Antigen-Antibody **reactions** (1)

By: Masheal Aljumaah OCT 2018

Learning **objectives:**

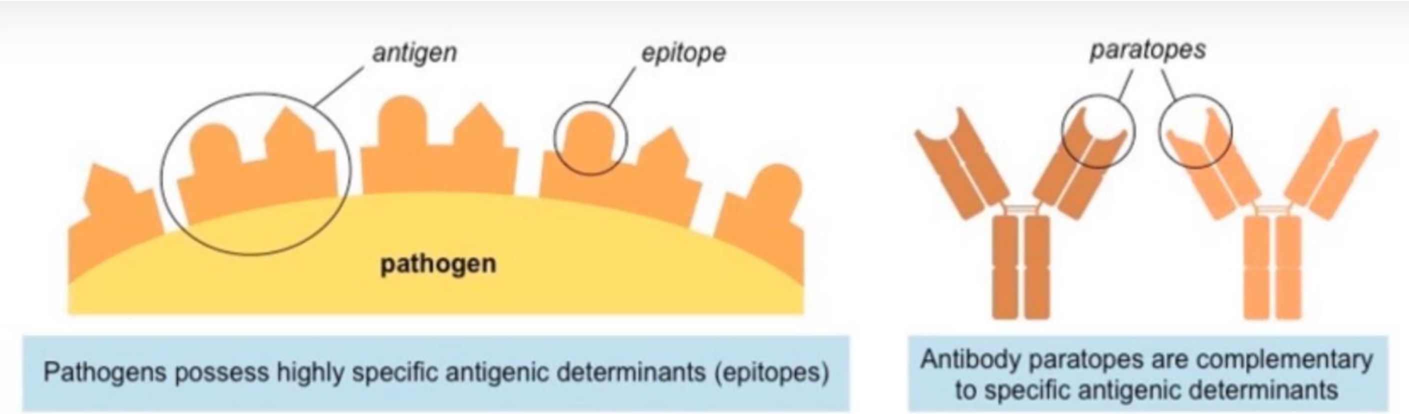
- introduction to Antigen Antibody reactions**.**
- **Antigen Antibody reactions part1:** Precipitation, Flocculation and Immunodiffusion.
- **Antigen Antibody reactions part 2:** Agglutination.
- **Antigen Antibody reactions part 3:** Complement Fixation Test.

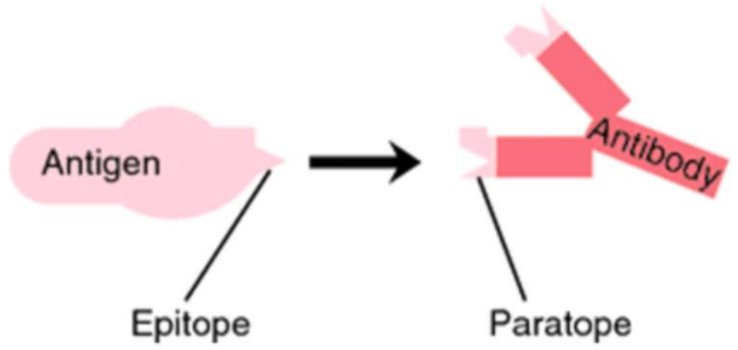
Key terms:

Epitope: also known as (antigenic determinant), is the part of an antigen that is recognized by the immune system, specifically by antibodies, B cells, or T cells. For example, the epitope is the specific piece of the antigen to which an antibody binds.

Key terms:

Paratope: also called an (antigen-binding site), is a part of an antibody which recognizes and binds to an antigen. paratope is produced by the complementarity determining regions of the light and heavy chains generating a specific three-dimensional shape. Any light chain can join with any heavy chain to produce a different paratope. Thus, theoretically, with 10^4 different light chains and 10^4 different heavy chains, 10^8 different specificities could be generated.


antigen
epitope
pathogen
paratopes
Pathogens possess highly specific antigenic determinants (epitopes)
Antibody paratopes are complementary to specific antigenic determinants


Antigen
Antibody
Epitope
Paratope

Key terms:

Affinity measures the strength of interaction between an epitope and an **antibody's** antigen binding site. It is defined by the same basic thermodynamic principles that govern any reversible biomolecular interaction: K_A = **affinity** constant.

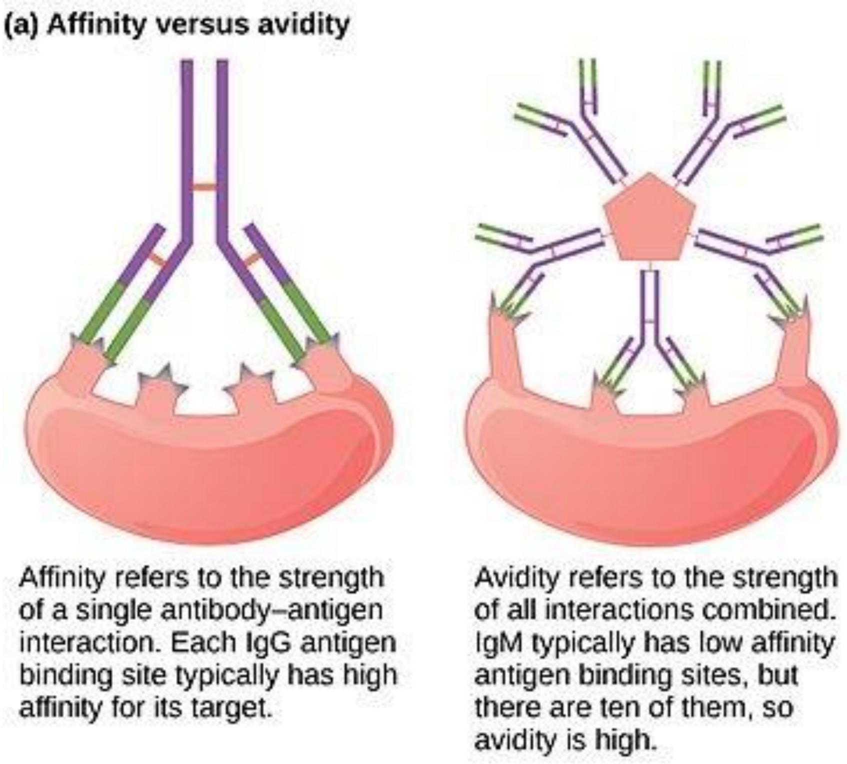
Key terms:

Avidity is a measure of the overall stability of the complex between antibodies and antigens and is governed by three factors, the intrinsic affinity of the antibody for the epitope, the valency of the antibody and antigen, and the geometric arrangement of the interacting components.(is the collective affinity of multiple binding sites(affinity+ Valence))

Key terms:

Valency of antibody:

refers to the number of antigenic determinants that an individual **antibody** molecule can bind.



Pentameric IgM lower affinity than IgG, but higher avidity of IgM is due to its higher valency, which enables it to bind effectively to the antigen

Key terms:

Sensitivity:

Ability to detect minute quantities of antigen/ antibody.

Specificity:

Ability to detect homologous antigen and no other.

Antigen Antibody reactions : 3 stages

1 Primary Stage

- No visible effect
- reversible
- Van der Waal's forces ionic bond and hydrogen bonding

2 Secondary Stage

- Demonstrable events

3 Tertiary Reaction

vivo chain reaction – neutralisation, destruction of injurious Ag, tissue damage

General features of Antigen antibody reactions

1. **SPECIFIC**
   Cross reactivity may occur.

