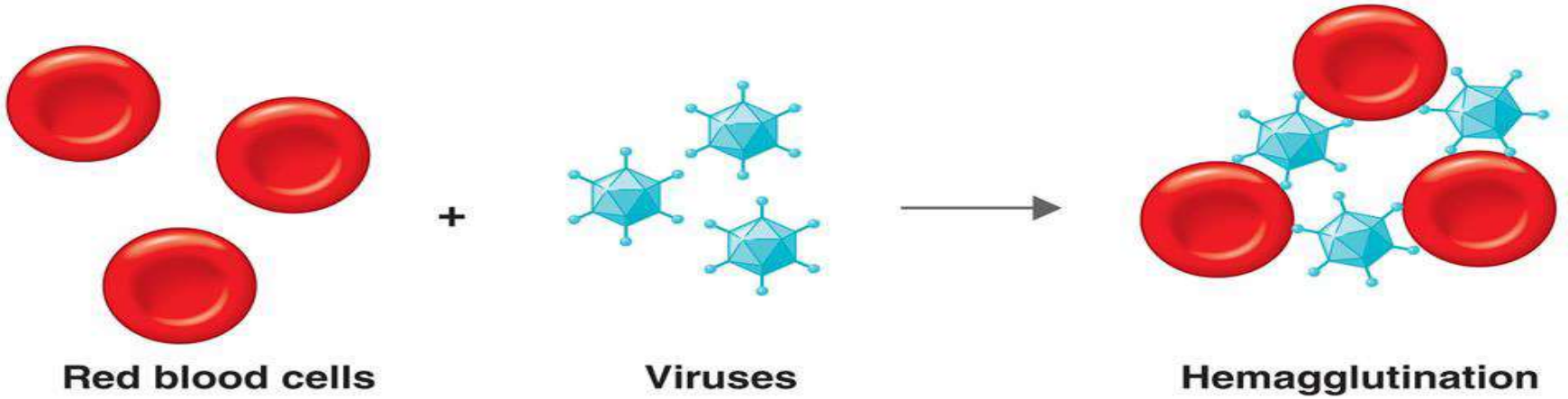


- **Latex agglutination test**
- •sample is mixed with latex beads coated with antigens

