Antigen-Antibody

reactions (2)

By: Masheal Aljumaah OCT 2018

Learning **objectives:**

- introduction to Antigen Antibody reactions**.**
- **Antigen Antibody reactions part1:** Precipitation, Flocculation and Immunodiffusion.
- **Antigen Antibody reactions part 2:** Agglutination.
- **Antigen Antibody reactions part 3:** Complement Fixation Test.

1.

Precipitation

PRECIPITATION

Soluble **antigen** and antibody electrolytes

Suitable temperature and pH

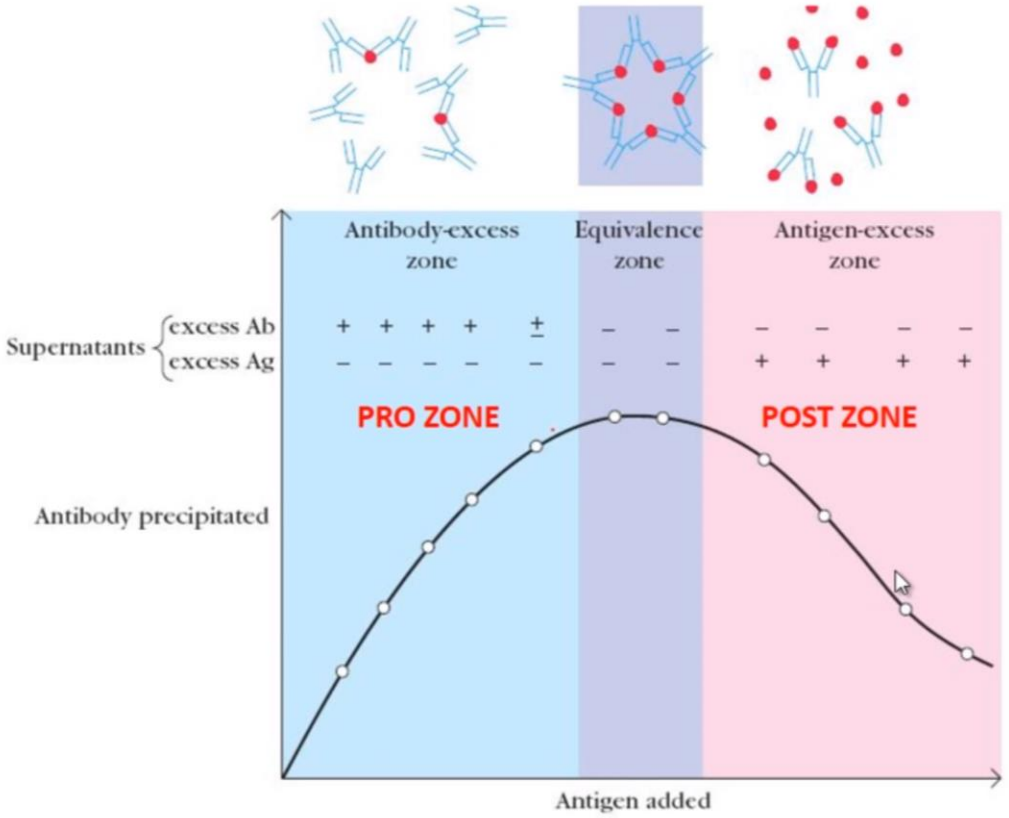
- Insoluble precipitate - **precipitation** → Liquid / Gel
- Suspended - floccules - **flocculation**

LATTICE THEORY

the interaction of multivalent antige n with multivalent antibody will, at o ptimum proportions of each (zone of equivalence), result in the formation of a lattice and a precipitate.

Ag excess = early infection.

Ab excess= late in infection

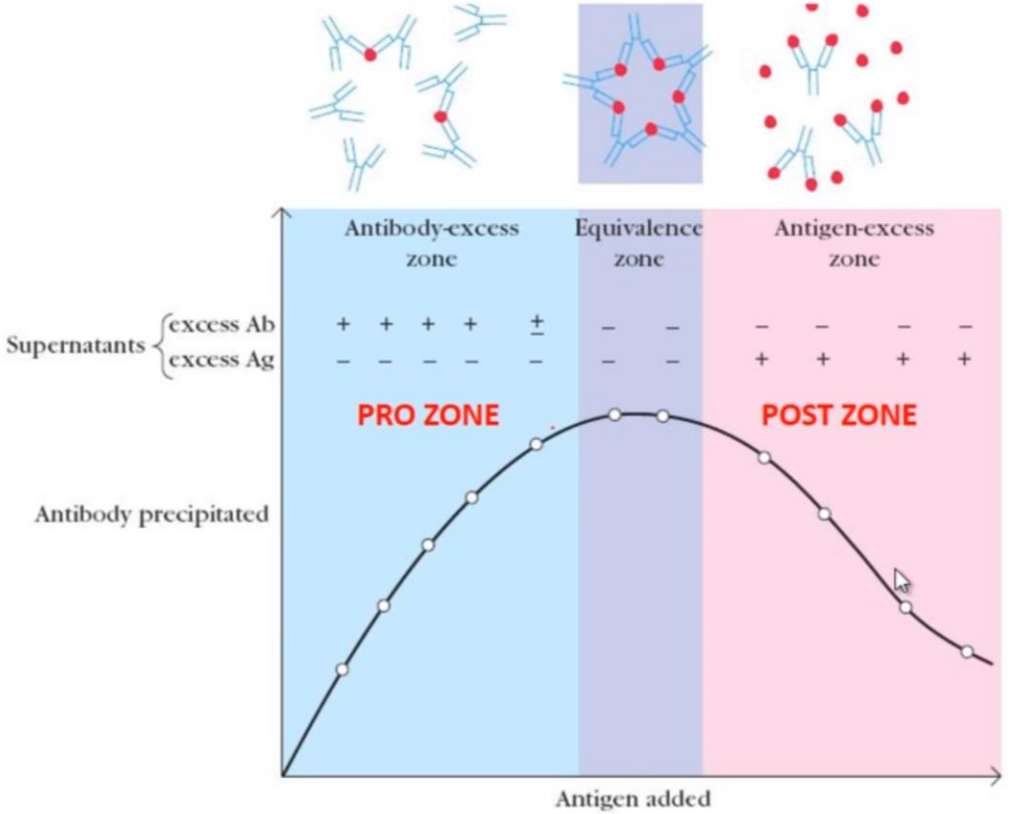


Precipitation Curve

Zone of antibody excess (Prozone)
precipitation is inhibited and antibody not bound to antigen can be detected in the supernatant