2. **ENTIRE MOLECULE**
   Entire molecule react

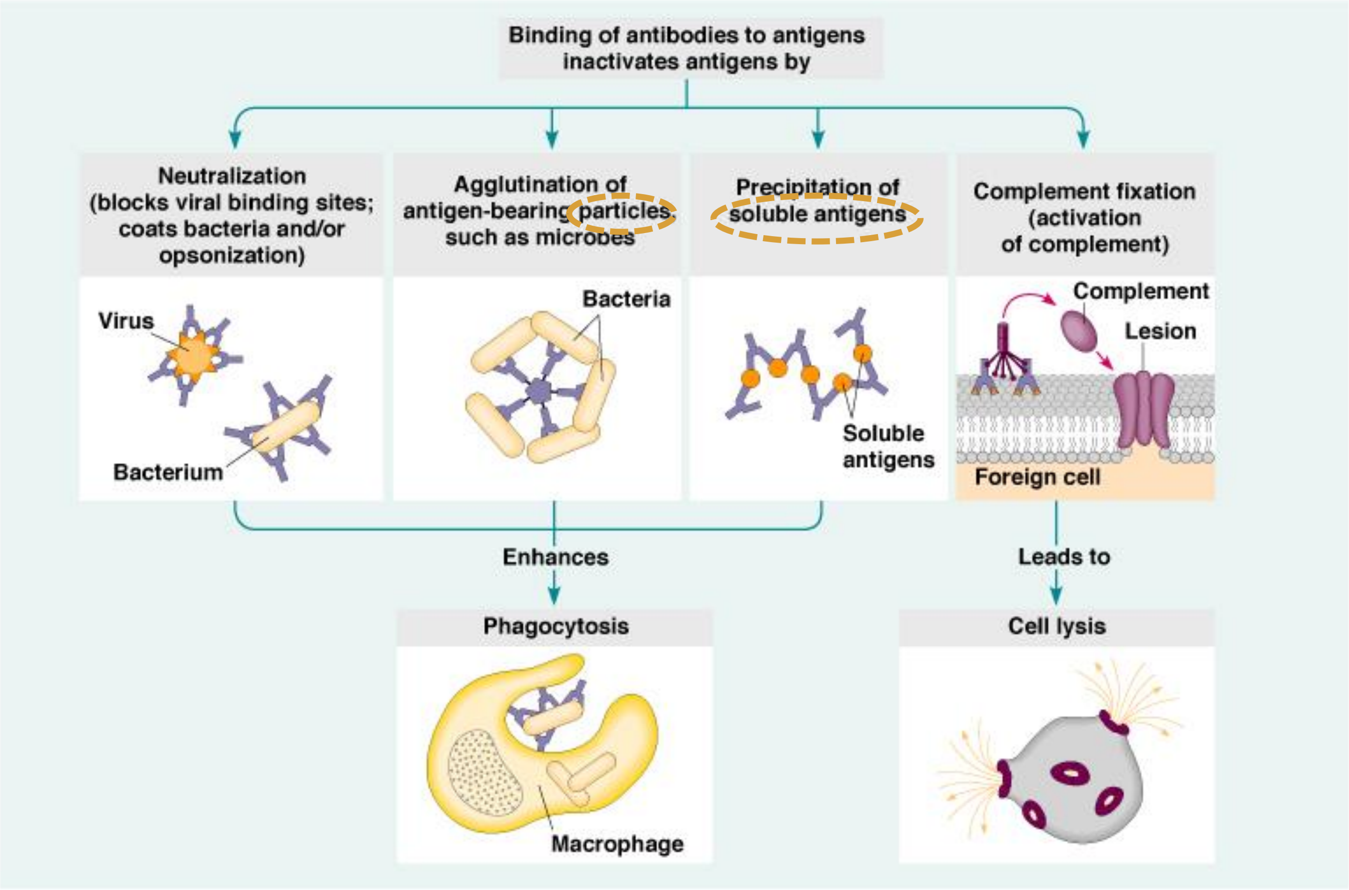
3. **NO DENATURATION**
   No denaturation of antigen or antibody.

4. **SURFACE ANTIGENS**
   Combination on surface antigens are immunologically relavant.

5. **REVERSIBLE**
   Combination is firm but reversible.

6. **AFFINITY & AVIDITY**

Consequences of Antibody Binding



1.

Precipitation

“

PRECIPITATION

Soluble antigen and antibody electrolytes

Suitable temperature and pH

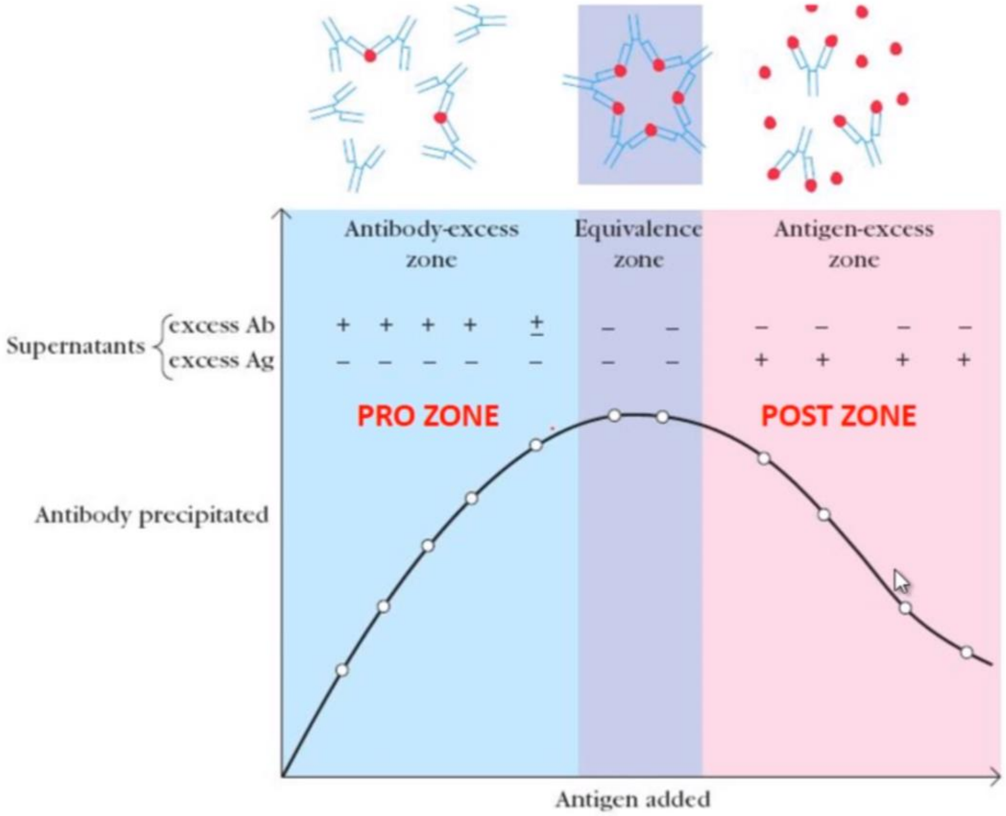
- Insoluble precipitate - **precipitation** → Liquid / Gel
- Suspended - floccules - **flocculation**

LATTICE THEORY

the interaction of multivalent antigen with multivalent antibody will, at optimum proportions of each (zone of equivalence), result in the formation of a lattice and a precipitate.

Ag excess = early infection.

