SEROLOGICAL REACTIONS

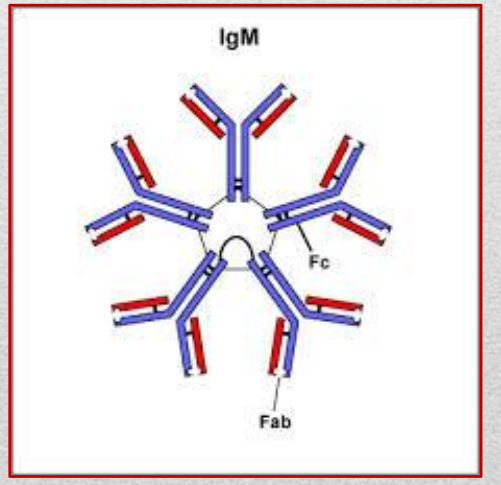
- OBJECTIVES
  - Definition of Antigen and Antibody
  - Antigen-antibody Interactions
  - Serological Reactions
  - Brucellosis Diagnosis

- Antigens are substances that are capable of stimulating an immune response.

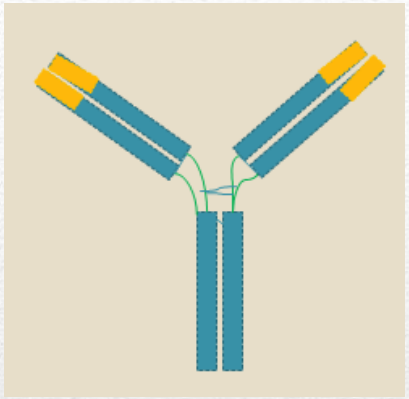
Antigen

Antibody

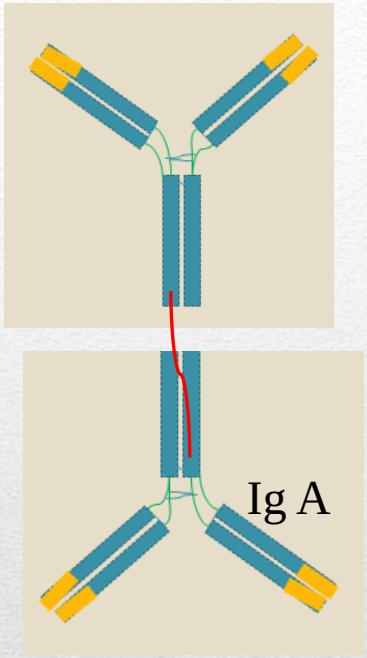
- Antibody is the substance produced in response to introduction of antigen which it reacts specifically in some observable way.



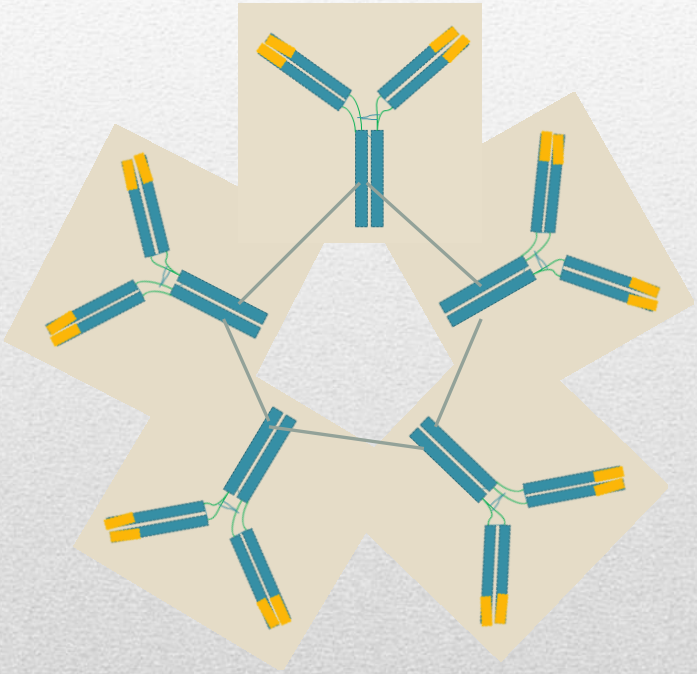
Antibody Types - Structures



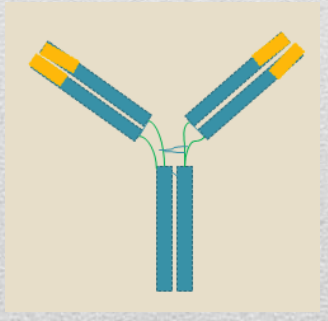
Ig G



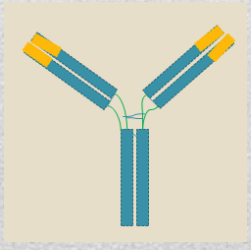
Ig A



Ig M



Ig E



Ig D

- Antibodies are important building blocks of an effective immune response and provides antigen specific immunity to against extracellular pathogens and dangerous soluble proteins such as toxins.
- It is found serum, stomach secretions and in other body fluids such as milk.

