

Serological methods for microbial diagnosis

Learning outcome

- **You should be able to:**
 1. Define the term antibody, antigen, Ab titer, prozone.
 2. Understand the different methods used for serological diagnosis, principle and applications.
 3. Specificity VS. sensitivity

Serology

- Can any one differentiate between antibody & antigen?
- *In vitro* reaction between Ab with Ag is called serology
- Specificity VS. sensitivity

Specificity VS. Sensitivity

False positive >>> a cross reaction with another molecule

High specificity prevent false positive

False negative >>> when there are no reaction even though the presence of Ab and Ag

High sensitivity prevent false negative

Different methods in serodiagnosis

1- Precipitation:

- Double diffusion
- single diffusion reaction

2- Agglutination reaction

- Direct (tube and slide agglutination)
- Indirect (Passive)
- Agglutination inhibition reaction

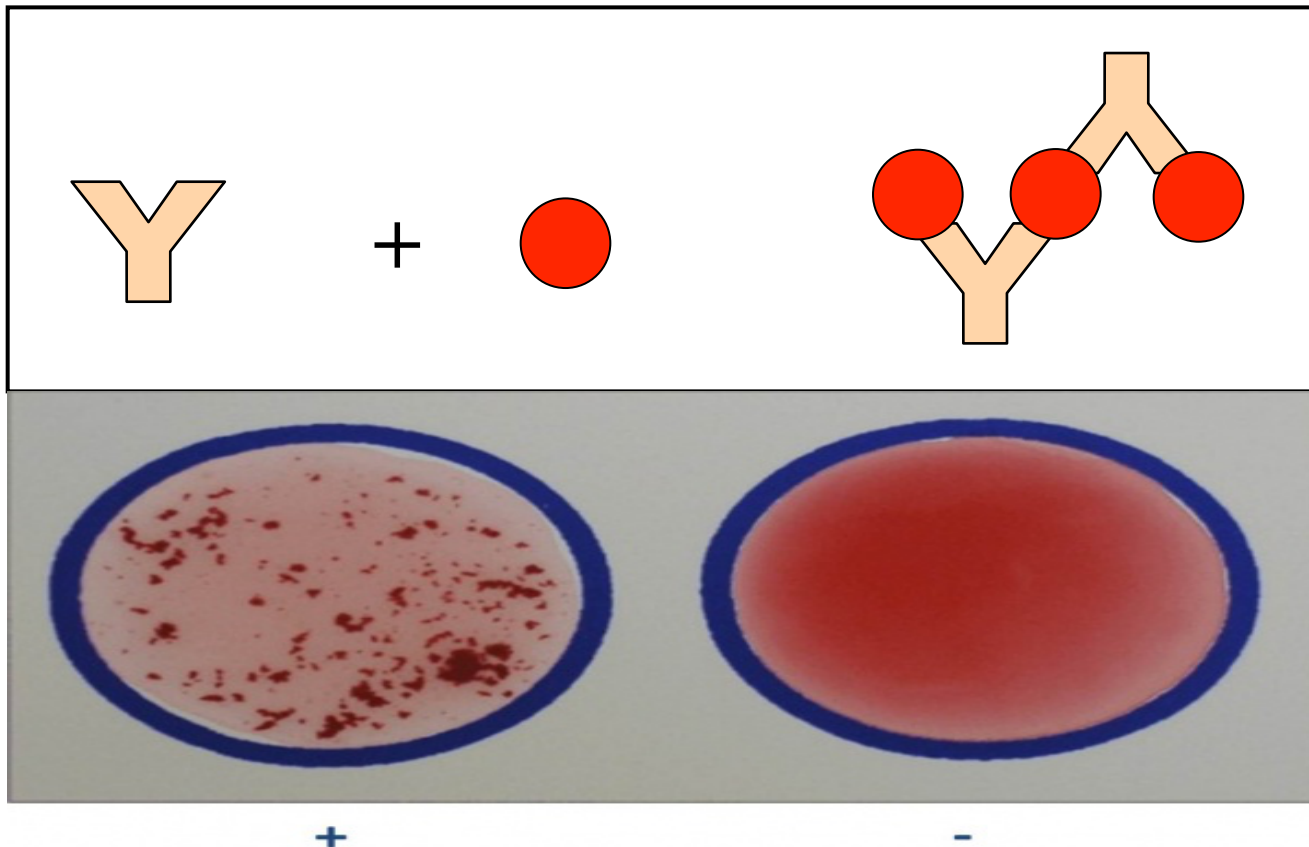
Different methods in serodiagnosis

3- Immunofluorescence

- Fluorescence methods
- Enzyme immunoassay (EIA)
- Radio immunoassay (RIA)