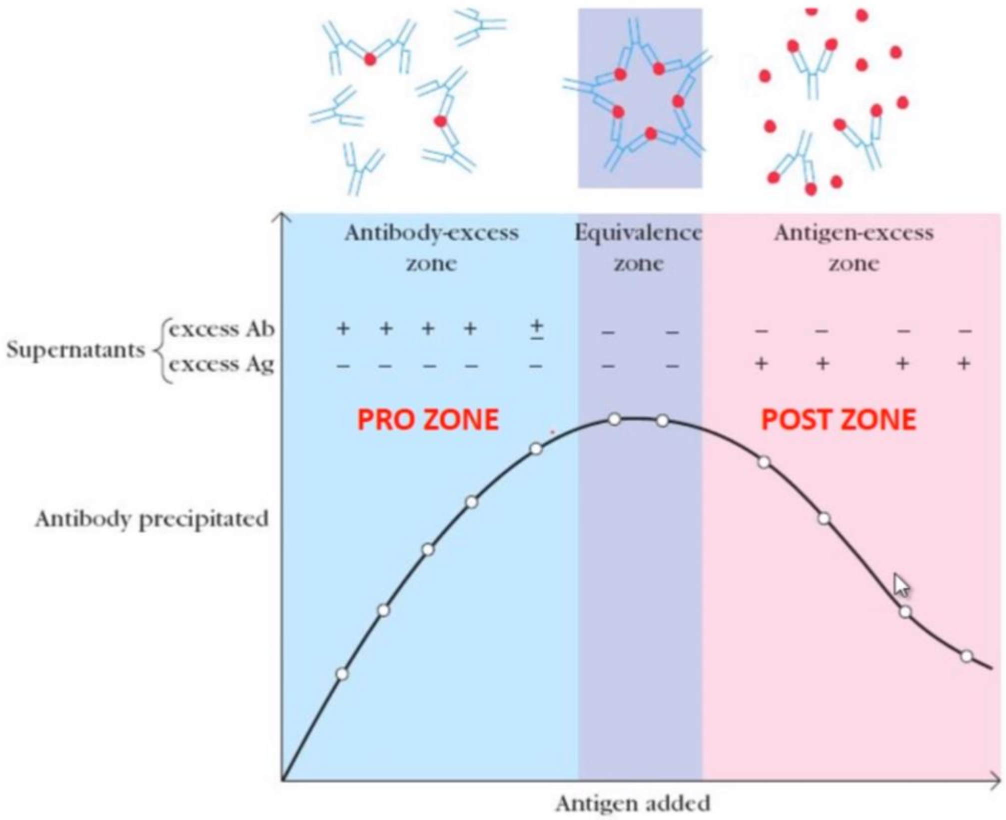
Ab excess= late in infection

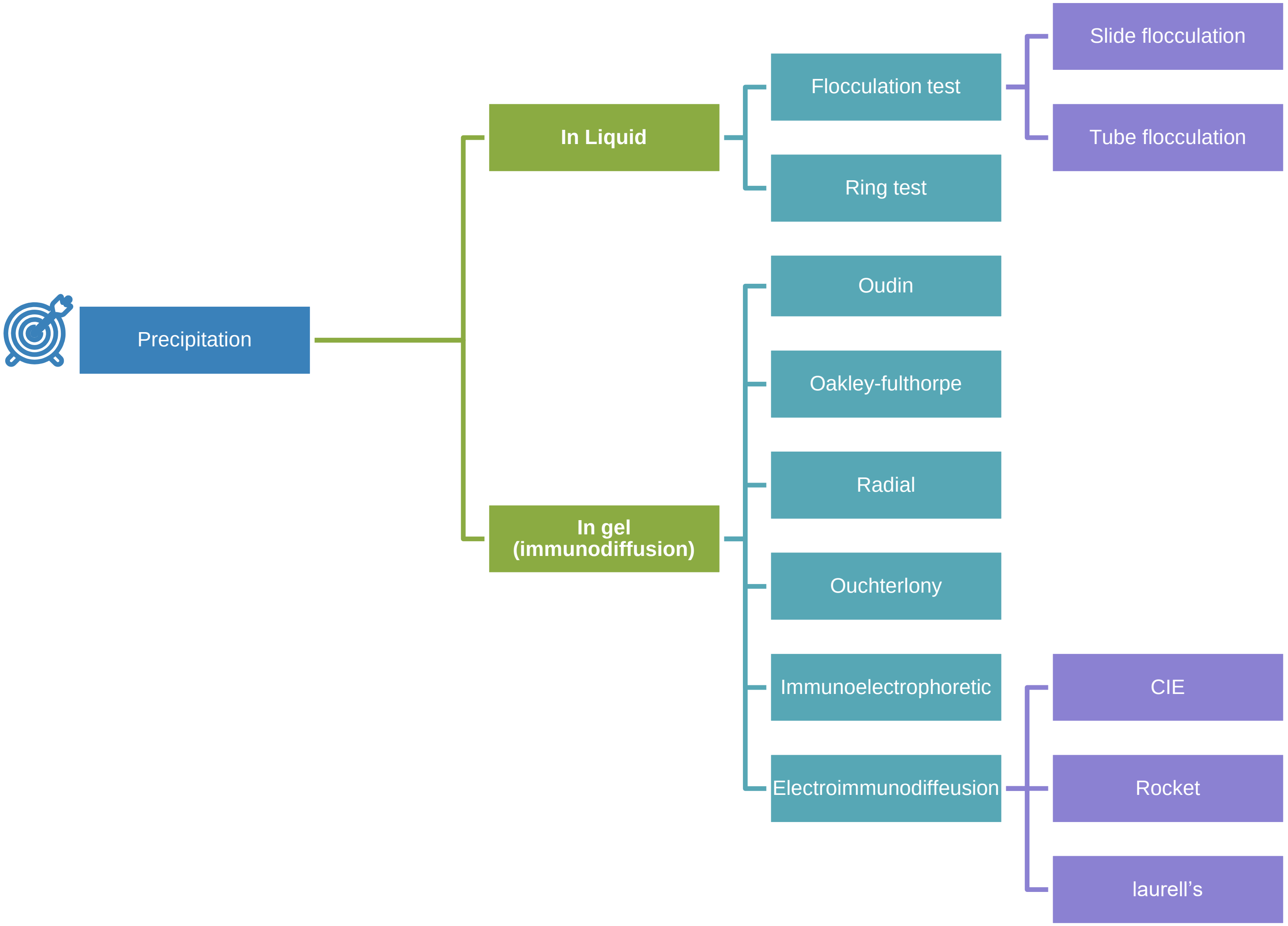


Zone of antibody excess (Prozone)
precipitation is inhibited and antibody not bound to antigen can be detected in the supernatant

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

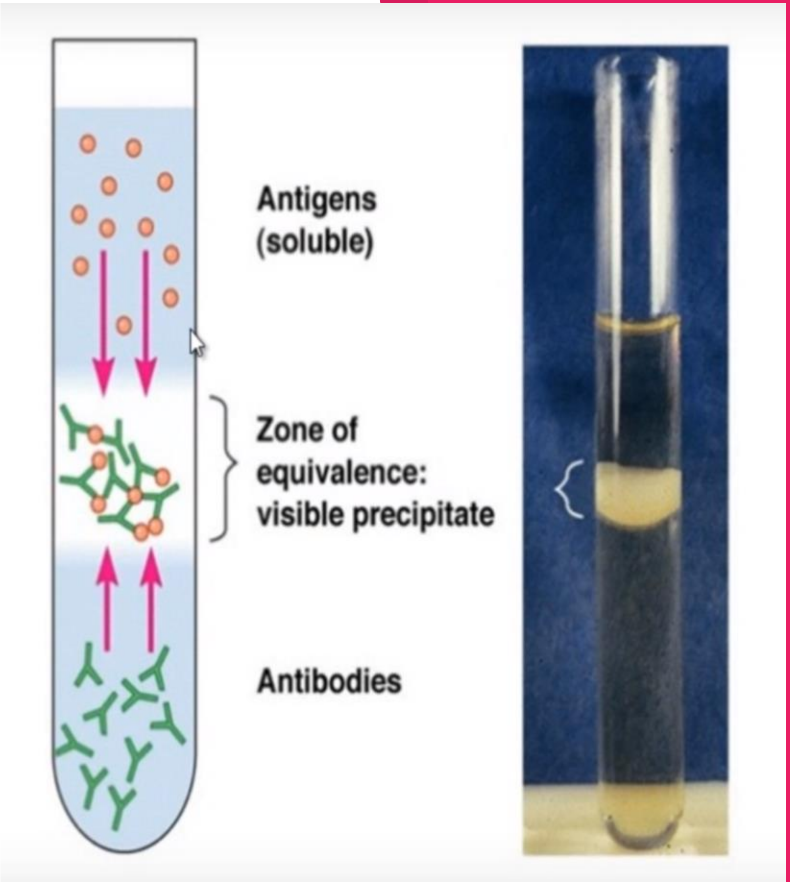
In liquid: Precipitation

(a) Ring Precipitate:

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

Example:

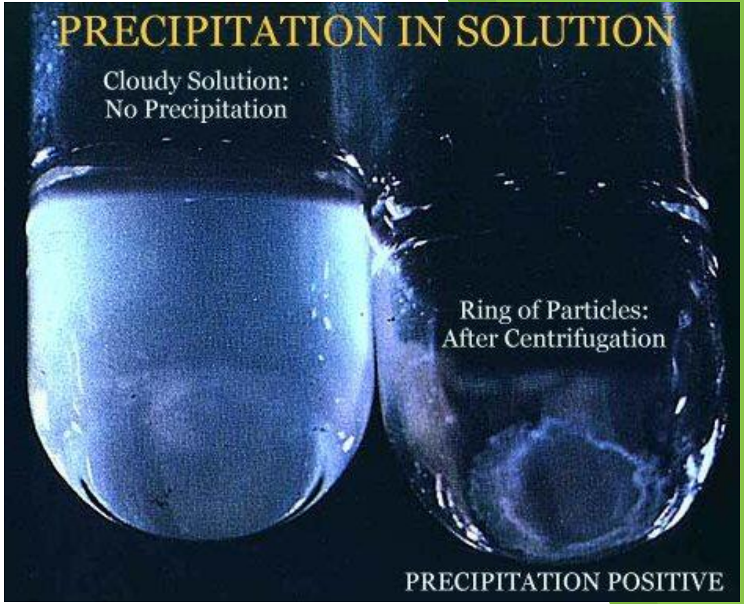
1. Ascoli's thermoprecipitin test → **Anthrax**
2. Lancefield grouping of streptococci



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

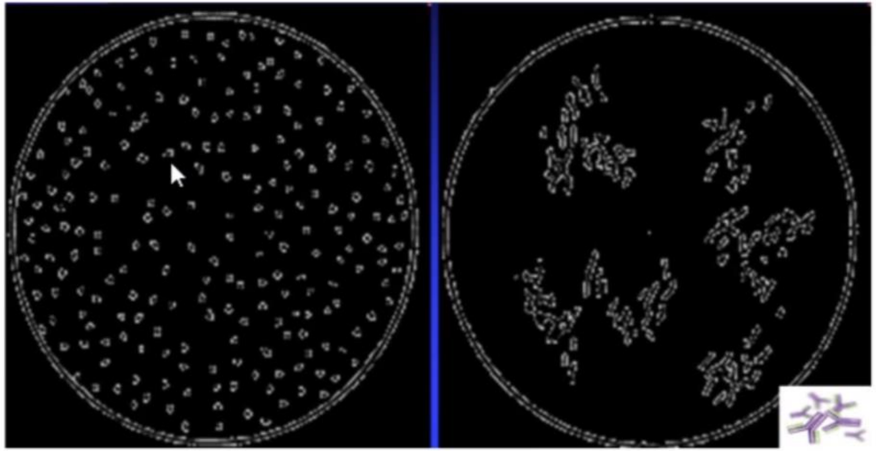
- Drop of antigen and antiserum on a slide – mixed by shaking – floccules appear

Example:

1. VDRL slide test – syphilis

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

VDRL



Negative VDRL test

Positive VDRL test

In liquid: Precipitation

(b) Flocculation test:

1- <u>Tube Flocculation test</u>

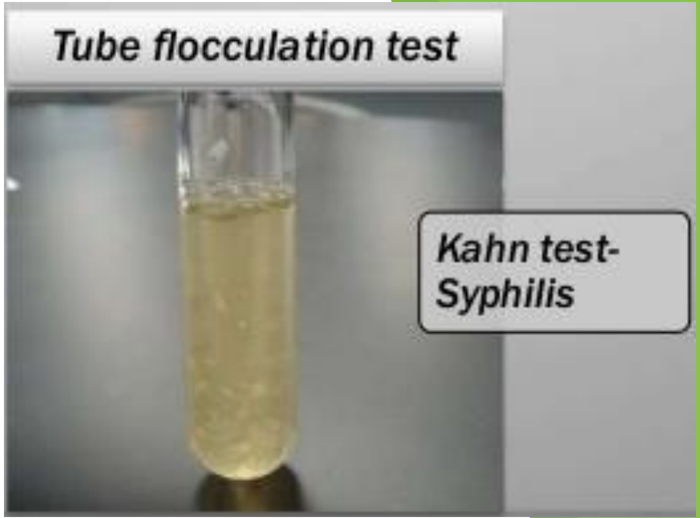
- Antigen and antiserum in a test tube– floccules appear

Example:

1. Kahn test for syphilis

↓

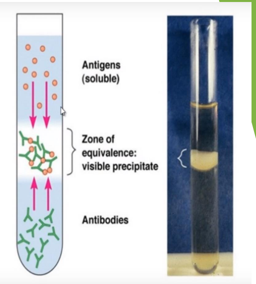
Kahn antigen – alcoholic extract of fresh beef heart with cholesterol + On reaction with syphilitic serum, floccules are formed which can be seen with the naked eye.



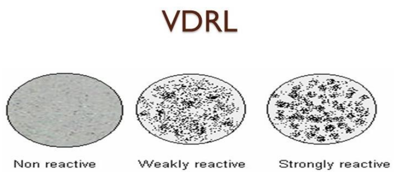
Precipitation

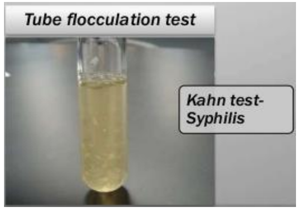
(a) **In Liquid**

- Flocculation test
  - Slide flocculation →
  - Tube flocculation →
- Ring test →



Lancefield grouping





In gel: Precipitation
(immunodiffusion)

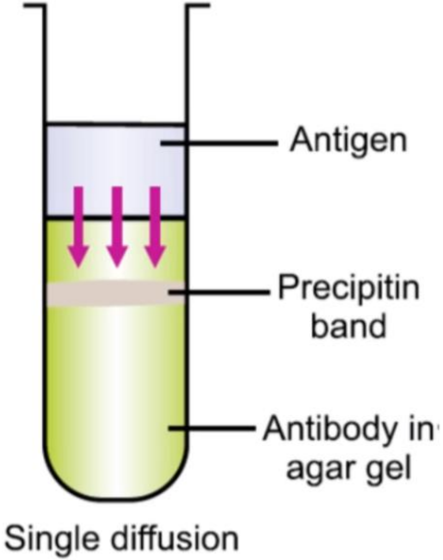
Why?