Zone equivalence
Maximal precipitation in which antibody and antigen form large insoluble complexes and neither antibody nor antigen can be detected in the supernatant;

Zone of antigen excess (Postzone)
Precipitation is inhibited & Ag. not bound to Ab. can be detected in the supernatant



- Precipitation
  - **In Liquid**
    - Flocculation test
      - Slide flocculation
      - Tube flocculation
    - Ring test
  - **In gel (immunodiffusion)**
    - Oudin
    - Oakley-fulthorpe
    - Radial
    - Ouchterlony
    - Immunoelectrophoretic
      - CIE
    - Electroimmunodiffeusion
      - Rocket
      - laurell's

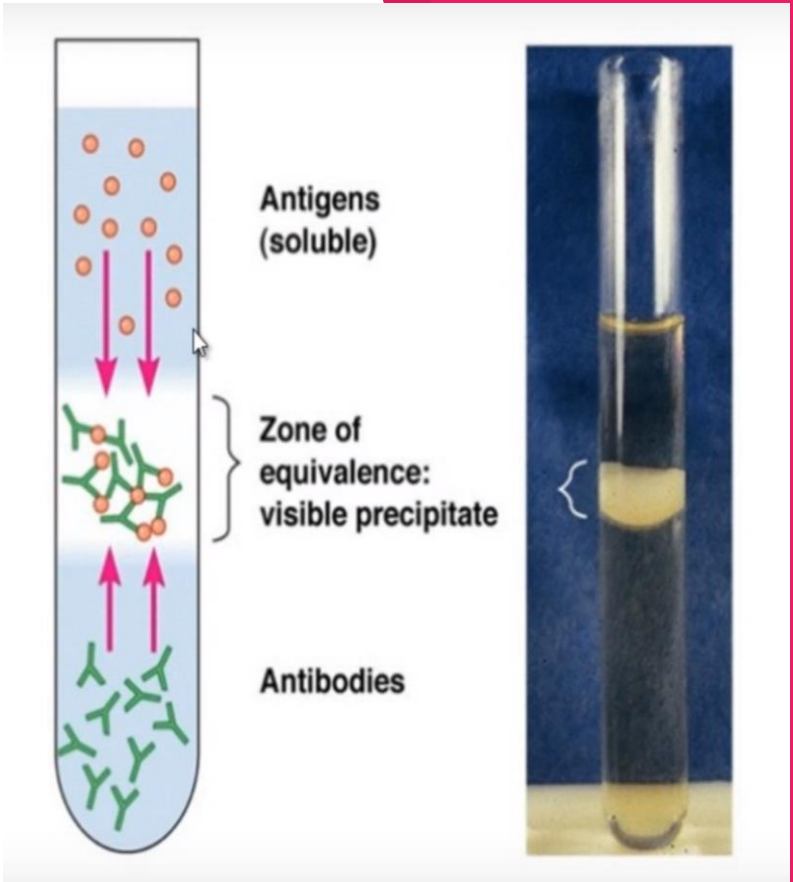
In liquid: Precipitation

(a) Ring Precipitate:

- layering antigen solution over column of antibody in a narrow tube
- Precipitate at the junction of two liquids

Example:

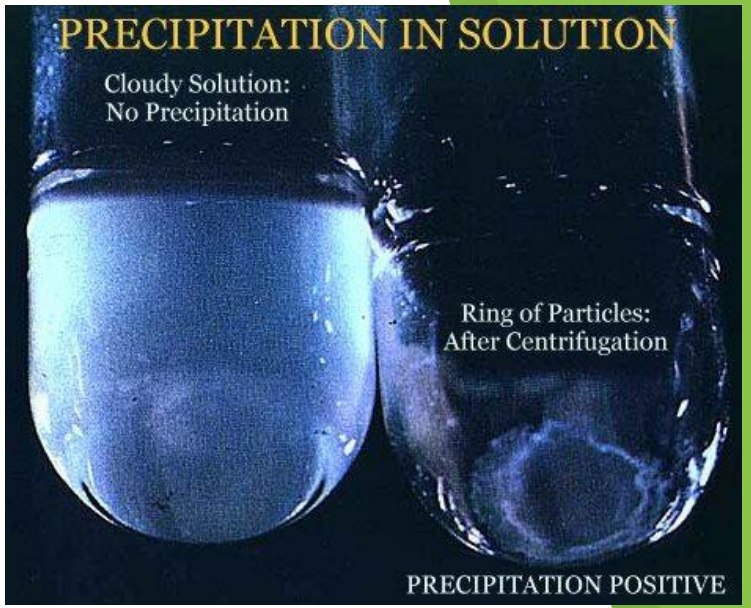
1. Ascoli's thermoprecipitin test ⟶ **Anthrax**
2. Lancefield grouping of streptococci



In liquid: Precipitation

Bottom Precipitate

Occurs when **Soluble Ag** interact with **soluble Ab** and **form a visible precipitate** that give **bottom ppt** after centrifugation.