(Slide) Agglutination Reaction

Principle



Slide agglutination Reaction

- A qualitative test.

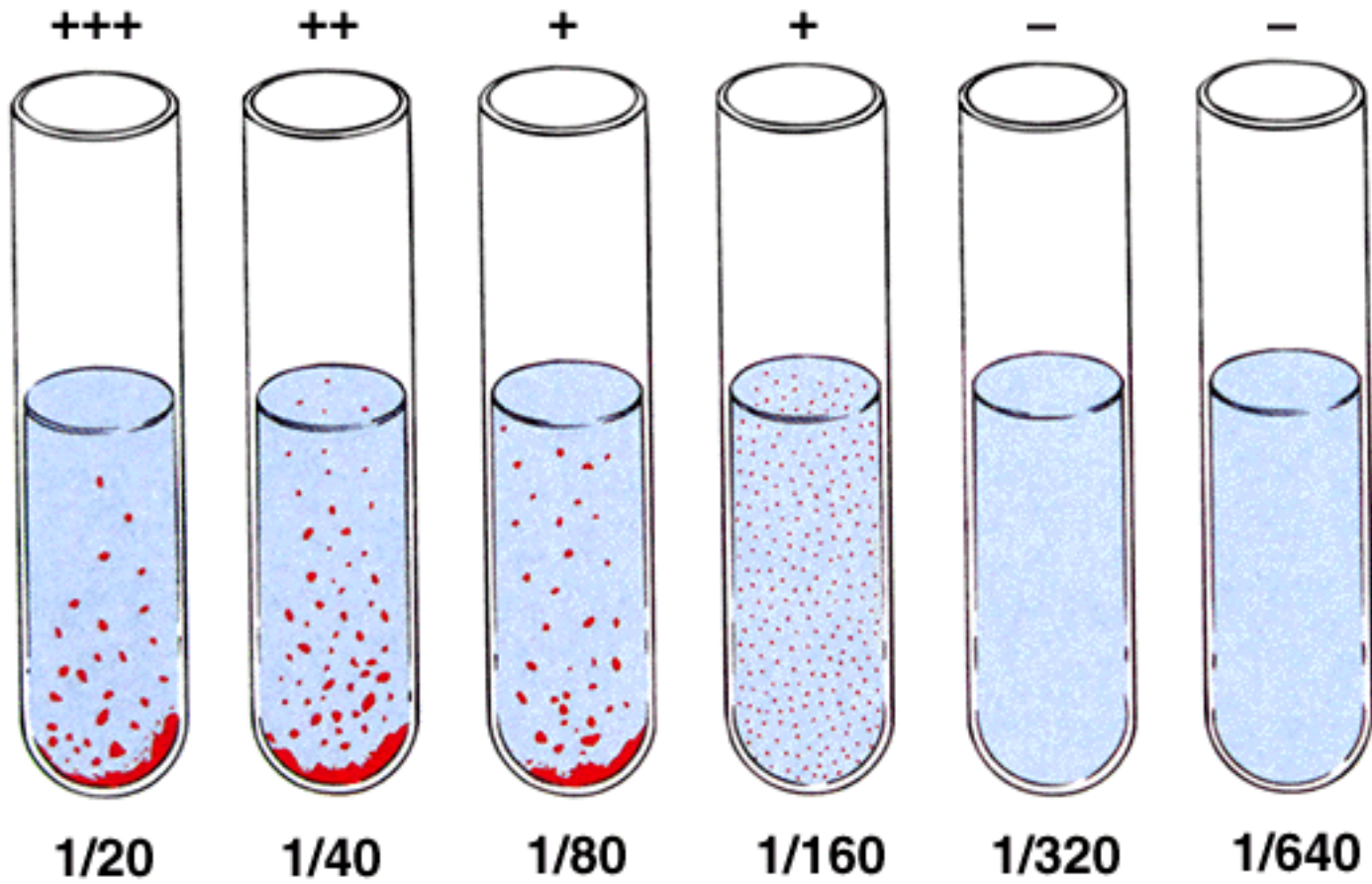
Applications:

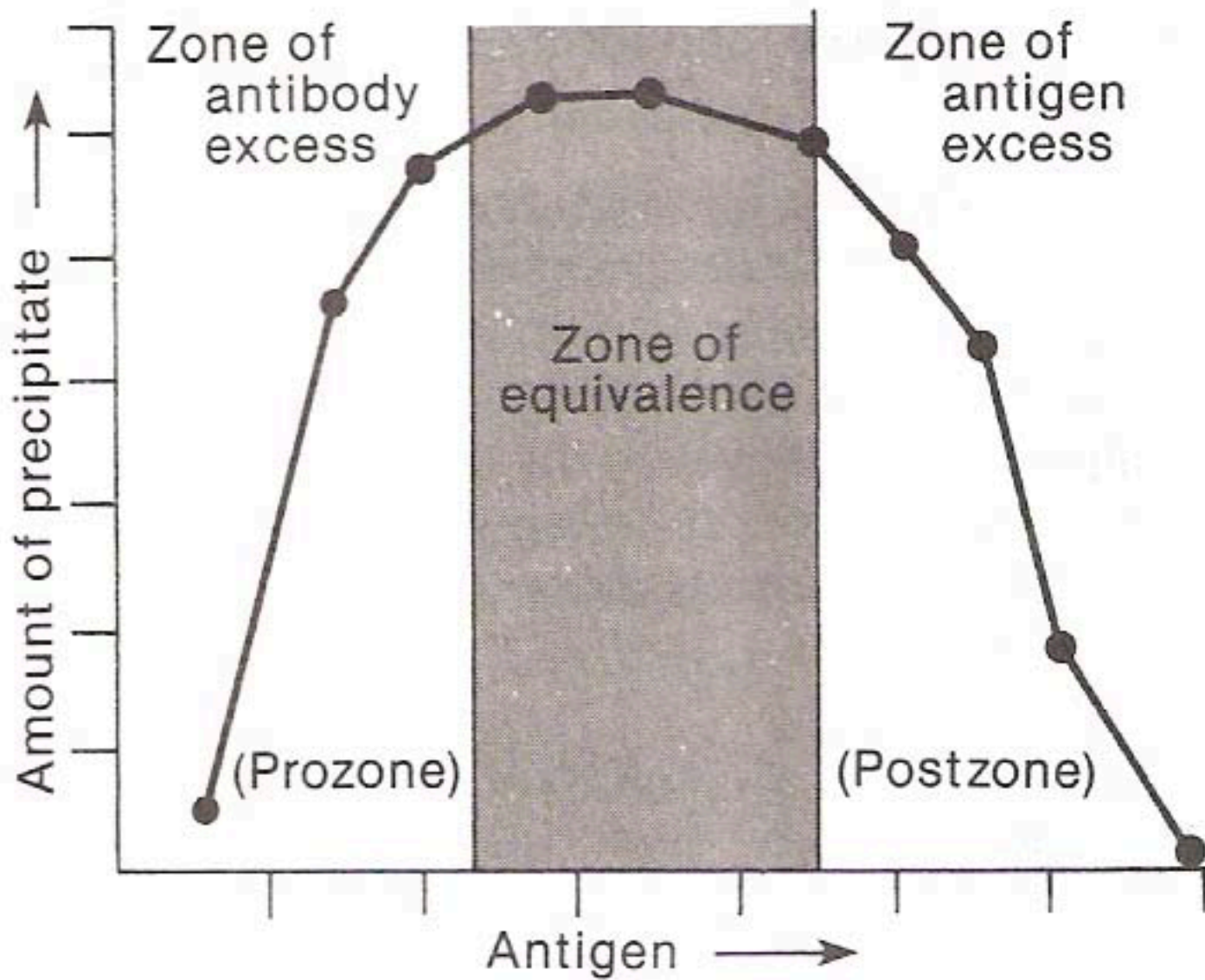
1. It may be used mostly to determine **blood Groups** (ABO types) or
2. if antibody to bacteria or their products is presented in blood as an indication of **bacterial infection.**