- 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)

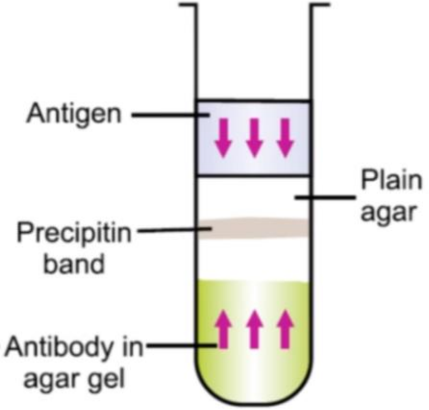
- Antibody - agar gel - test tube
- Antigen solution layered over it
- Antigen diffuses towards the agar gel, forming a line of precipitation



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

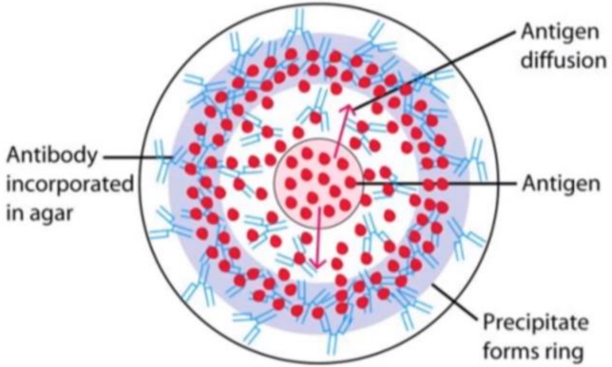


Double diffusion

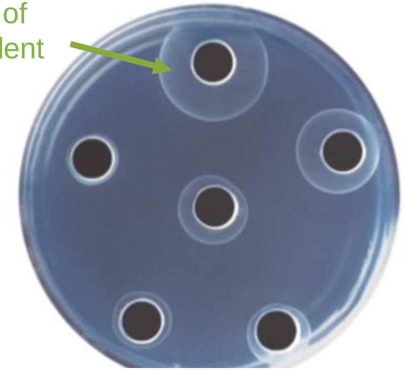
In gel: Precipitation
(immunodiffusion)

(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



Zone of equivalent



In gel: Precipitation
(immunodiffusion)

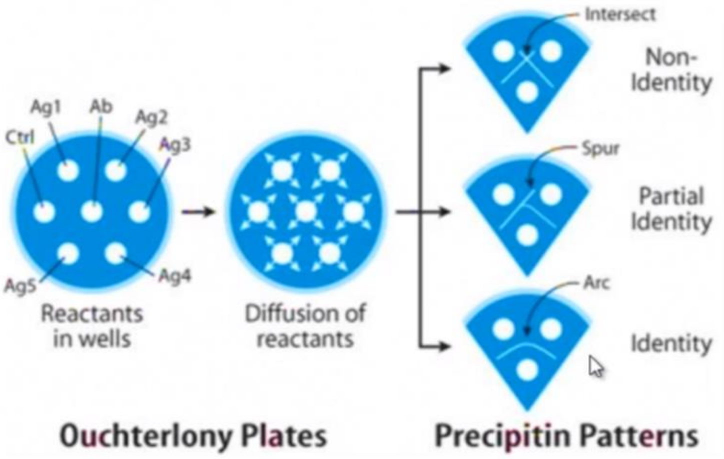
(d) **OUCHTERLONY IMMUNODIFFUSION (double diffusion – two dimensions)**

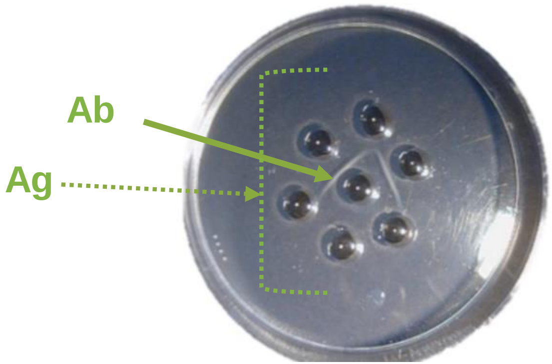
Most widely employed

- 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*



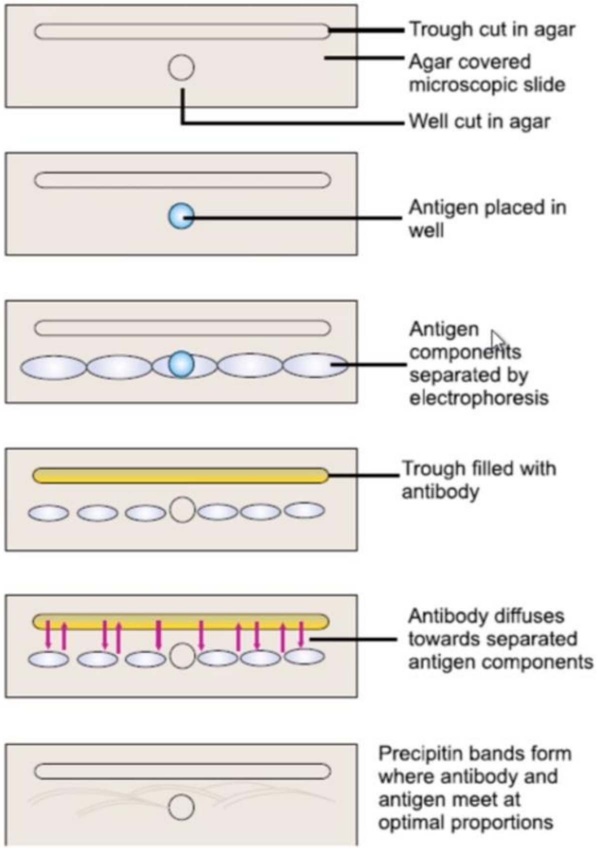


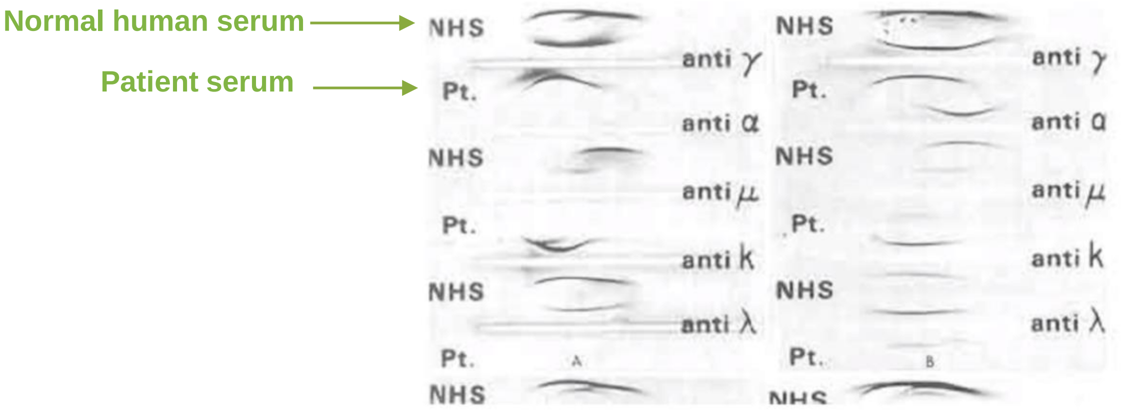
In gel: Precipitation
(immunodiffusion)