Antigen-Antibody Interactions

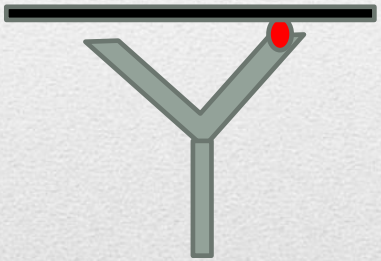
1. Antigen-antibody binding is specific.
2. Antigen-antibody binding is chemical event (weak chemical bonds). Antigens have specific chemical groups that determine their specificity against their antibodies. These chemical groups, named 'epitope', are found in the aminoterminal ends of antibodies and unite specifically with the so-called 'Paratope' merger zones.
3. Electrolytic medium is required for antigen-antibody binding.
4. Antigen and antibody bind at different ratios. Bonds can occur in one or more regions depending on the degree of closeness of the two molecules.

Epitope = Antigenic determinant

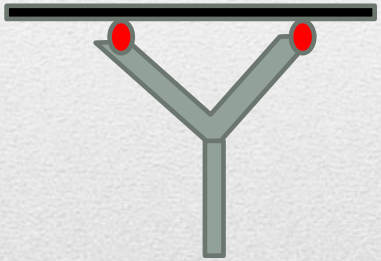
Antigen binding site = Paratope

- These are 2 terms related to antigen-antibody binding strength.

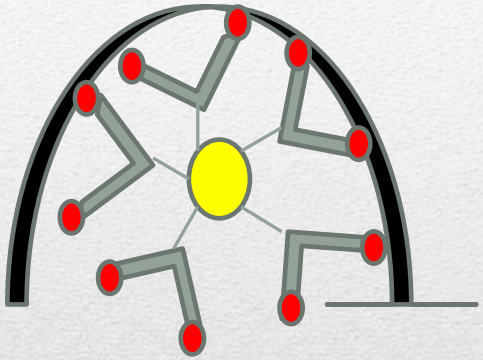
- Affinity measures the strength of interaction between an epitope and paratope.
- Some isotypes of antibodies are multivalent and bind to multiple antigens. The strength of that *overall* connection is the avidity.



Affinity



Affinity



Avidity

- Serological reactions are interactions between antigen and antibody in vitro conditions.
- These methods are based on antigen-antibody specific binding.

(Detection of suspected antigen with known antibody or suspected antibody with known antigen)

In vitro serological responses can be applied for 2 purposes.

1. Laboratory diagnosis of infectious diseases
   a) Direct etiological diagnosis
   b) Indirect etiological diagnosis

2. Recognition of microorganisms and their antigens

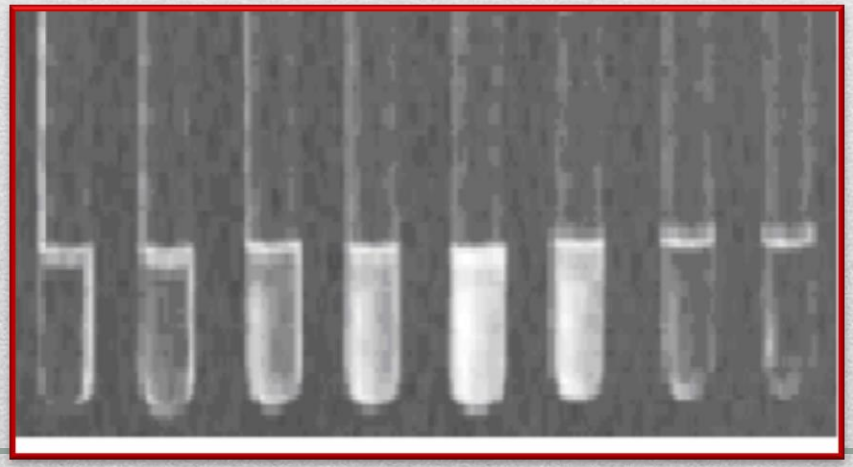
It is used to distinguish primary and secondary infection.

