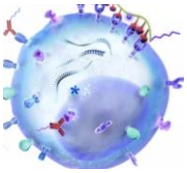


# IMMUNOLOGY - SEROLOGY

COURSE CODE | IS – LEC WEEK 1 – MIDTERMS

## Chapter 1: ANTIGEN – ANTI BODY BINDING

- **Affinity –** Affinity refers to the strength of binding between a single antigenic site and an antibody site

- **Avidity –** Measure of the overall strength of binding between multiple antigens and antibodies.

**Divalent IgG:** 2 binding sites
**Multivalent Pentameric IgM:** 10 binding sites

**PRECIPITATION:** Soluble antigen + antibody (in proportions) → visible precipitate.

**Optimal Precipitation** = Achieved at antigen-antibody equivalence.

## ZONE OF PRECIPITATION

- **Prozone:** Excess antibody
- **Zone of Equivalence:** Equal proportion of antigen and antibody
- **Postzone:** Excess antigen

## MEASUREMENT BY LIGHT SCATTERING

- **Immunoturbidimetry:** Measures turbidity or cloudiness of a solution.
- **Nephelometry:** Measures the light that is scattered at a particular angle relative to the incident beam.

## I. PASSIVE IMMUNODIFFUSION TECHNIQUES

**1. Radial Immunodiffusion (RID)**

- Principle: Single immunodiffusion technique; formation of a precipitation ring around antigenwell

**Setup:**

- Antibody (Ab) agarose gel.
- Antigen (Ag) into the well.

**Interpretation:** Diameter of the ring is proportional to the concentration of the antigen.

**Two Principal Methods:**

1. **Fahey (Kinetic Diffusion) Method:** Rings measured at 19 hours before reaching zone of equivalence (F – FASTER)
2. **Mancini (Endpoint Diffusion) Method:** Diffuses for 24–72 hours (M - MATAGAL).

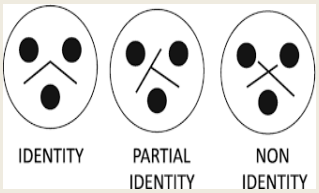
**Sources of Error:** Overfilling of the sample wells.

2. **Ouchterlony Technique**
**Type:** Double diffusion method.

- **Application:** Used for autoantibody detection in autoimmune diseases.
- **Setup:** Multiple wells containing Ab and Ag loaded in gel.

Interpretation:

- **Identity:** Smooth curve → Same epitopes.
- **Non-Identity:** Crossed lines → Different epitopes.
- **Partial Identity:** Spur formation → Similar antigens.




**Simpler antigen** = less epitope reactivity. The spur points toward the simpler antigen.

## II. ELECTROPHORETIC TECHNIQUES

1. **Rocket Electrophoresis**

- **Also Known As:** Laurell Technique (developed by Carl Laurell).

**Method:**

- Ab in agarose gel.
- Unknown Ag placed in the well and driven by an **electric current.**

**Result:** Rocket-shaped precipitation

2. **Immunoelectrophoresis**
**Method:**
- Antibody (Ab) placed in the trough.
- Unknown Antigen (Ag) loaded in the well.

**How It Works:**
- Serum is electrophoresed (electrophoresis).
- Ab placed on trough parallel line (reaction)

**Purpose:** Screening major Ig classes (IgG, IgA, IgM) in blood or urine.

**Results Interpretation:**
**Normal Result:**
- Uniform, symmetrical arcs.
- No distortion, thickening, or unexpected doubling of arcs.
- Balanced proportions of kappa and lambda light chains.

**Abnormal Result:**
- **M-spike (Monoclonal Gammopathy):** Sharp, distorted, bending arc.
- **Waldenström Macroglobulinemia:** Increased localized thickening in the IgM arc.
- **Hypogammaglobulinemia / Immunodeficiencies:** Faint or missing arcs for IgG, IgA, or IgM.
- **Polyclonal Increase:** Broad thickening across an entire immunoglobulin region.

3**. Immunofixation Electrophoresis (IFE)**
- Similar to immunoelectrophoresis, except antiserum is applied **directly** to the gel surface.
- Diffusion path is shorter. faster execution than standard Immunoelectrophoresis

## Chapter 2: AGGLUTINATION

**AGGLUTINATION**: Particulate antigen + antibody → clumping.
- **Example:** Blood bank direct agglutination (similar to precipitation, except antibody occurs with suspended particulate antigen).

**Two Phases of Agglutination:**

- **Sensitization:** Binding of antigen to antibody (no visible reaction).
- **Lattice Formation:** Cross-linking between multiple antigens and antibodies (visible reaction).

**ENHANCEMENT**

**IgG Antibodies:** Require anti-human globulin (AHG) to view clumping.

**Coombs Reagent:** Anti-human immunoglobulin used to enhance agglutination.

## TYPES OF AGGLUTINATION REACTIONS

1. **Direct Agglutination**
- Tests that have as their endpoint the agglutination of a particulate antigen naturally found on the cell/particle.
- **Agglutinin:** Antibody that causes cells antigens to clump together.

2. **Hemagglutination –** Involves RBC agglutination reaction

## QUALITATIVE AGGLUTINATION TESTS

**1. Passive (Indirect) Agglutination**
- Uses particles that are coated with antigens not normally found on their surface.
- Antigen attaches to a particular "carrier" particle.
- **Carriers Used:** Latex, gelatin, erythrocytes.
- Binds high concentration.

**Reverse Passive Agglutination**
- Same concept as passive agglutination, but antibody is **attached to the carrier** particle instead.

**Agglutination Inhibition**
**Step 1:** Soluble Ag in patient sample is incubated with known Ab reagent.
**Step 2:**
- **Agglutination (−):** Negative result (Agglutination occurs)
- **Inhibit (+):** Positive result (no agglutination occurs).