Tube or micro-plate reaction

Principle

Patient	1/2	1/4	1/8	1/16	1/32	1/64	1/128	1/256	1/512	1/1024	Pos.	Neg.	Titer
1	●	●	●	●	●	●	○	○	○	○	●	○	64
2	●	●	●	○	○	○	○	○	○	○	●	○	8
3	●	●	●	●	●	●	●	●	●	○	●	○	512
4	○	○	○	○	○	○	○	○	○	○	●	○	<2
5	●	●	●	●	●	○	○	○	○	○	●	○	32
6	○	○	●	●	●	●	●	○	○	○	●	○	128
7	●	●	●	●	●	○	○	○	○	○	●	○	32
8	●	●	○	○	○	○	○	○	○	○	●	○	4





Tube agglutination reaction

- A semi quantitative test

Applications

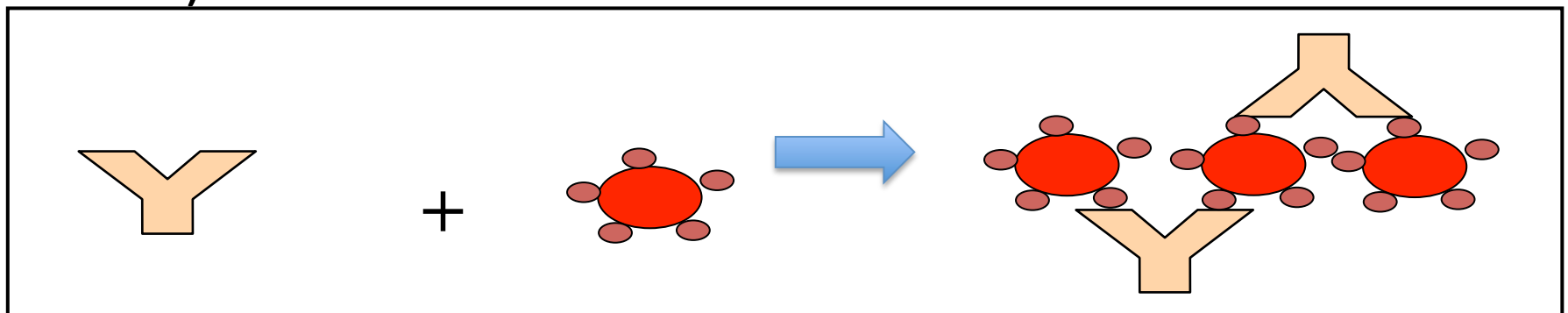
1- Brucellosis screening

2- Widal test: a diagnostic procedure for detection of Salmonella infections, where the presence of antibodies against Salmonella H and O antigens can be demonstrated in the patients' serum. It can help to diagnose Typhoid fever