In liquid: Precipitation

(b) Flocculation test:

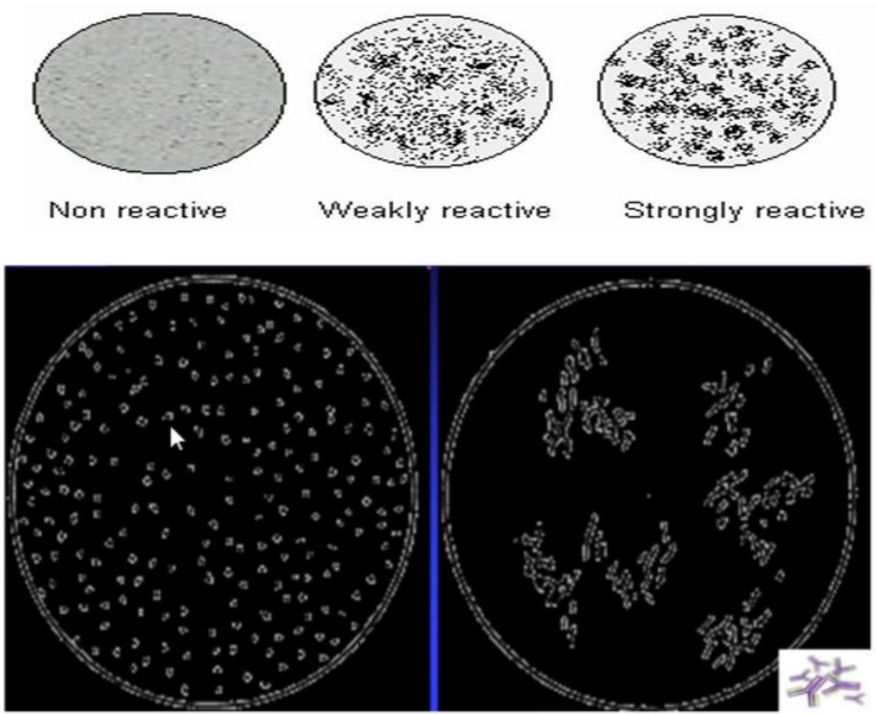
1- Slide Flocculation test

- Drop of antigen and antiserum on a slide – mixed by shaking – floccules appear

Example:

1. VDRL slide test – syphilis

(The **v**enereal **d**isease **r**esearch **l**aboratory)



In liquid: Precipitation

(b) Flocculation test:

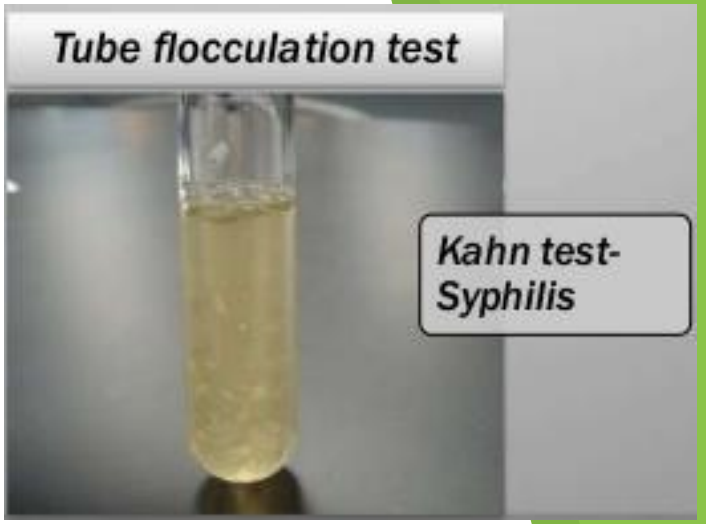
1- Tube Flocculation test

- Antigen and antiserum in a test tube– floccules appear

Example:

1. Kahn test for syphilis

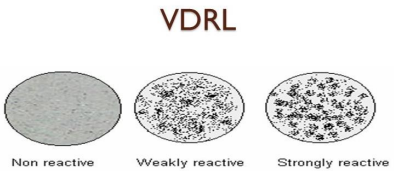
Kahn antigen – alcoholic extract of fresh beef heart with cholesterol + On reaction with syphilitic serum, floccules are formed which can be seen with the naked eye.



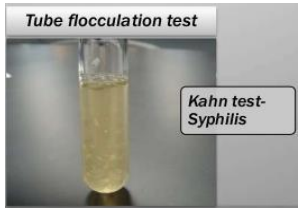
Precipitation

(a) **In Liquid**

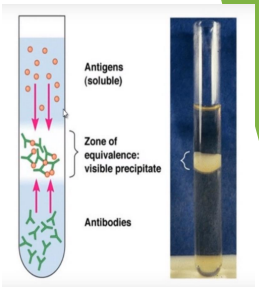
- Flocculation test
  - Slide flocculation → VDRL



  - Tube flocculation →



- Ring test →



Lancefield grouping

In gel: Precipitation
(immunodiffusion)

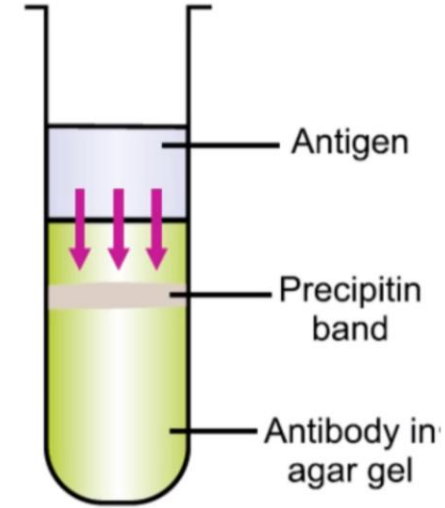
Why?

- Visible, distinct **band** of precipitation → preserved for a long period of time
- **Different antigens** observed → Each Ag will form a different band.
- **Cross-reaction** and **non-identity** between different antigens

In gel: Precipitation
(immunodiffusion)

(a) Oudin Immunodiffusion (Single diffusion - one dimension)

- Antibody - agar gel - test tube
- Antigen solution layered over it
- Antigen diffuses towards the agar gel, forming a line of precipitation

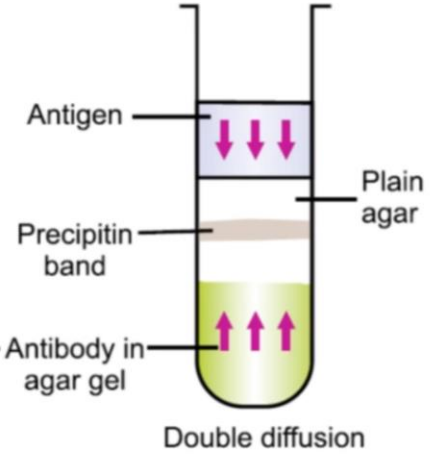


Single diffusion

In gel: Precipitation
(immunodiffusion)