IgG (-)
IgM (+)
↓
Newly acquired infection

IgG (+)
IgM (-)
↓
Previously passed and acquired immunity

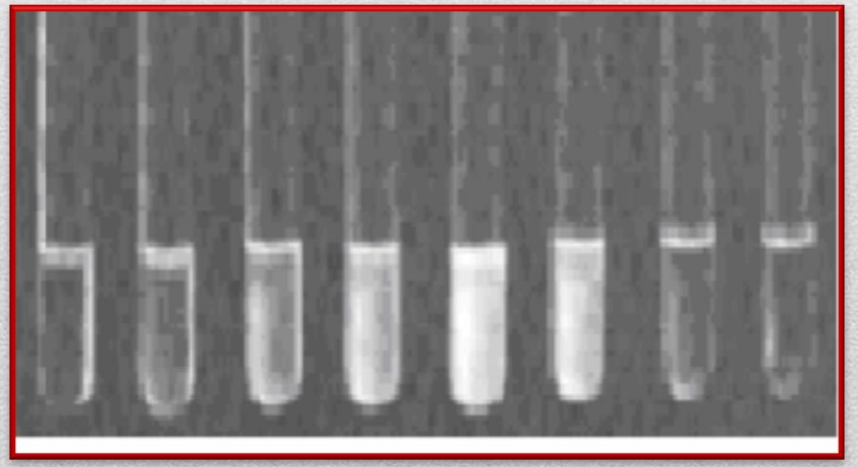
Antigen-Antibody Interactions

- Take a series of tubes
- place a certain amount of antibody (eg 1 ml of immune serum),
- add increasing amounts of water-soluble antigen respectively.
- add physiological saline to them (Equalize volumes)
- wait for a while in the appropriate environment.



1 2 3 4 5 6 7 8

- At the end of time the first tubes are clear, there is a growing blur in the latter and the blurring is gradually diminishing the next ones and that the last tubes are still clear.



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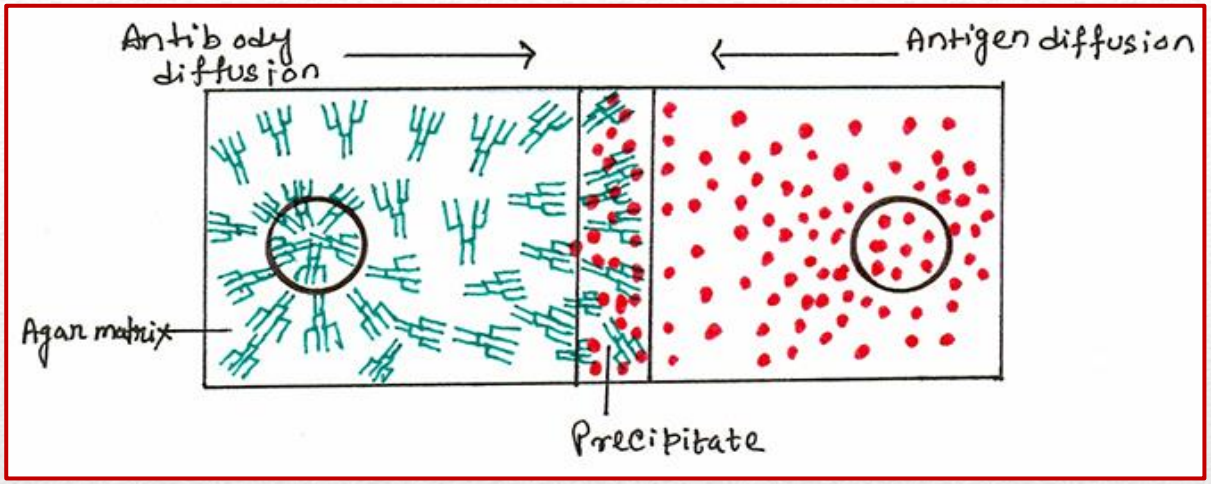
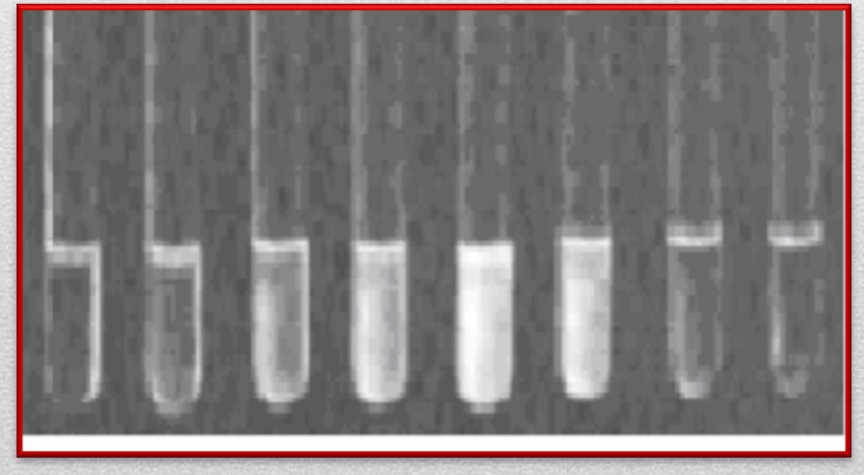


- When the tubes are centrifuged after a while and the upper liquid part is examined, the antigens and antibodies are not found in the supernatant which is the most abundant turbidity tube.
- The region in which the antigen and the antibody are exactly joined together is called **the optimal zone**.

- ✓ The region of excess antibody where the tubes before the optimal junction tube is called **the prezone (pre-zone).**
- ✓ After the optimal tubing, the region where there is no change in some tubes based on the excess antigen is called **the postzone (post-zone)**.