(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

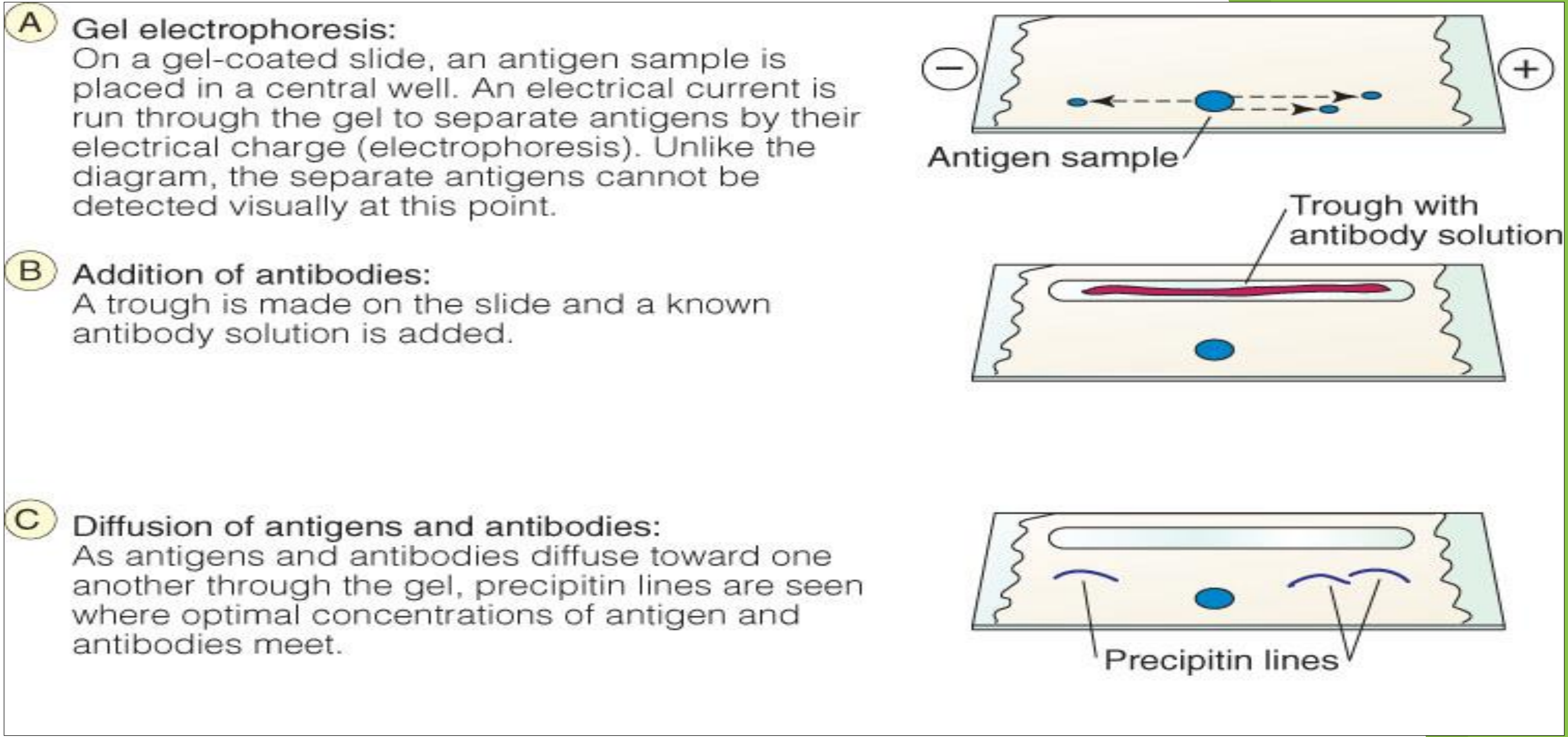




- Immunoelectrophoresis (IEP)

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

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



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.

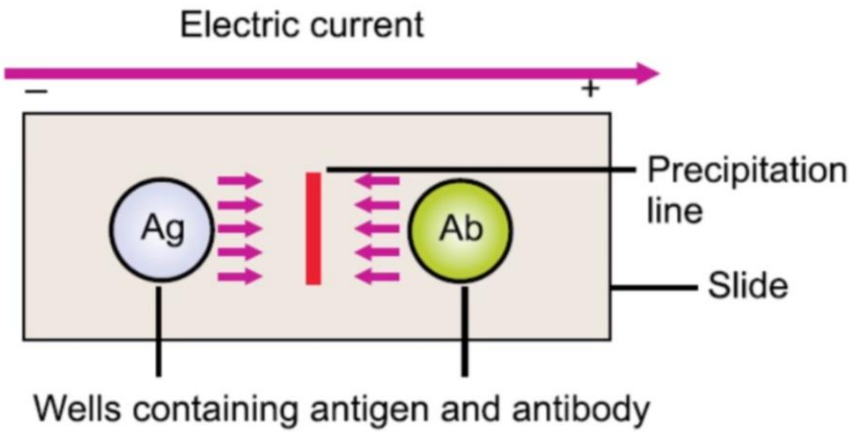
In gel: Precipitation
(immunodiffusion)

(e) ELECTROIMMUNODIFFUSION → (3 techniques)

1. Counter immunoelectrophoresis (CIE)

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

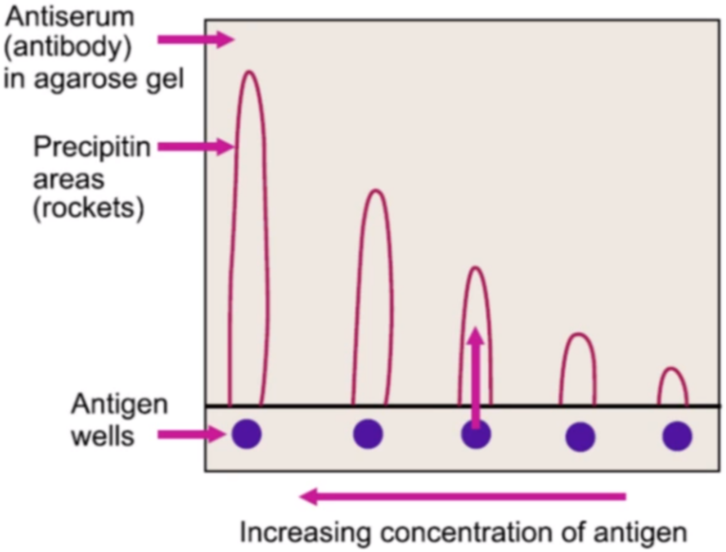
Example: ∝-fetoprotein,cryptococcal antigen