(b) Oakley–Fulthorpe Immunodiffusion (Double diffusion - one dimension)

- Antibody incorporated in gel
- Above this column of plain agar
- Antigen layered on top of this
- Antigen and antibody move towards each other

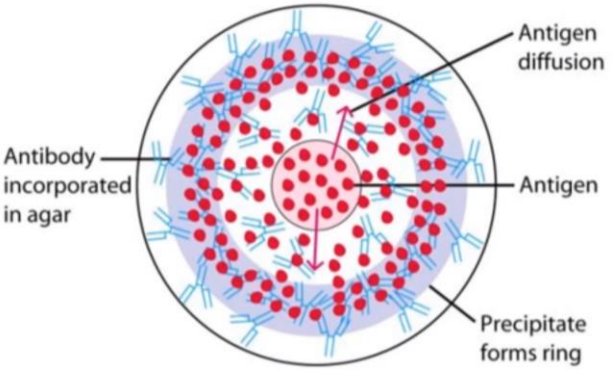


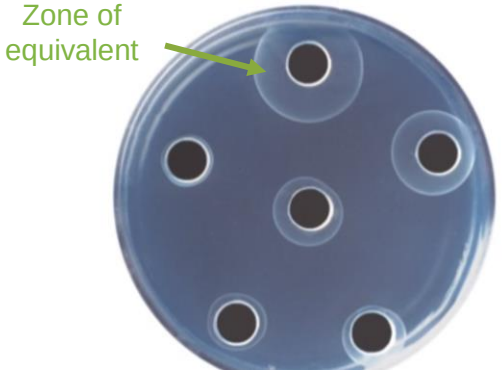
Double diffusion

In gel: Precipitation
(immunodiffusion)

(c) RADIAL IMMUNODIFFUSION (single diffusion in two dimensions)

- Antiserum in gel - slide/Petri dish
- Antigen added to wells cut on surface
- Diffusion radially from well
- Ring-shaped bands of precipitation





In gel: Precipitation
(immunodiffusion)

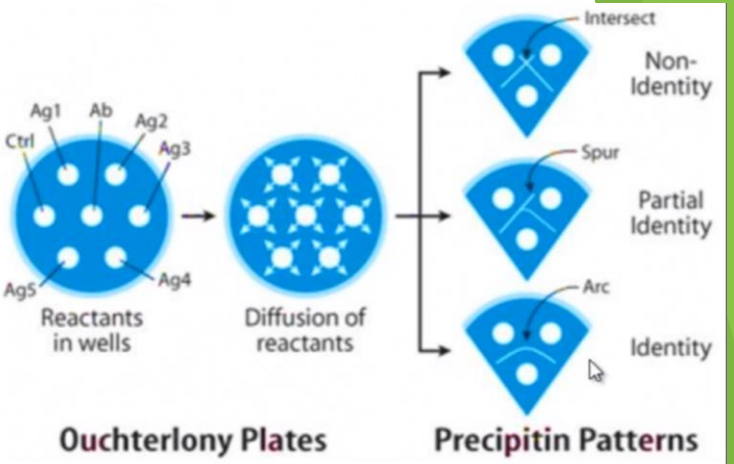
(d) OUCHTERLONY IMMUNODIFFUSION (double diffusion – two dimensions)

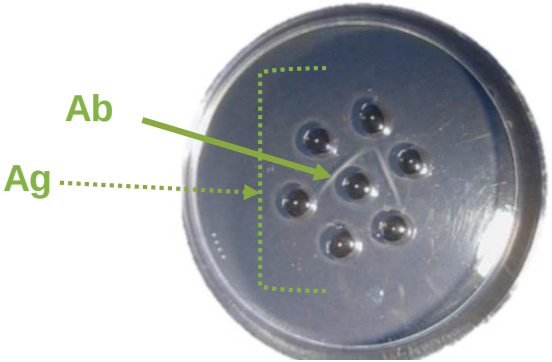
Most widely employed

- Agar gel on a slide
- Wells cut using a template
- Antiserum in central well
- Antigen in surrounding wells

Example:

1. Elek's gel precipitation test for *C.diphtheriae*



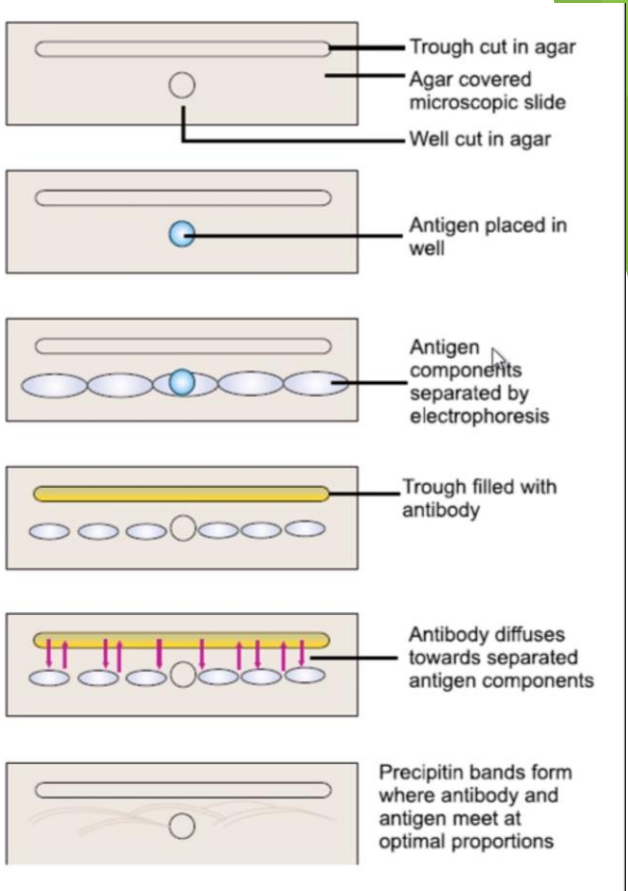


In gel: Precipitation
(immunodiffusion)