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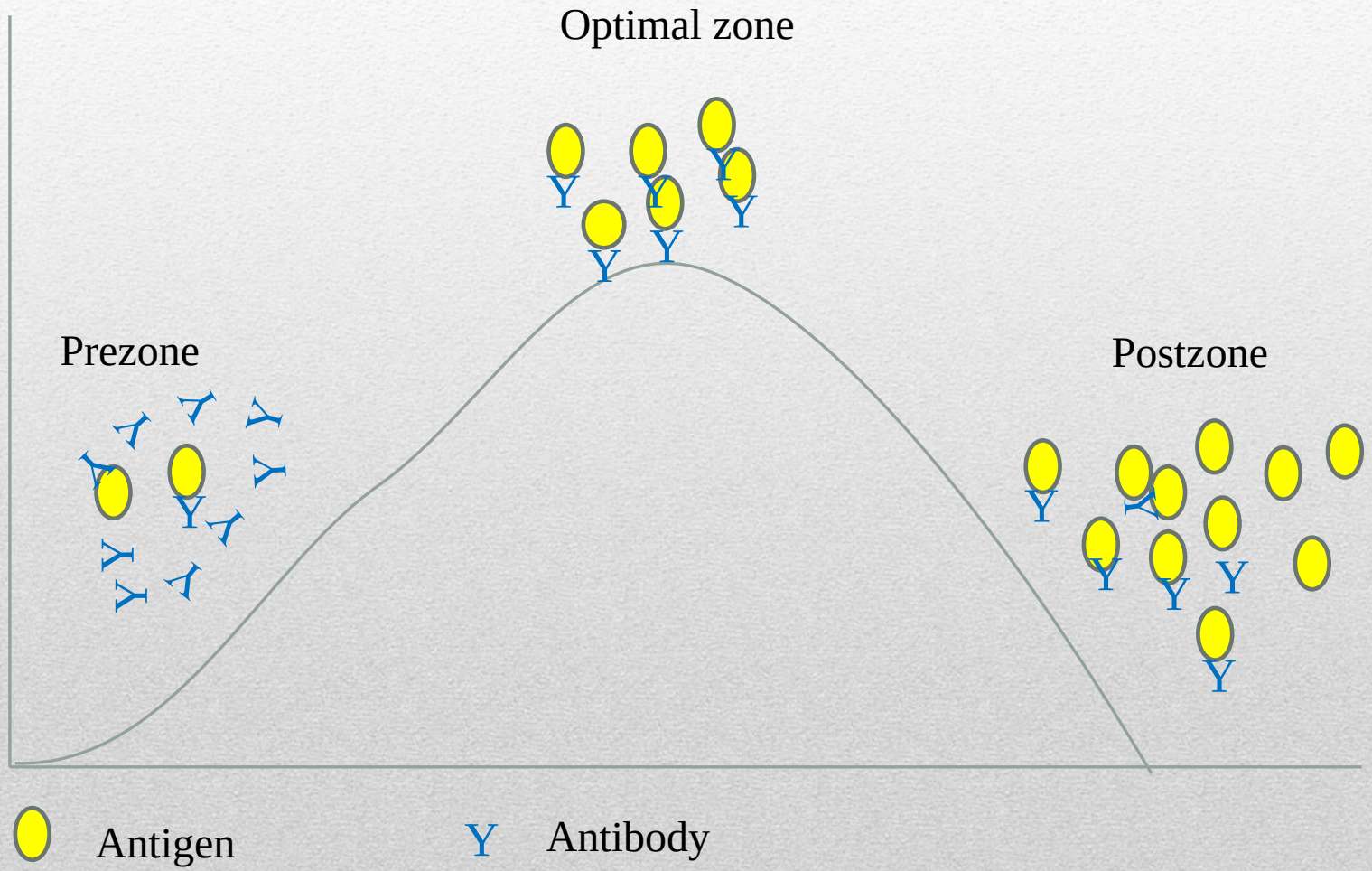
Antigen-Antibody Interactions



- Precipitation
- Agglutination
- Erythrocyte-Containing Agglutination Assays
- Toxin-Antitoxin Reactions
- Neutralization Tests
- Fluorescent Antibody Test
- Enzyme-Linked Immunosorbent Assay
- Radioimmunoassay
- Opsonositofajik Test
- Complement Fixation Test

This lesson

SEROLOGICAL REACTIONS

When a soluble antigen combines with its antibody in the presence of electrolytes (NaCl) at a suitable temperature and pH, the antigen-antibody complex forms an insoluble precipitate. (occurs as a collapse event after a blur)

- Antigen ⟹ Presipitinogen
- Antibody ⟹ Presipitin

PRECIPITATION

- Precipitation application methods:

1. Ring test
2. Dilution test in tube
3. Precipitation in gel
    a. Single diffusion
    b. Double diffusion
    c. Immunoelectrophoresis
    d. Counter-immunoelectrophoresis