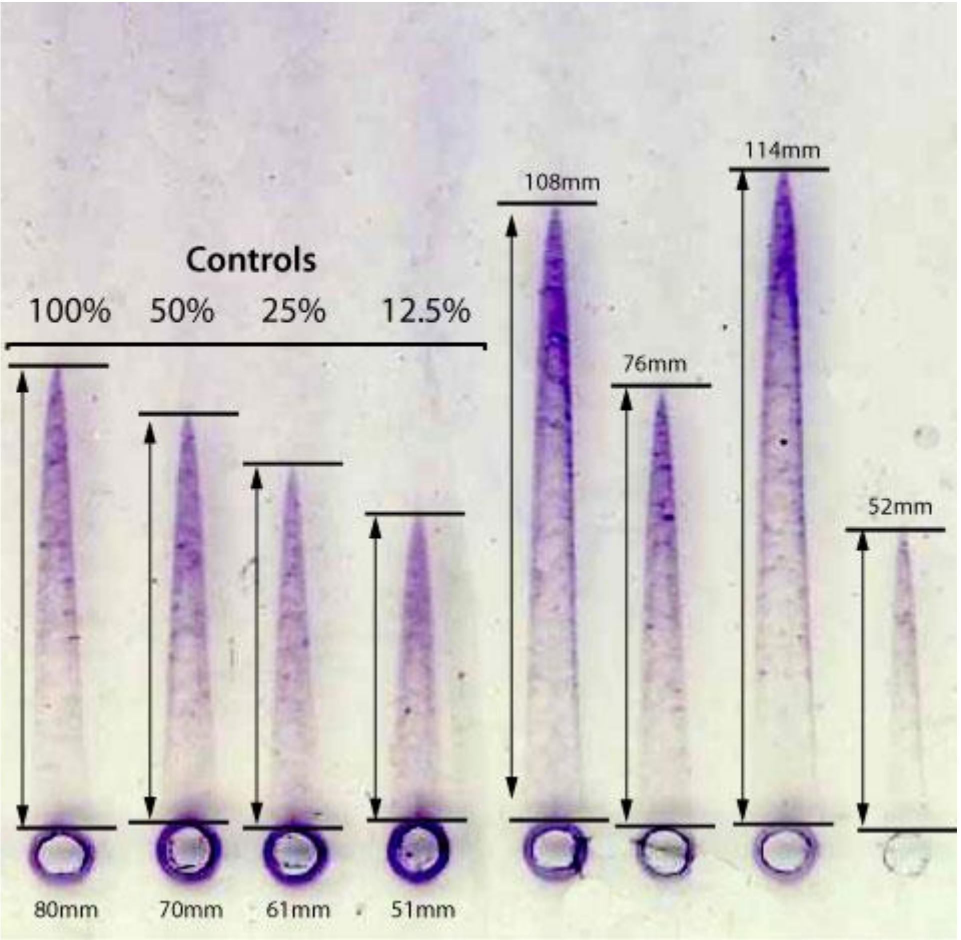


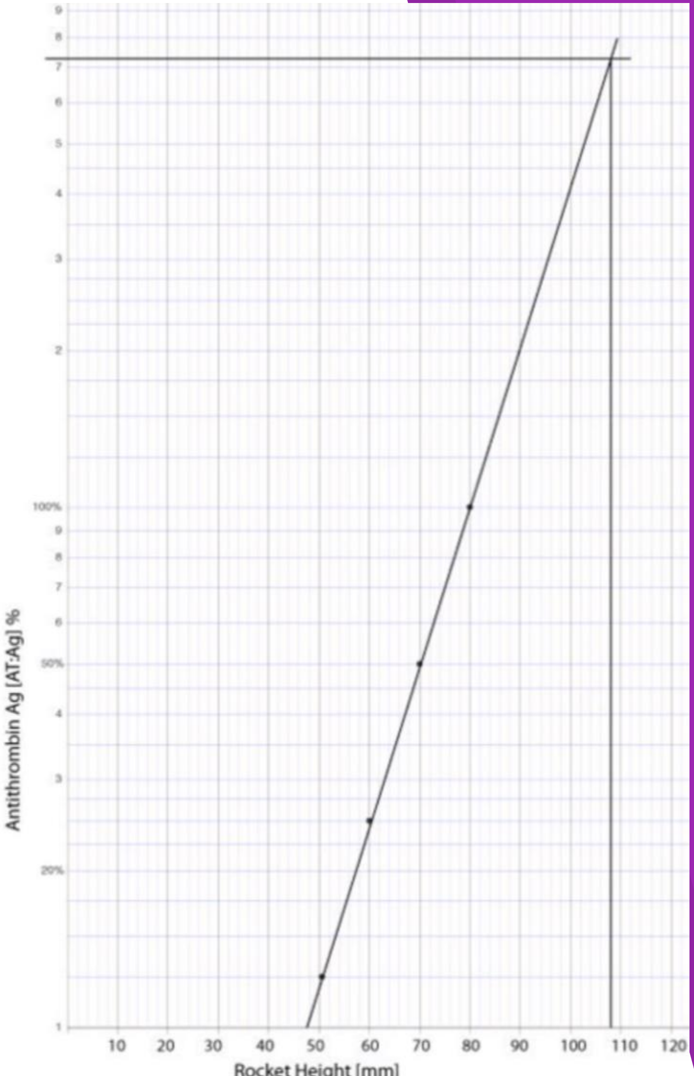
In gel: Precipitation
(immunodiffusion)

(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%
108mm
114mm
76mm
52mm
80mm
70mm
61mm
51mm

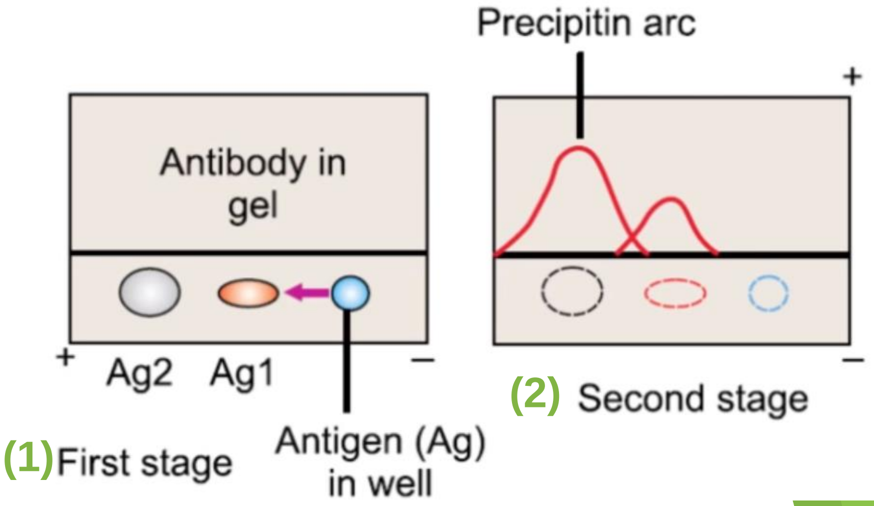

Antithrombin Ag [AT:Ag] %
100%
50%
20%
Rocket Height [mm]
10
20
30
40
50
60
70
80
90
100
110
120

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 → **Single** diffusion, **One** dimension
- Oakley-fulthorpe → **Double** diffusion, **One** dimension
- Radial → **Single** diffusion, **Two** dimension
- Ouchterlony → **Double** diffusion, **Two** dimension
- Immunoelectro phoresis
- Electroimmunodiffeusion
  - CIE
  - Rocket → **One** dimensional, **single** electrophoresis
  - laurell's → **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

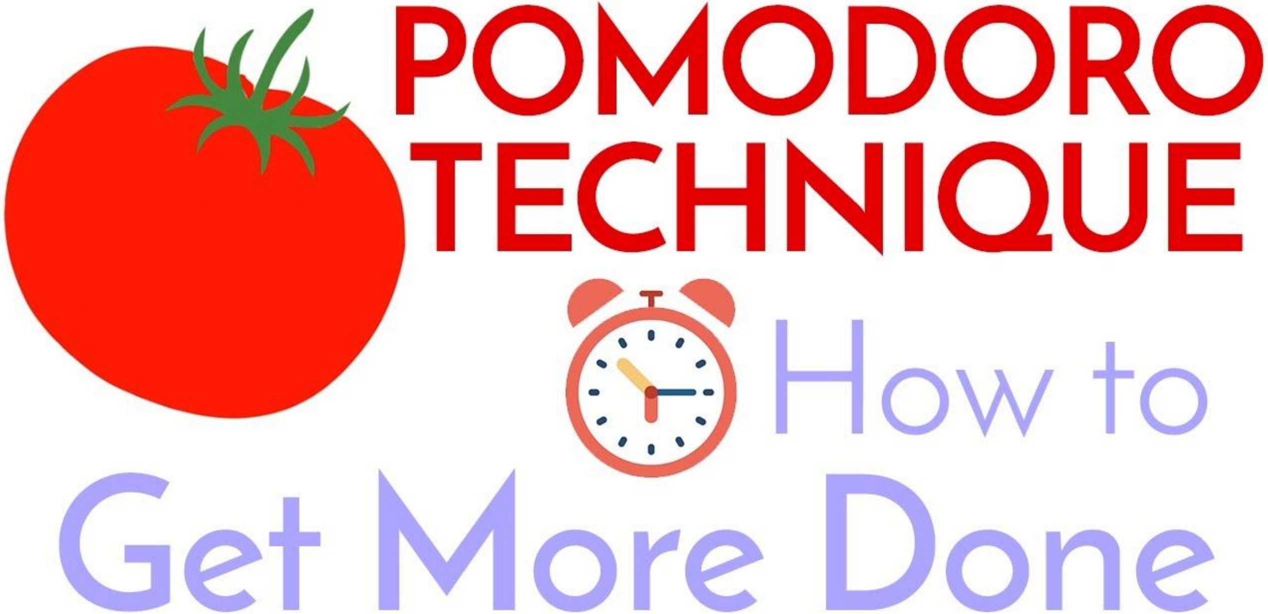
Passing light through a cloudy solution. (Net decrease in light intensity)