Passive Agglutination/Hemagglutination

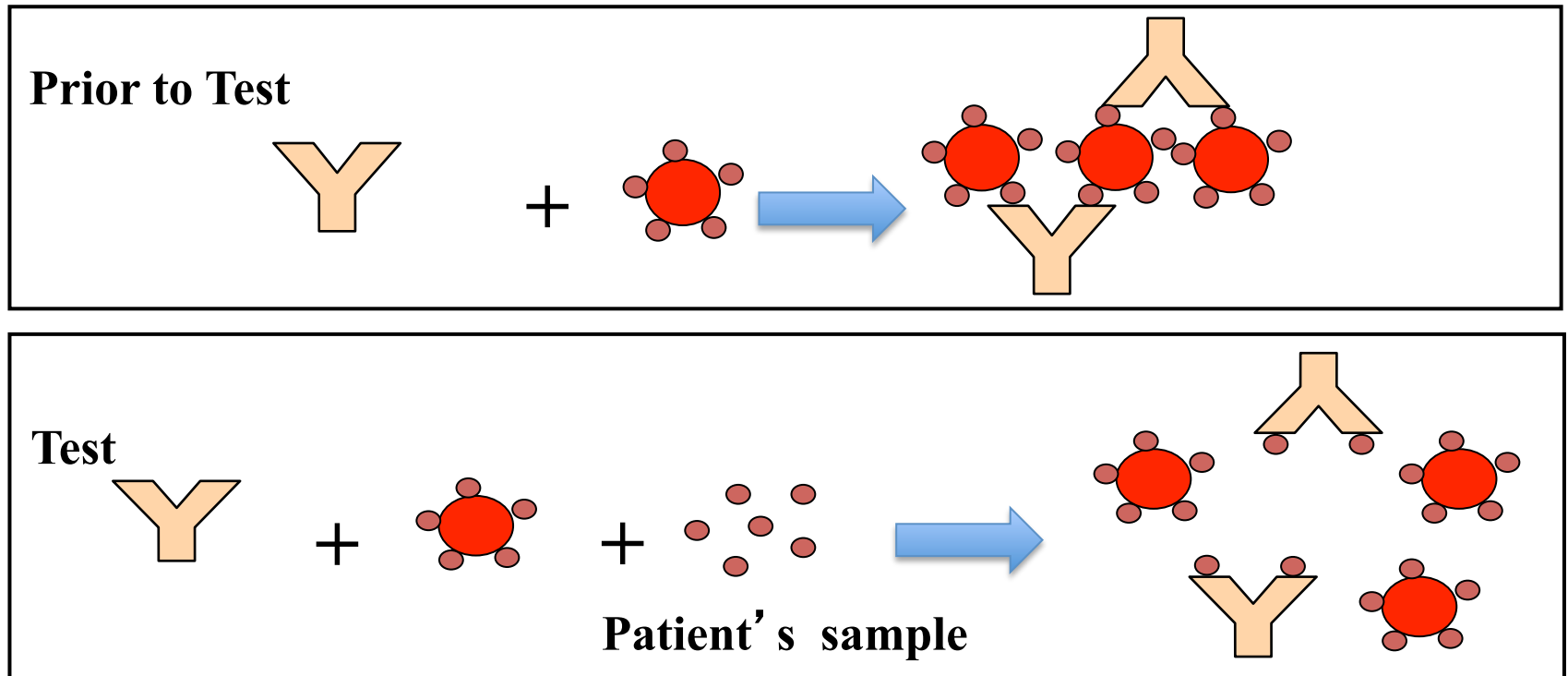
Definition -agglutination test done with a soluble Ag or Ab coated onto a particle (latex beads, charcoal) which serve as inert carrier

Applications Measurement of antibodies to soluble antigens (RF, pregnancy test, Ab to group A St. and CRP)