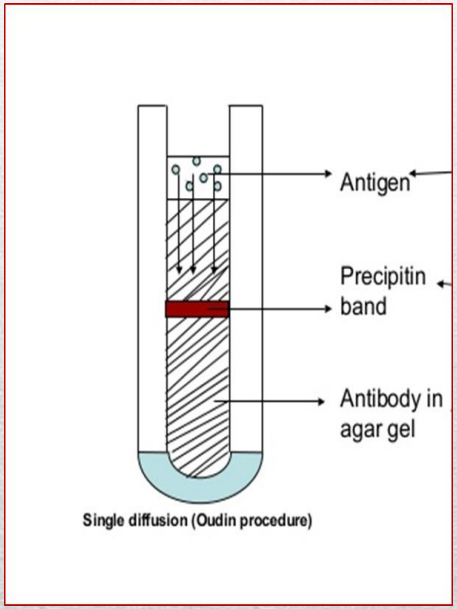
Ring test

- For testing,
  - put a small amount of antiserum in a thin tube, the antigen is added slowly.(not mix)
  - After a short incubation, if there is an annular precipitate where the antibody and antigen are in contact, the result is positive.

Precipitation in gel- Single diffusion

- Oudin procedure

- Gelatin mixed antiserum is added into a thin tube.
- After the gelatin is frozen, the suspected antigen solution is spread on a layer.
- If the antibody and the antigen are suitable, a precipitat occurs at a certain distance from the surface and the region where the optimal density is present.

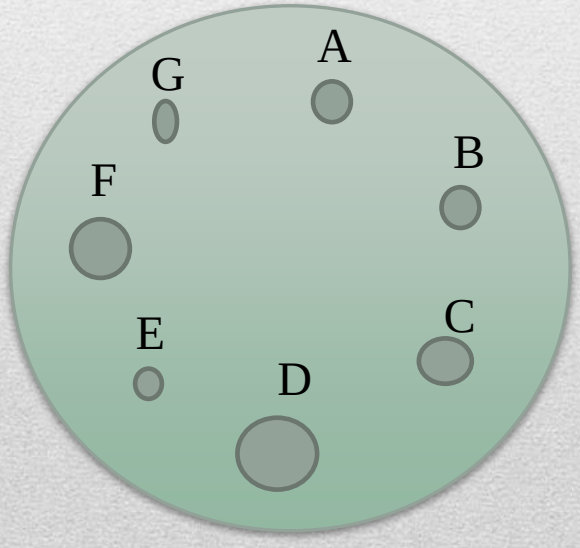


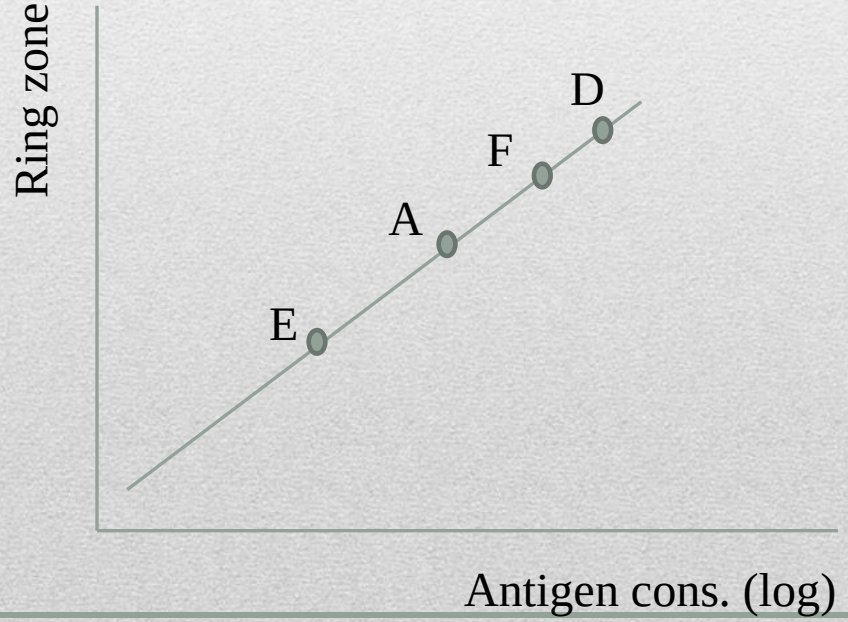
Precipitation in gel- Single diffusion

- Mancini method (Radial immunodiffusion)
- Antibody is incorporated into agar and antigen is measured into a well.
- As the antigen diffuses with time, precipitation rings form depending on the antigen concentration.

Mancini Method

By calibrating the method, such radial immunodiffusion is used to measured unknown material.





Double diffusion

- Ouchterlony method

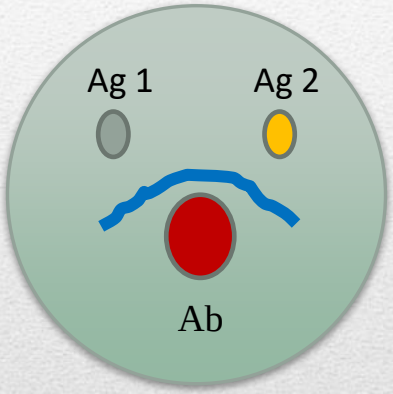
➢ Antigen and antibody are placed in different wells in agar and allowed to diffuse and form concentration gradients.