(d) IMMUNOELECTROPHORESIS (IEP)

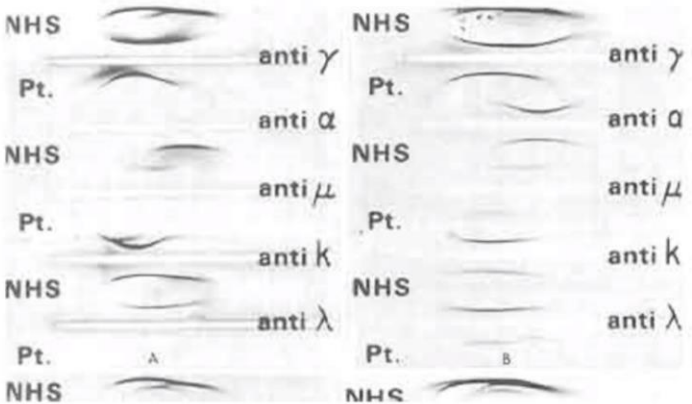
Why? (to speed up the process)

- Electrophoretic separation of a composite antigen into its constituent proteins
- Followed by immunodiffusion against its antiserum
- Result - Separate precipitation lines between each protein and its antibody



Normal human serum →

Patient serum →



- Immunoelectrophoresis (IEP)

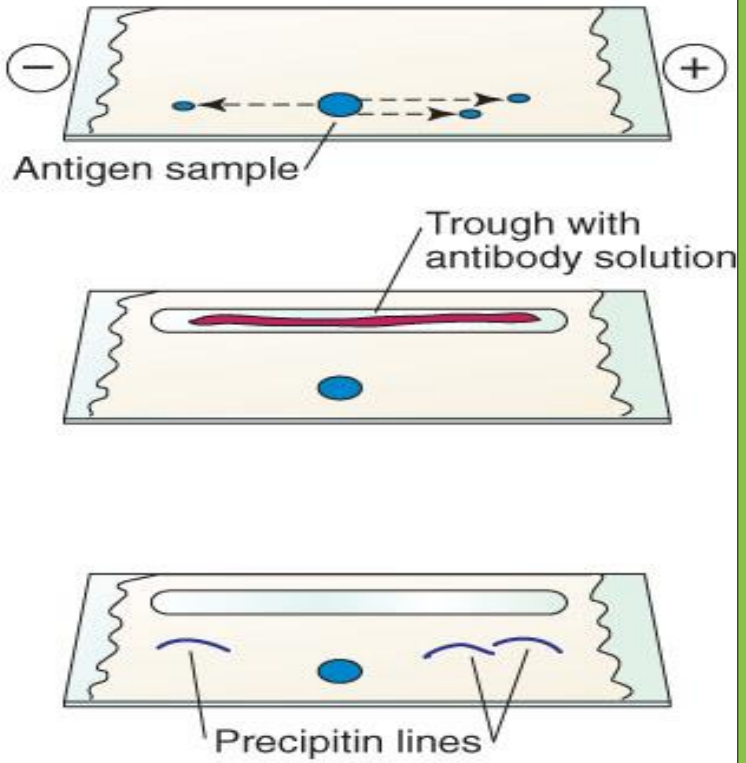
NHS = "normal human serum", pt = Patient serum

- Note that there is an abnormality or bowing to the precipitin line of the patient's serum with certain anti-immunoglobulin isotype antibodies.
- On the left bowing occurs with anti-gamma and anti-kappa antibodies.

A **Gel electrophoresis:**
On a gel-coated slide, an antigen sample is placed in a central well. An electrical current is run through the gel to separate antigens by their electrical charge (electrophoresis). Unlike the diagram, the separate antigens cannot be detected visually at this point.

B **Addition of antibodies:**
A trough is made on the slide and a known antibody solution is added.

C **Diffusion of antigens and antibodies:**
As antigens and antibodies diffuse toward one another through the gel, precipitin lines are seen where optimal concentrations of antigen and antibodies meet.



In **electro-immunodiffusion, diffusion is combined with electrophoresis**
Electrophoresis separates antigen molecules according to differences in their electrical charges and molecular weight then specific **antibodies diffuse** and react with separated antigen forming precipitin bands.

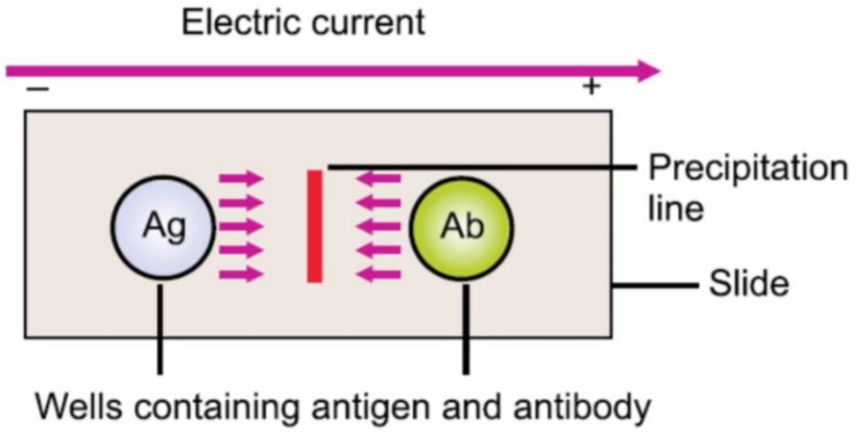
In gel: Precipitation
(immunodiffusion)

(e) ELECTROIMMUNODIFFUSION → (3 techniques)

1. Counter immunoelectrophoresis (CIE)

- Simultaneous electrophoresis of antigens and antibody in gel in opposite directions