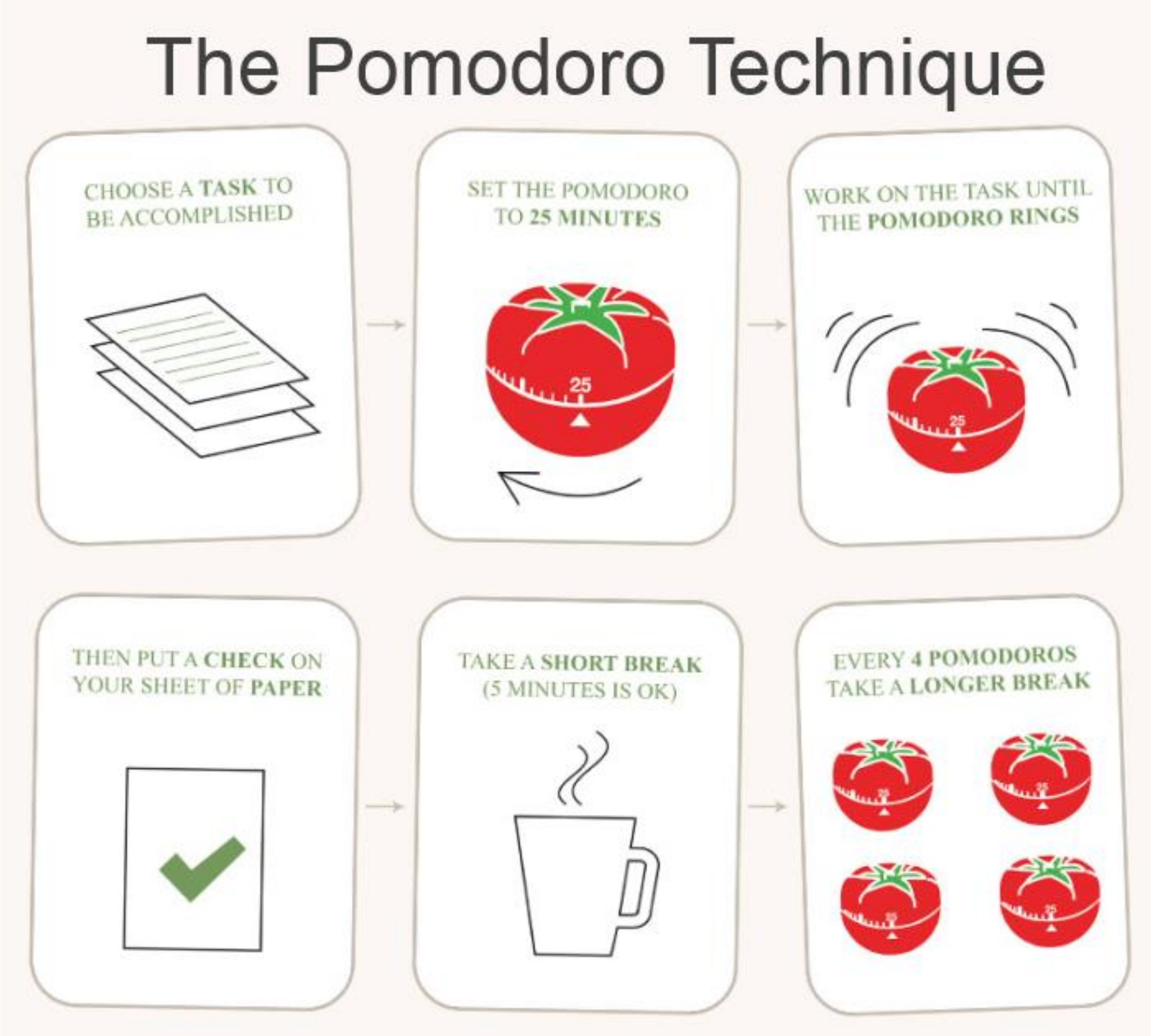
Nephelometry

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

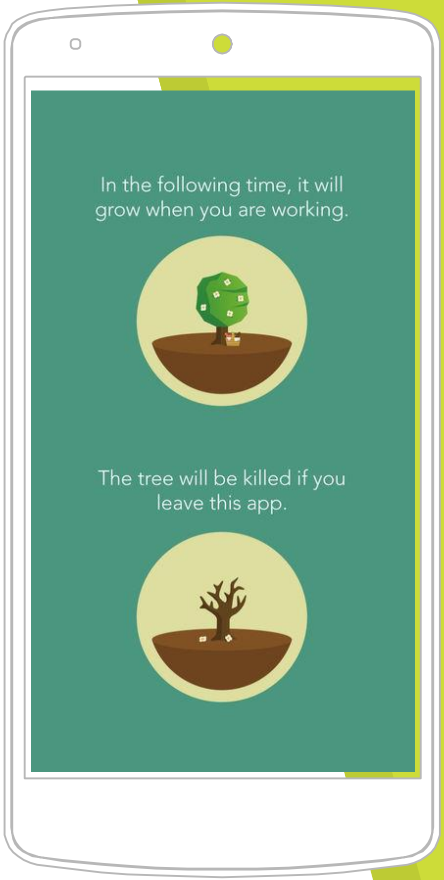

The Pomodoro Technique
CHOOSE A TASK TO BE ACCOMPLISHED
SET THE POMODORO TO 25 MINUTES
25
WORK ON THE TASK UNTIL THE POMODORO RINGS
THEN PUT A CHECK ON YOUR SHEET OF PAPER
TAKE A SHORT BREAK (5 MINUTES IS OK)
EVERY 4 POMODOROS TAKE A LONGER BREAK

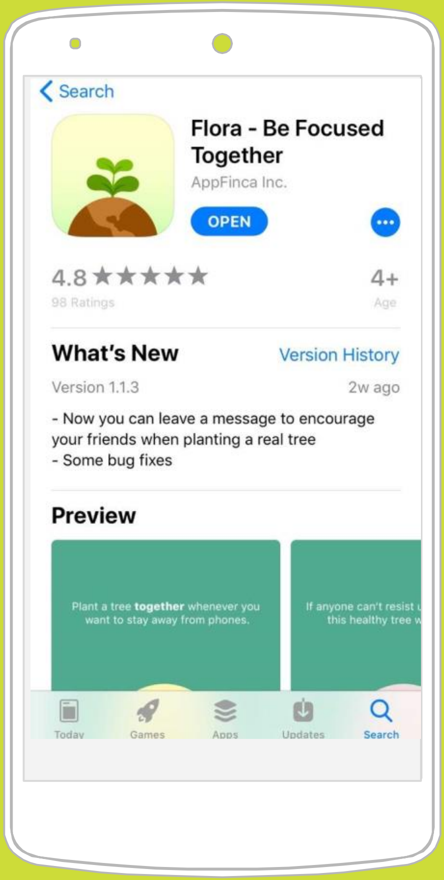
Pomodoro timer

http://www.tomatotimers.com/

Forest app

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





“ Assignment

- Pick **<u>one</u>** 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