➢ Where optimal proportions (see zone of equivalence) occur, lines of precipitate form.

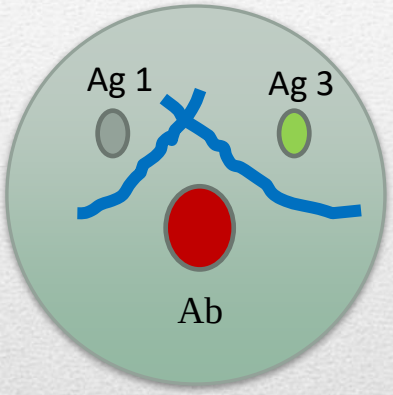
➢ This method indicates whether antigens are identical, related but not identical or not related.

Ouchterlony Method

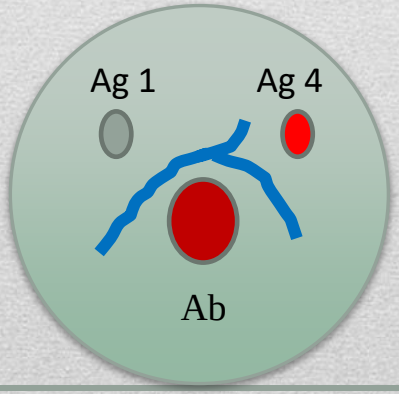
Identical



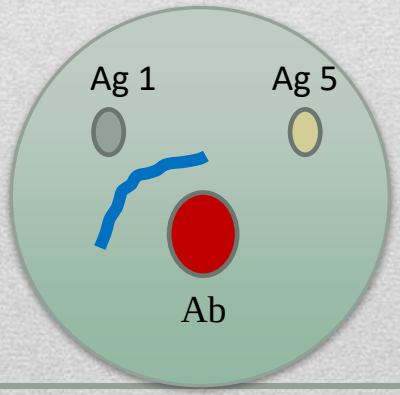
Antigen are different



Crossreactive



Antigen are different

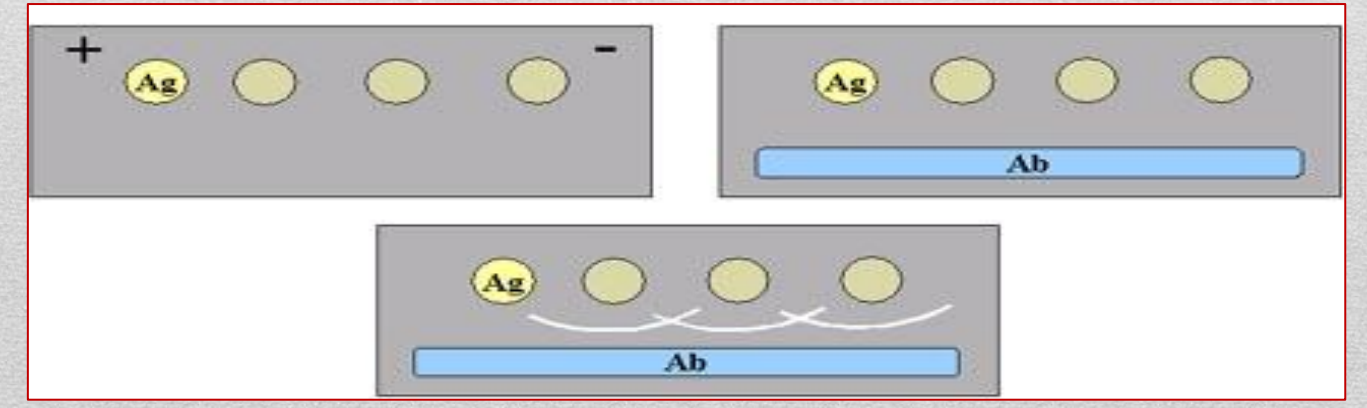


Electrophoresis

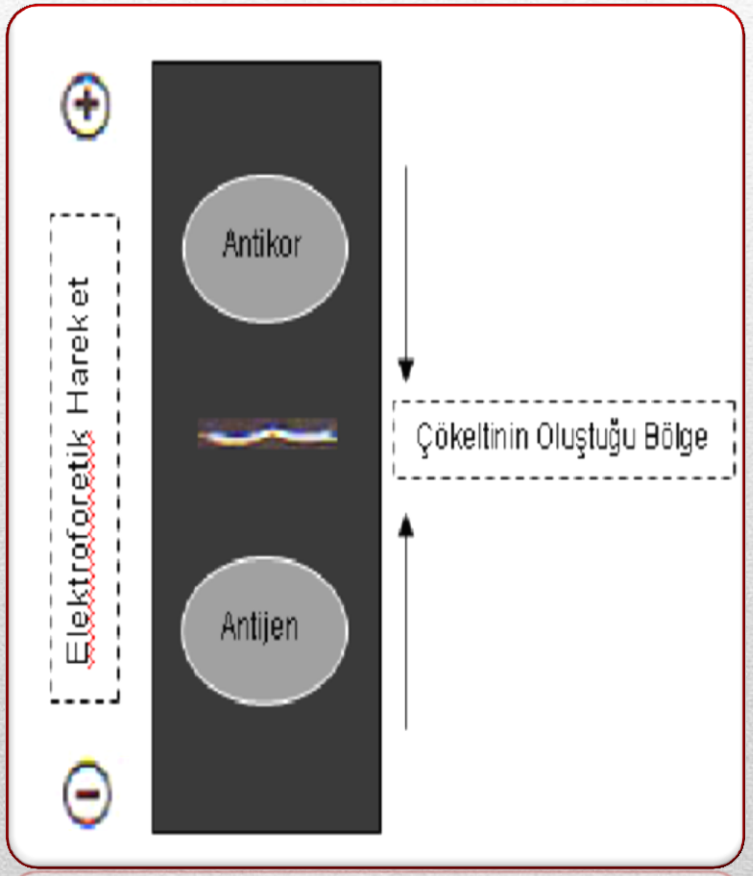
- Process of separating proteins in a mixture utilizing their different net electrical charges.
- Size and shape can cause frictional drag

Immunoelectrophoresis

- It is used as a rapid and qualitative technique to detect the presence or absence of antigens in body fluids.
- This method detects viral or bacterial antigens in the serum.
- It is based on two-direction spread precipitation in electrophoresis gel.



Counter-immunoelectrophoresis



- This method relies on movement of antigen toward the cathode,
- and of antibody toward the anode,
- during the passage of electrical current through agar.
- The meeting of the antigen and antibody is greatly accelerated by this method and is made visible in 30-60 minutes.
- This line can be painted with special methods if desired.

AGGLUTINATION

- Antigens naturally present on the surface of cells
  - such as bacteria in suspension, erythrocytes, leukocytes or
    - antigens artificially adhered to the surfaces of synthetic particles such as erythrocytes, latex, bentonite
- combine with their antibodies in the electrolytic environment to form visible particles. This is called agglutination.

Antigen $\longrightarrow$ Agglutinogen
Antibody $\longrightarrow$ Agglutinin

- When a particulate antigen is mixed with its antibody in the presence of electrolytes at a suitable temperature and pH,
  - the particles are clumped or agglutinated.

Antigen → Agglutinogen