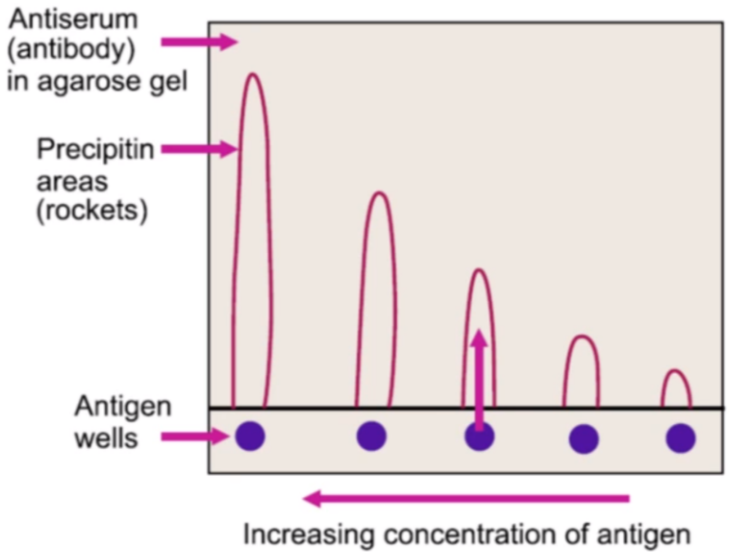
Example: ∝-fetoprotein,cryptococcal antigen

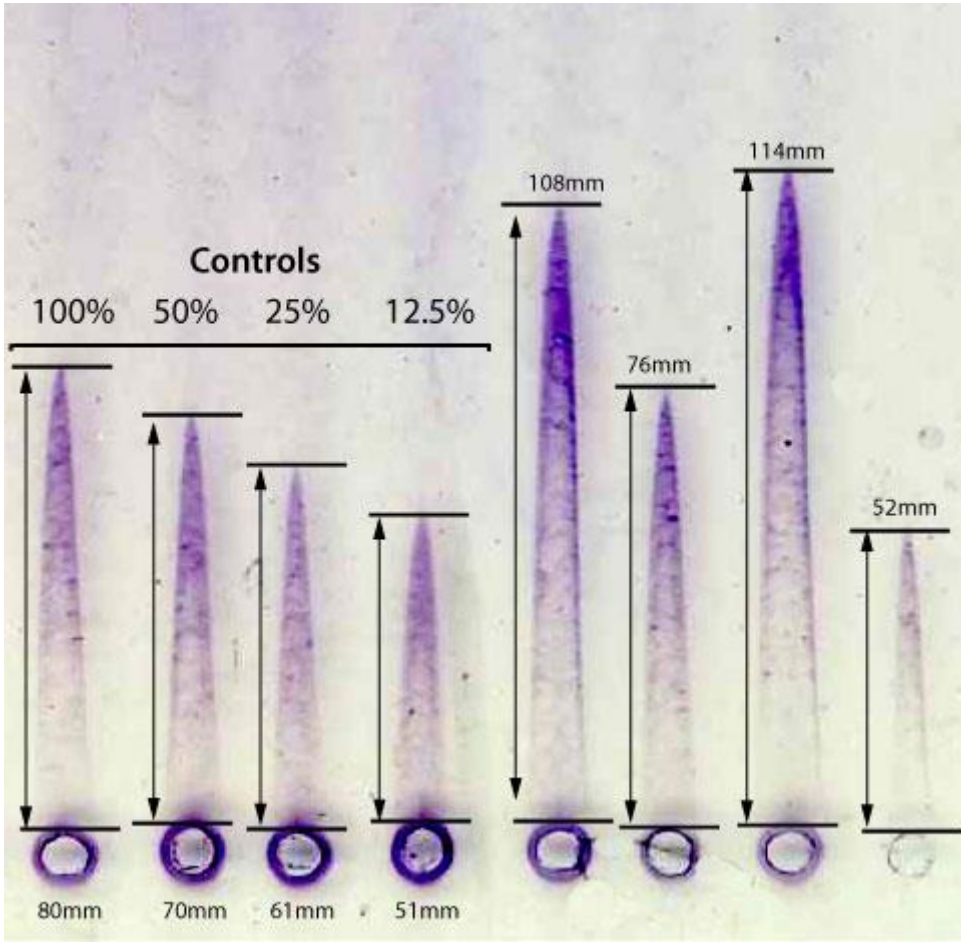


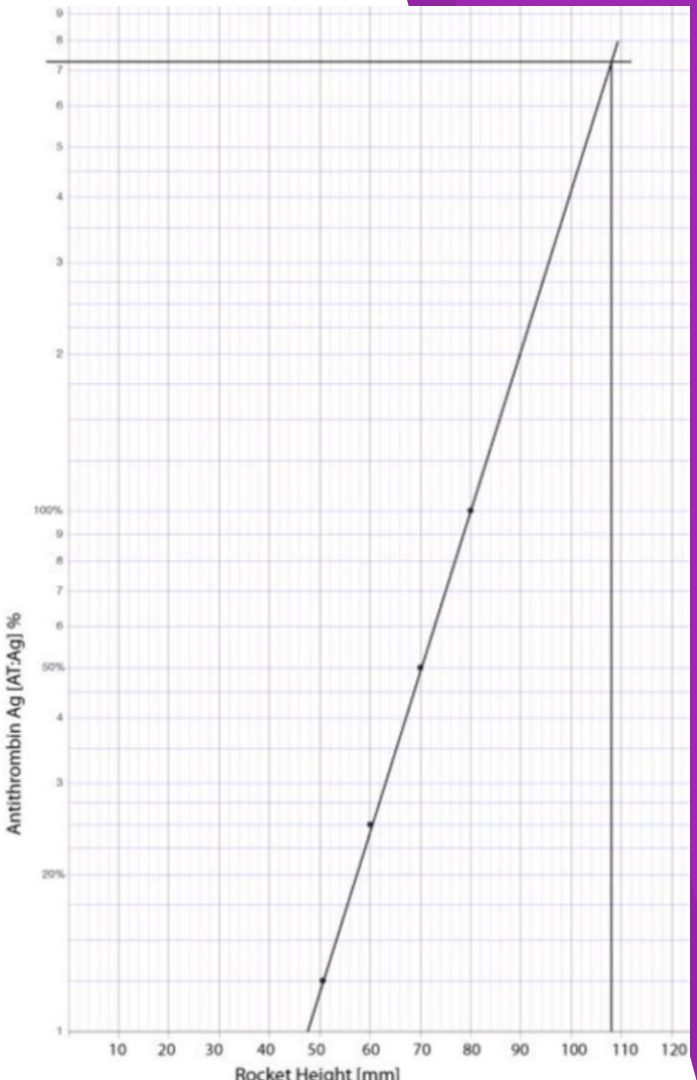
In gel: Precipitation
(immunodiffusion)