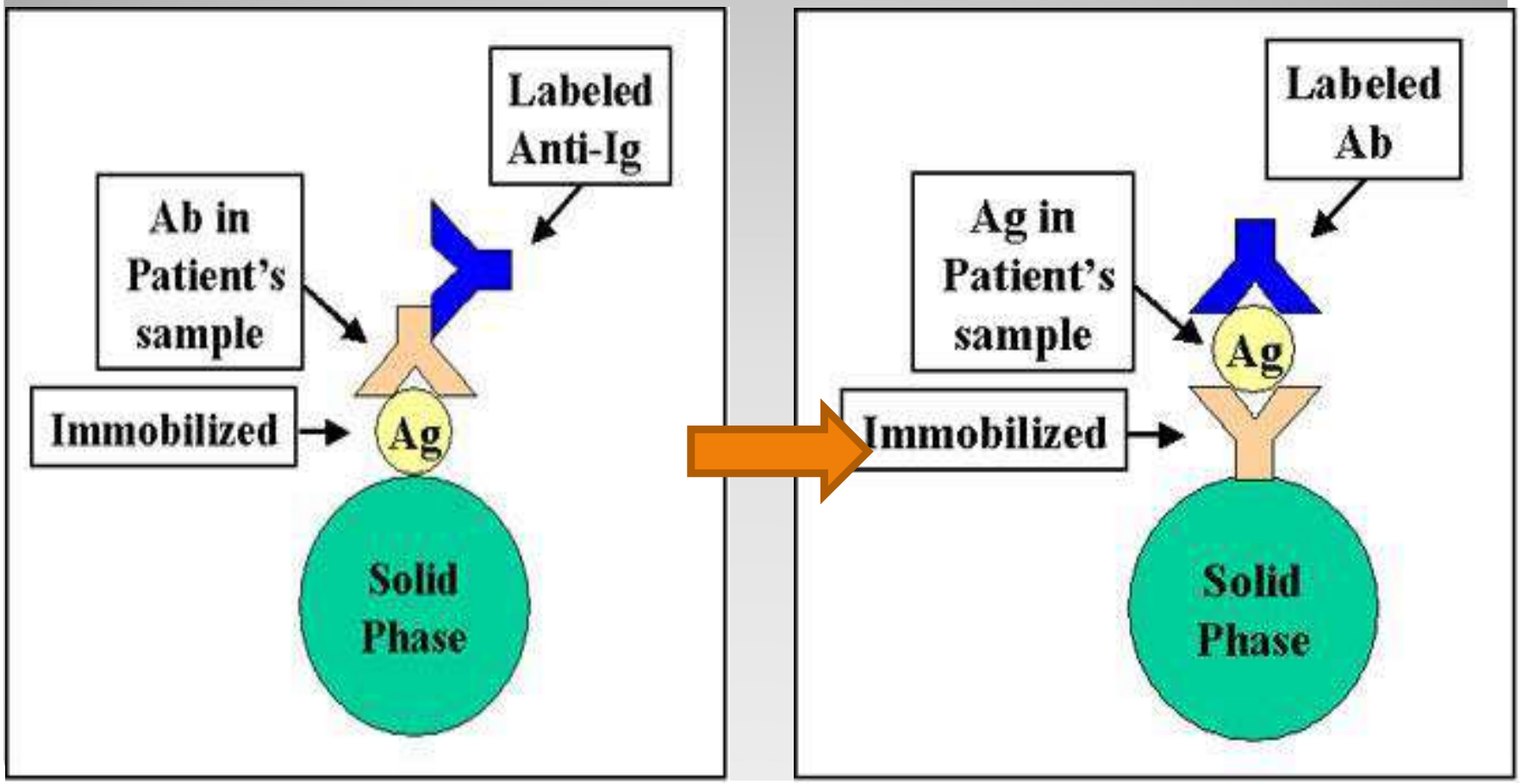




- **Hemagglutination**
- –Hemagglutination reactions involve agglutination reactions using red blood cells.
- –Hemagglutination reactions are used in blood typing, the diagnosis of certain diseases, and the identification of viruses.
- –Viral hemagglutination occurs when spikes on the virus cause agglutination of red blood cells - there is no antigen-antibody interaction