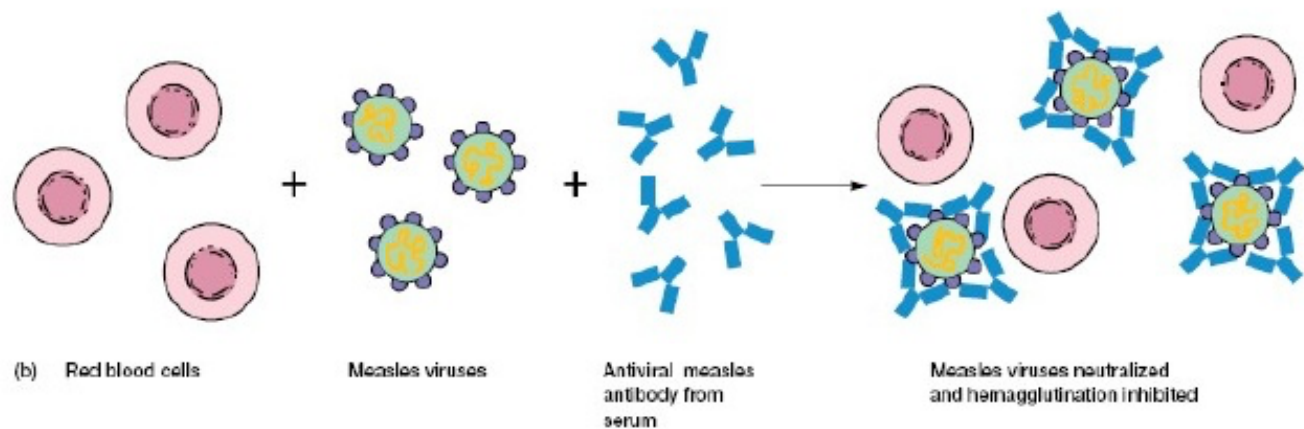
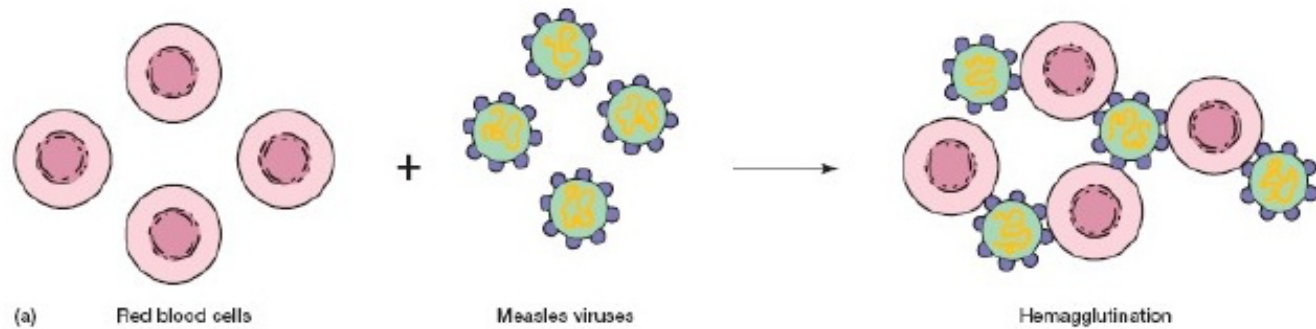
Agglutination/Hemagglutination Inhibition

Definition - test based on the inhibition of agglutination due to competition with a soluble Ag



Agglutination/Hemagglutination Inhibition

- Applications
- Measurement of soluble Ag (Human Chorionic Gonadotropin HCG)
- Widely used for viral diagnosis such as measles, mumps and influenza viruses and others



Viral Hemagglutination.

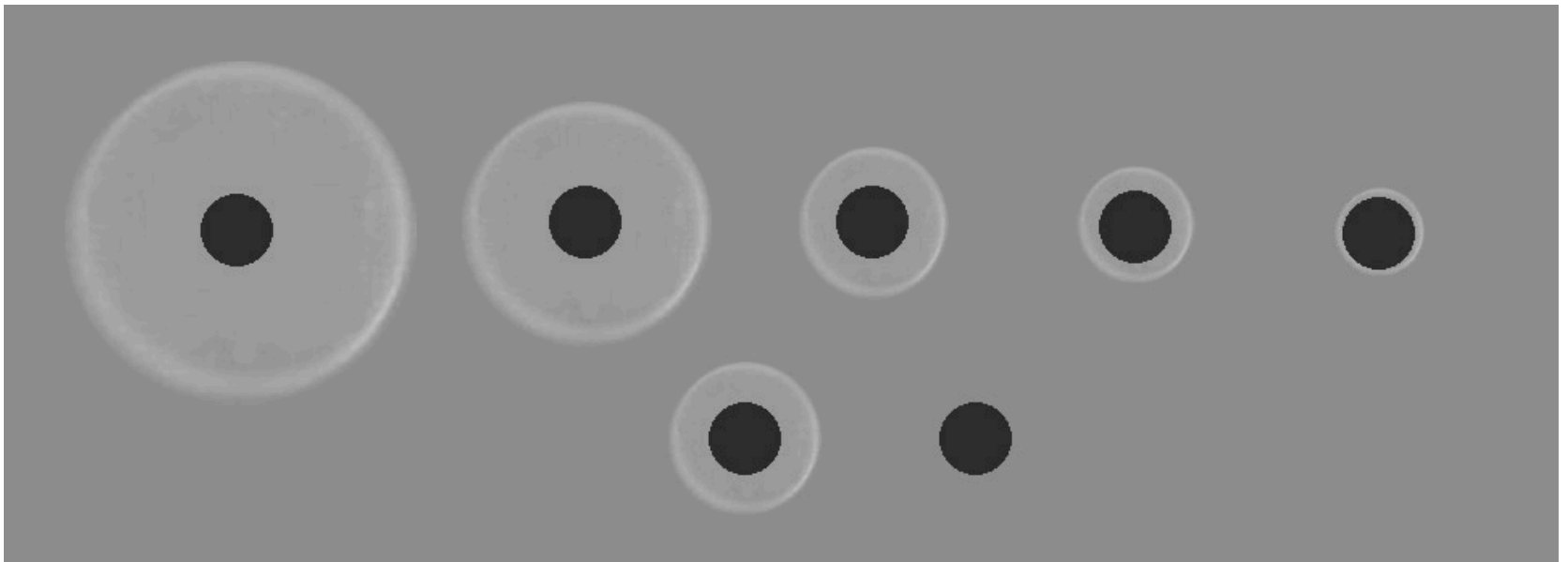
(b) Certain viruses can bind to red blood cells causing hemagglutination.