(f) ELECTROIMMUNODIFFUSION
2. Rocket electrophoresis
(One dimensional, single electroimmunodiffusion)

- Quantitative estimation of antigens
- Antigen - Increasing concentration placed in wells - punched in set gel
- Antigen electrophoresed into antibody containing agarose
- Pattern of immunoprecipitation - Rocket




Controls
100%
50%
25%
12.5%
80mm
70mm
61mm
51mm
108mm
76mm
114mm
52mm

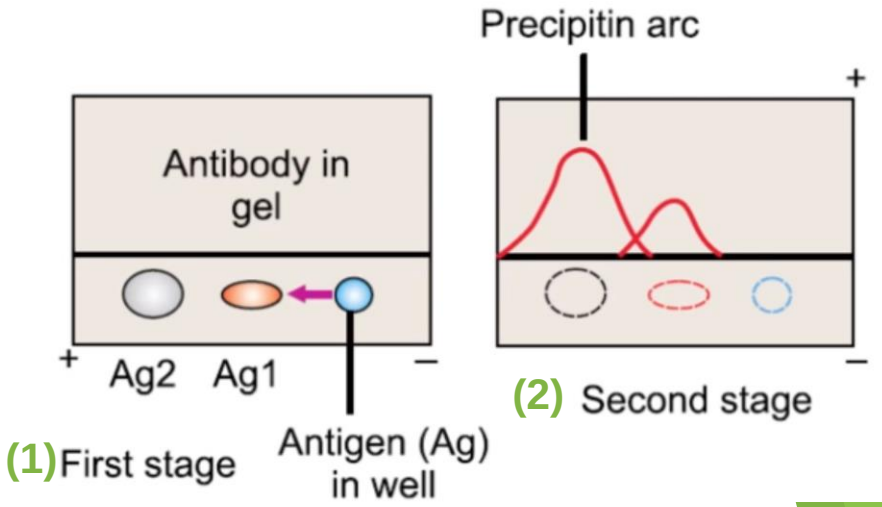

Antithrombin Ag [AT-Ag] %
Rocket Height [mm]
10
20
30
40
50
60
70
80
90
100
110
120

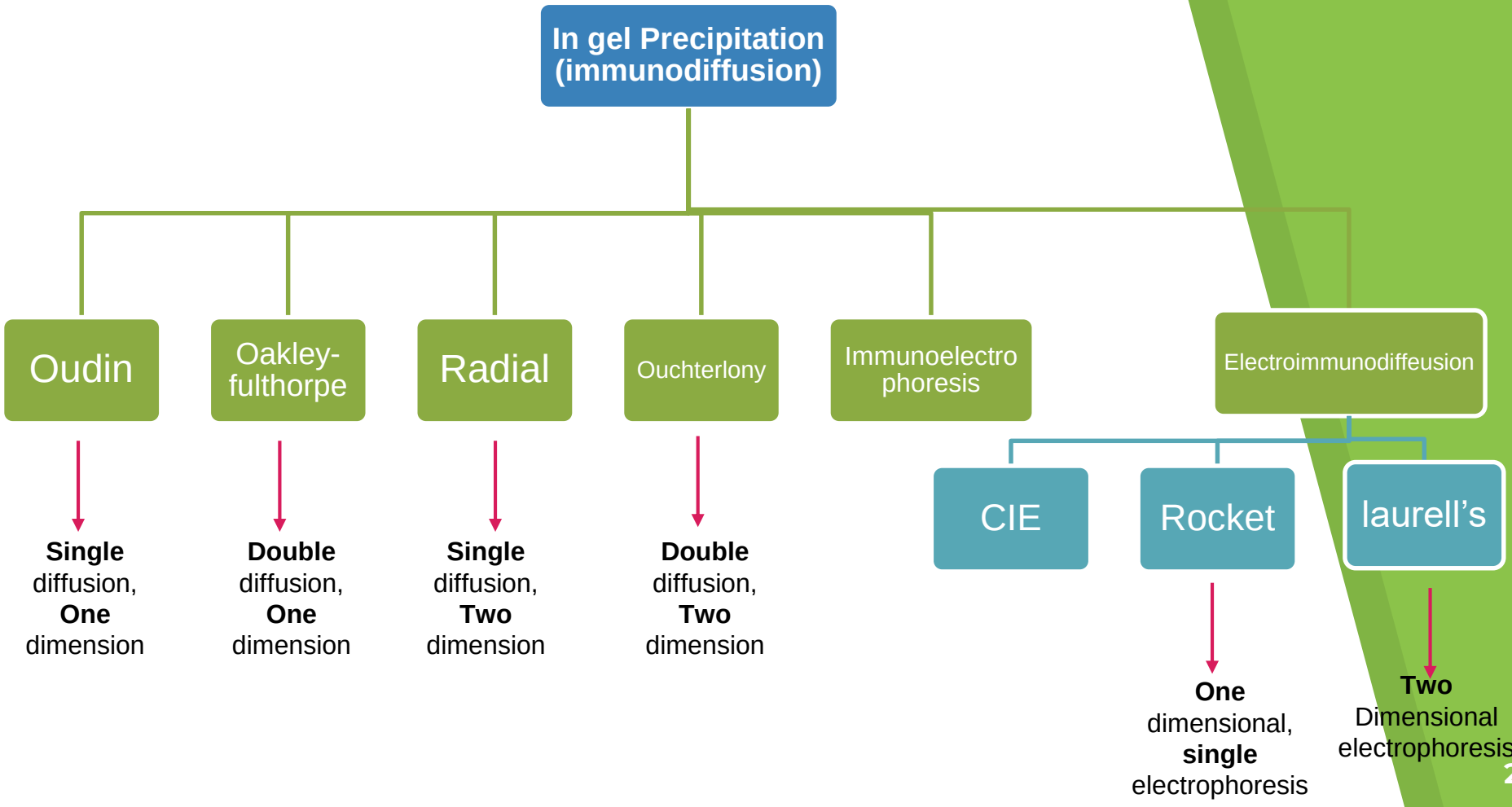
In gel: Precipitation
(immunodiffusion)

