Antigen-Antibody reactions (2)
Learning objectives:

❖ introduction to Antigen Antibody reactions.

❖ **Antigen Antibody reactions part 1**: Precipitation, Flocculation and Immunodiffusion.

❖ **Antigen Antibody reactions part 2**: Agglutination.

❖ **Antigen Antibody reactions part 3**: Complement Fixation Test.
1. Precipitation
Quotations are commonly printed as a means of inspiration and to invoke philosophical thoughts from the reader.

**PRECIPITATION**

*Soluble antigen* and antibody electrolytes
Suitable temperature and pH
- Insoluble precipitate - *precipitation*
- 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
**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
**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 venereal disease research laboratory)
(b) Flocculation test:

1- Tube Flocculation test

- Antigen and antiserum in a test tube—flocules appear

Example:

1. Kahn test for syphilis

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

Flocculation test
  - Slide flocculation
  - Tube flocculation

In Liquid

Ring test

Lancefield grouping

(a)
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
**In gel: Precipitation (immunodiffusion)**

(c) **RADIAL IMMUNODIFFUSION**
(specific for 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

[Diagram of radial immunodiffusion with labeled zones: Antigen incorporated in agar, Precipitate forms ring, 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 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.
In electro-immunodiffusion, diffusion is combined with electrophoresis. Electrophoresis separates antigen molecules according to differences in their electrical charges and molecular weight, and 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*: $\alpha$-fetoprotein, cryptococcal antigen

Electric current

Wells containing antigen and antibody
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

![Diagram showing antigen wells and precipitin areas](image)
In gel: Precipitation (immunodiffusion)

3. Laurell’s two-dimensional electrophoresis

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

![Diagram of antibody in gel with antigen mixture and precipitin arc]
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

- Immunoelectrophoresis

- Electroimmunodiffusion
  - CIE
  - Rocket
  - Laurell’s
    - 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

1. Choose a task to be accomplished.
2. Set the pomodoro to 25 minutes.
3. Work on the task until the pomodoro rings.
4. Then put a check on your sheet of paper.
5. Take a short break (5 minutes is ok).
6. 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?

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