(c) If serum containing specific antibodies to the virus is mixed with the red blood cells, the antibodies will neutralize the virus and inhibit hemagglutination (a positive test).

Precipitation Reactions

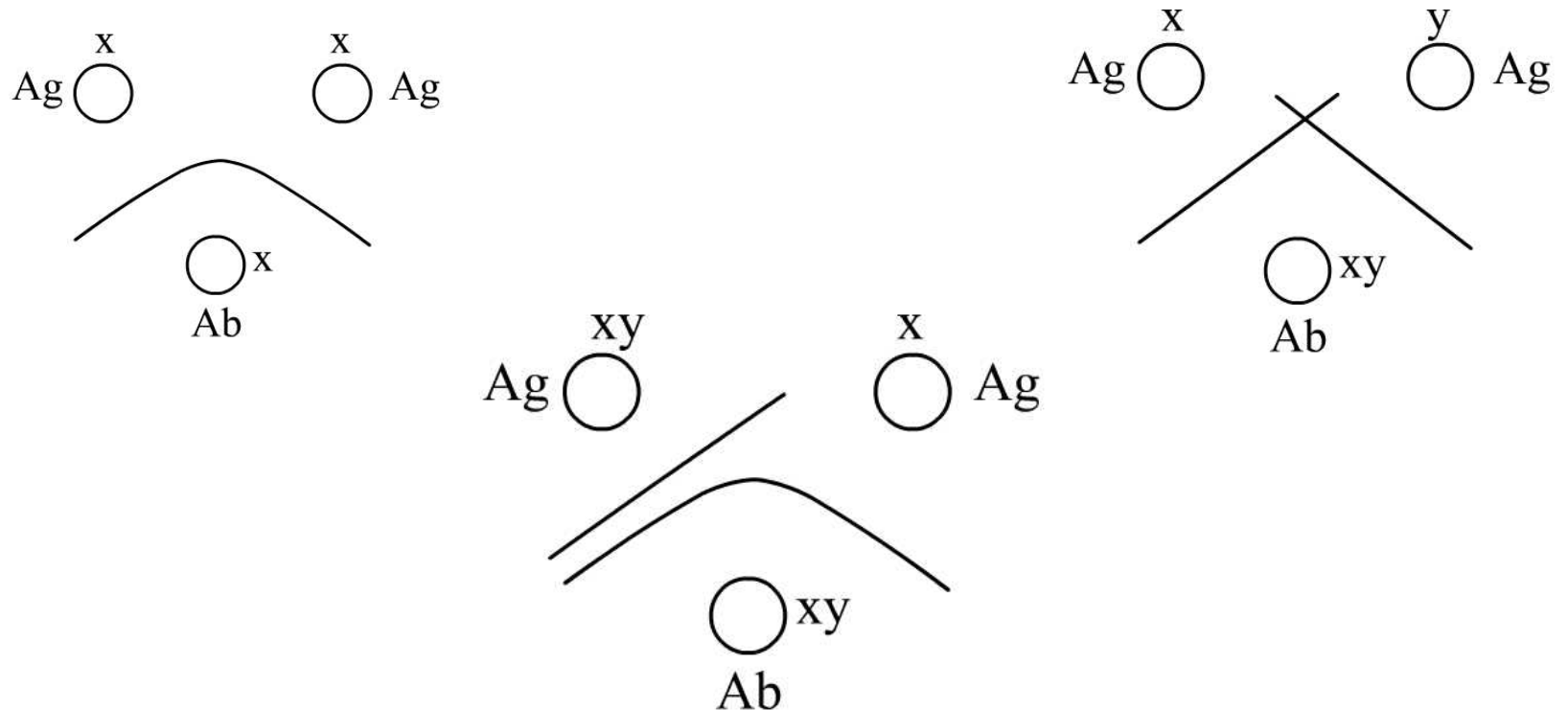
- This reaction occurs when soluble antigen binds its specific soluble antibody and produce visible precipitate (insoluble complex)
- It occurs when a maximally when there is optimal proportion of Ab and Ag
- Can be done in agar and referred to as immunodiffusion