Antibody → Agglutinin

The agglutination reactions can be used for 2 purposes.

1. The identification of bacteria
2. The diagnosis of infectious diseases

- Agglutination reactions used in the diagnosis of infectious diseases

  - Salmonellosis diagnosis ⟶ Gruber-Widal test
  - Brucellosis Diagnosis ⟶ Wright test
  - Ricketiosis diagnosis ⟶ Weil-Felix test
  - Leptospirosis diagnosis ⟶ Micro Agglutination
  - Treponema pallidum immobilization test

Erythrocyte-Containing Agglutination Assays

Hemagglutination Assays

- Agglutination assays in which erythrocyte is used as a particulate antigen are called hemagglutination assays.
- If the antigen interacts directly with the carrier, red blood cells causing hemagglutination.'active hemagglutination'
- If the carrier, either latex beads or red blood cells, is first "sensitized" coated with antigen or antibody'passive hemagglutination'.
  - Erythrocytes here only act as antigen carrier.

Heterophile Antibody Tests

- Common same structure in some living things which are different from each other in hereditary structure (Mice, dogs, cats, chickens, guinea pigs, etc.) are called heterophile antigens ,and antibodies against them are also called heterophile antibodies.

Heterophile Antibody Tests

- Infectious mononucleosis diagnosis - Paul-Bunnel Test
- Atypical pneumonia diagnosis - Cold agglutinin test
- Passive Hemaglutination and Particle Hemagglutination
- Virus Hemagglutination and Hemagglutination Inhibition Test

- Malta fever, Gibraltar fever, Bang's disease, Mediterranean fever
- Brucellosis is a bacterial disease caused by members of the *Brucella* genus that can infect humans and animal.
- Symptoms of the disease include intermittent fever, sweating, chills, aches, and mental depression. The disease can become chronic and recur, particularly if untreated.
- Antibodies occur in the disease starting from the 2nd week and are stable for a long time.