(g) ELECTROIMMUNODIFFUSION

3. Laurell's two-dimensional electrophoresis

- Antigen mixture electrophoretically separated in a direction perpendicular to the final rocket




In gel Precipitation (immunodiffusion)
Oudin
Oakley-fulthorpe
Radial
Ouchterlony
Immunoelectro phoresis
Electroimmunodiffeusion
CIE
Rocket
laurell's
Single diffusion, One dimension
Double diffusion, One dimension
Single diffusion, Two dimension
Double diffusion, Two dimension
One dimensional, single electrophoresis
Two Dimensional electrophoresis

Measurement of Precipitation by Light

Antigen-antibody complexes, when formed, will precipitate in a solution resulting in a turbid or cloudy appearance that can be measured by:

Turbidimetry

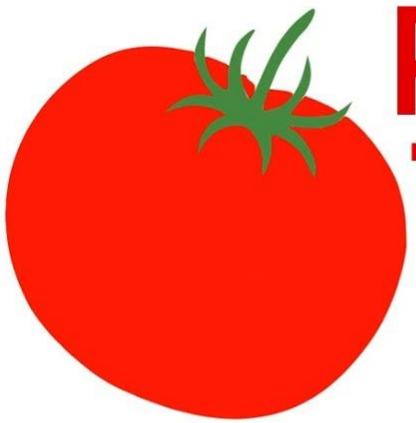
Passing light through a cloudy solution. (Net decrease in light intensity)

Nephelometry

Measuring light scattered at a particular angle after being passed through a solution i.e. **indirect measure**.
Amount of light scattered correlates to the concentration of the solution

Usage of turbidimetry and nephelometry

- measurement of serum proteins‘ concentration (immunoglobulins, acute-phase proteins, complement components C3, C4, transferrin, albumin,…)
- Rapid.
- fully-automated techniques
- for large quantity of samples



POMODORO TECHNIQUE

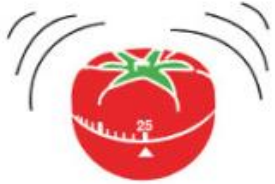
How to Get More Done

The Pomodoro Technique

CHOOSE A **TASK** TO BE ACCOMPLISHED

SET THE POMODORO TO **25 MINUTES**

WORK ON THE TASK UNTIL THE **POMODORO RINGS**



THEN PUT A **CHECK** ON YOUR SHEET OF **PAPER**

TAKE A **SHORT BREAK** (5 MINUTES IS OK)

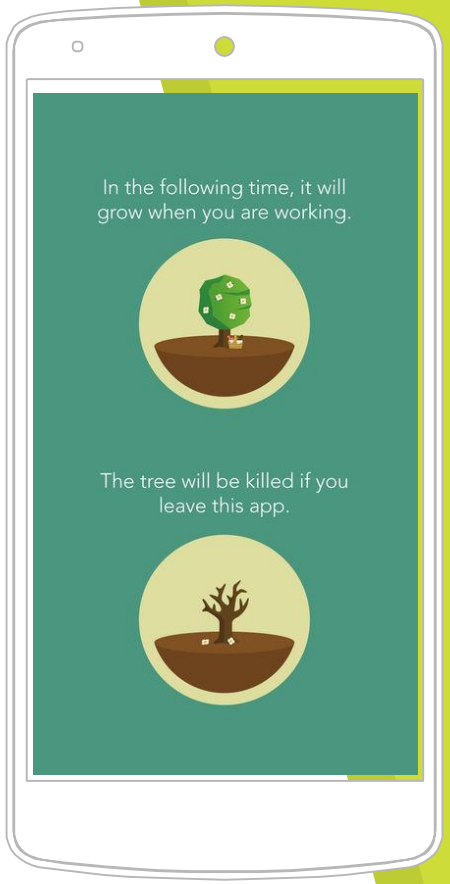
EVERY **4 POMODOROS** TAKE A **LONGER BREAK**

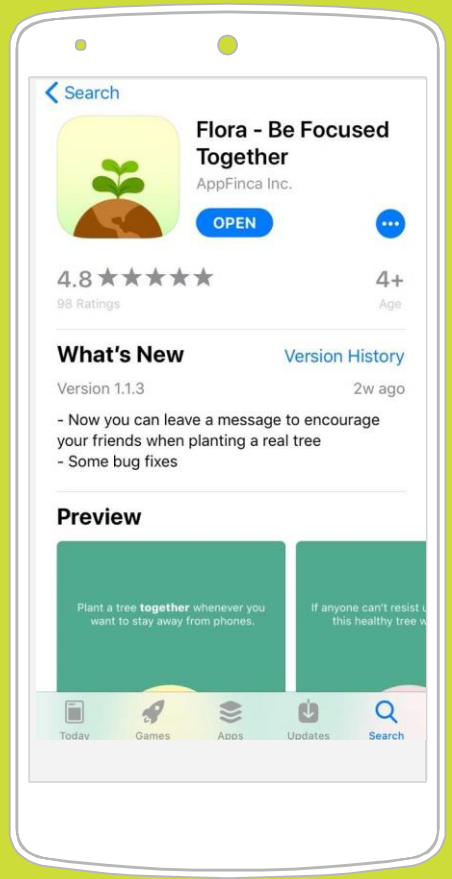
Pomodoro timer

http://www.tomatotimers.com/

Forest app

You can even help in planting real trees! Real forests!





Assignment

- Pick **one** precipitation application and write briefly about it.

- which immunoglobulin class is the most efficient to produce precipitation reaction?

a- IgG

b- IgM

c-IgA

THANKS!

Any questions?

You can find me at third floor office 87

maljumaah1@ksu.edu.sa