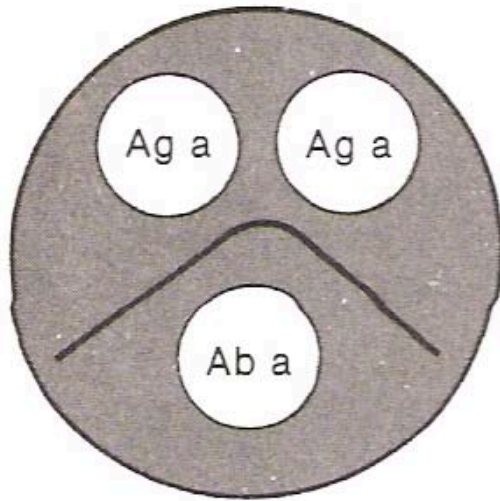
Single Immunodiffusion Reaction



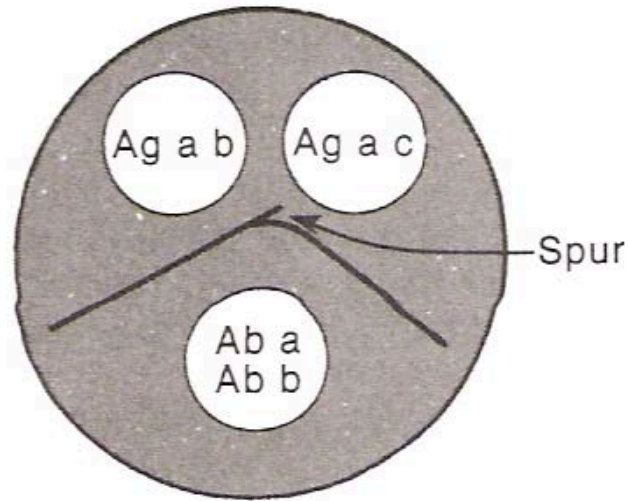
Double Immunodiffusion Reaction

The result is determined by the existence or non-existence of characteristic white band of precipitate

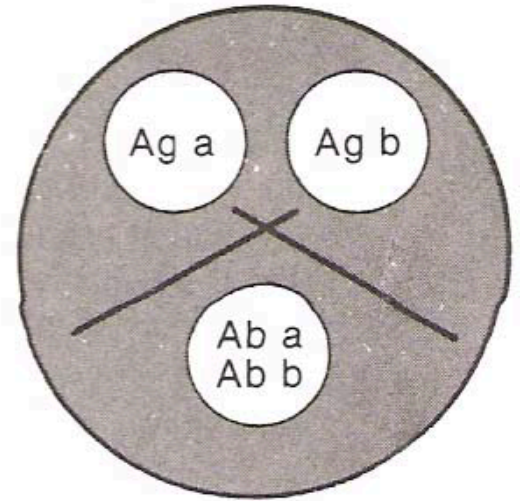




Identity



Partial identity



Nonidentity

**By the end you will be able to
answer these questions**