ELISA


Labeled
Anti-Ig
Ab in
Patient's
sample
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Ag in
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sample
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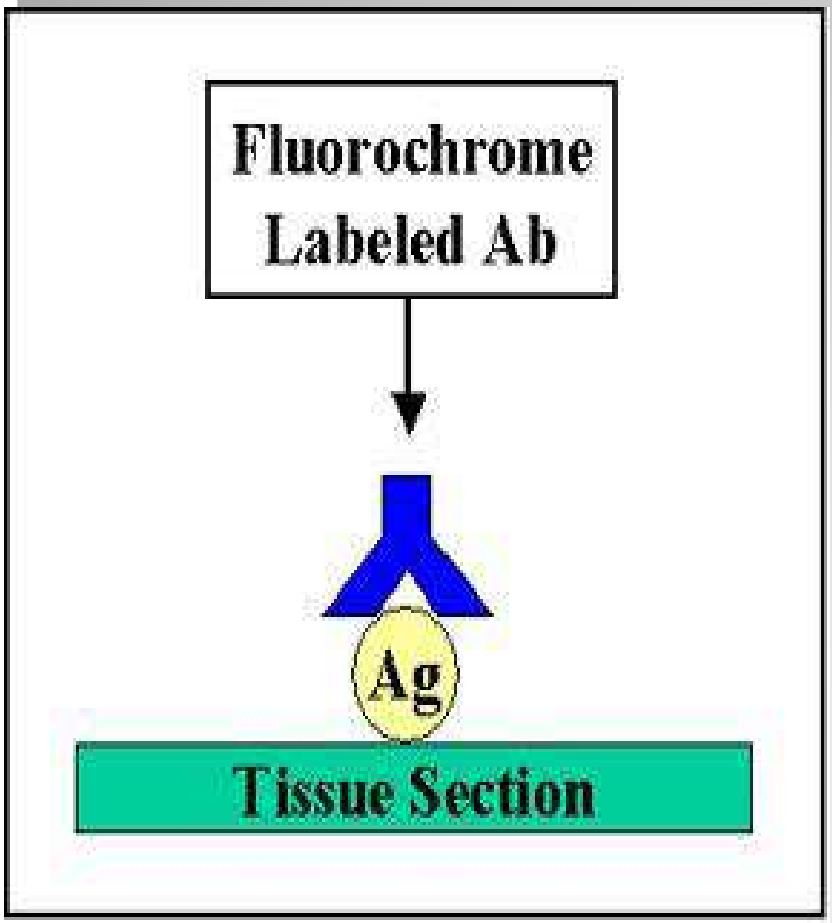
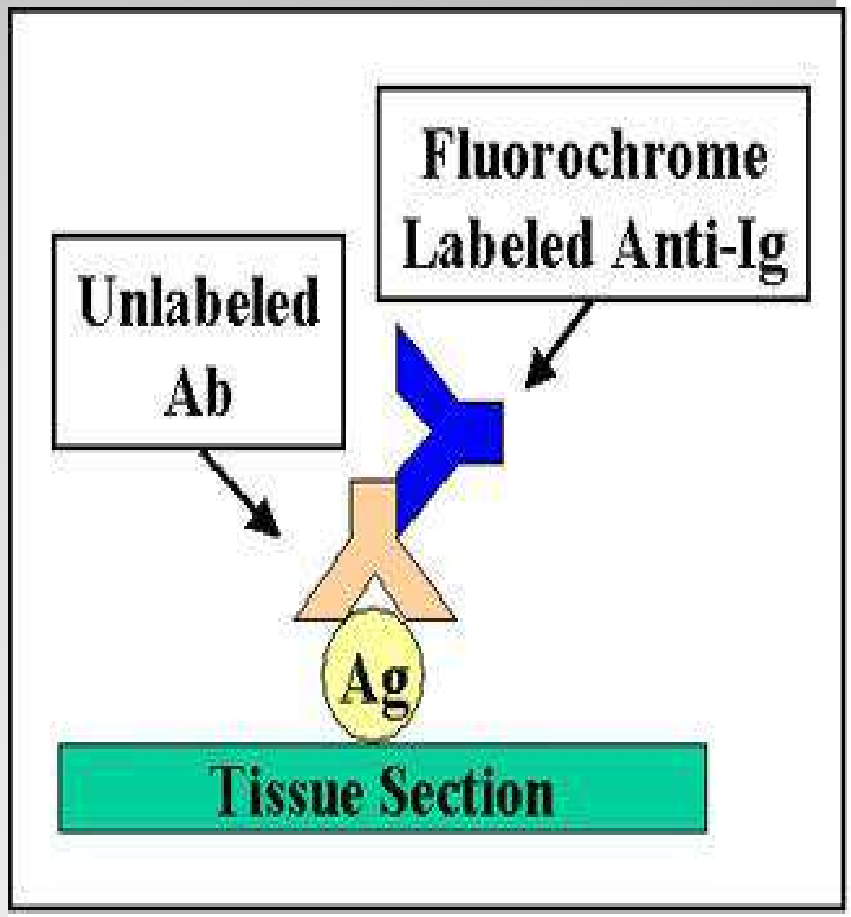
Phase

Immunofluerescence





THANK YOU