Brucellosis Diagnosis

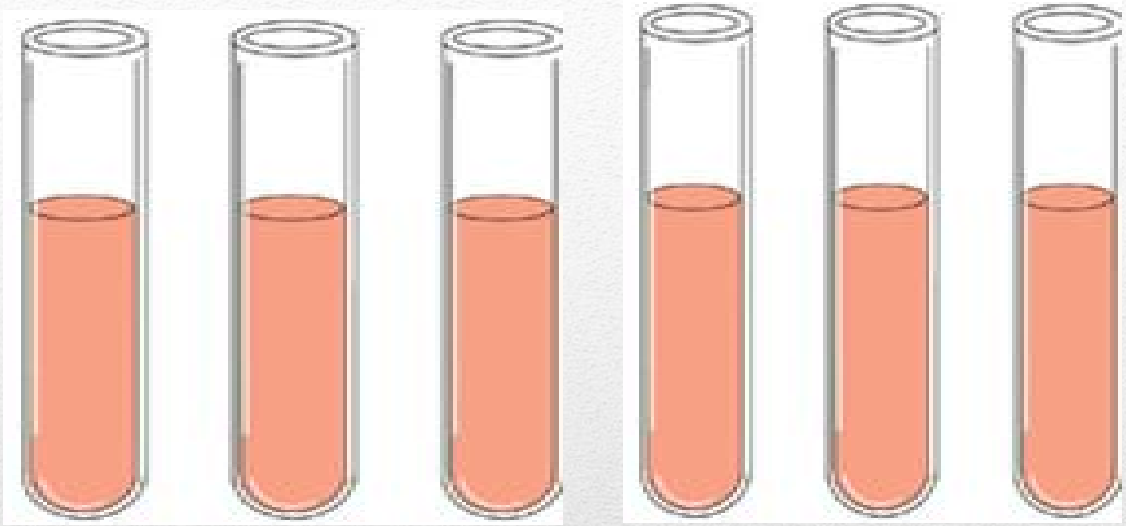
- In the experiment standard, heat-killed, phenolic bacterial suspension is used as the antigen.
- Agglutinin can be found in the serum of normal people, veterinarian, butcher, shepherd, occupational occupants at 1/80 or 1/100 titre.

(Agglutination results above 1/100 are important.)

Wright Agglutination Test Procedure

- Serum (+) containing tube
- Serum (-) containing tube
- Serum physiological (electrolytic environment)
- Antigen (*Brucella abortus*) containing tube
- 6 small tubes (for serum (+))
- 6 small tubes (for serum (-))

Wright Test



0,9 ml sp + 0,1 ml serum(+)

0,5 ml sp + 0,5

0,5 ml sp +

0,5 ml sp +

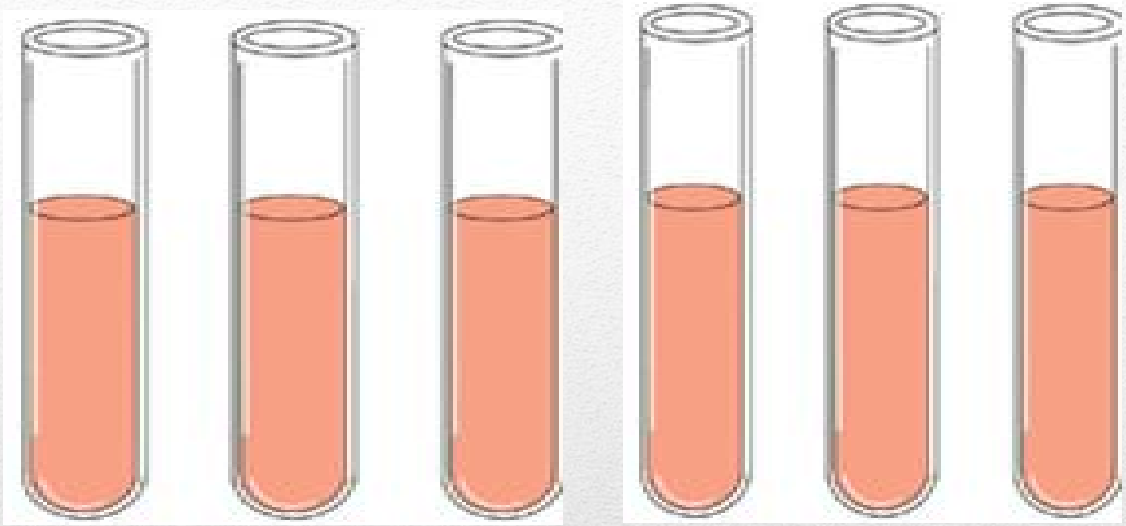
0,5 ml sp +

0,5 ml sp

outside

-	+ 0,5 ml antigen	+ 0,5 ml antigen	+ 0,5 ml antigen	+ 0,5 ml antigen	+ 0,5 ml antigen
1/10	1/40	1/80	1/160	1/320	

Wright Test



0,9 ml sp + 0,1 ml serum(-)

0,5 ml sp + 0,5

0,5 ml sp +

0,5 ml sp +

0,5 ml sp +

0,5 ml sp

outside

-	+ 0,5 ml antigen	+ 0,5 ml antigen	+ 0,5 ml antigen	+ 0,5 ml antigen	+ 0,5 ml antigen
1/10	1/40	1/80	1/160	1/320	

Brucellosis Diagnosis

- 1. tube Antibody control tube
- 6. tube Antigen control tube
- Diagnosis: At the end of the experiment, agglutination of more than 1/100 conc ⟹ Result (+)