

7) Immunoassays and serology

I. Fundamentals of Serology

- **Definition:** The study of **in vitro** reactions between antigens and antibodies.
- **Immune Specificity:** The core principle where an antibody reacts **only** with its inducing antigen.
- **Cross-reactions:** These can occur between related antigens, which may limit the specificity of a test.
- **Titers:** A **titer** is the **highest dilution** yielding a positive reaction. A **higher denominator** (e.g., 1/64 vs 1/4) indicates a **higher concentration** of antibodies.
- **Diagnostic Criteria:**
 - A fourfold or greater rise in IgG antibody titer between acute and convalescent samples is required for diagnosis.
 - **IgM** titers suggest a **current/recent infection**.
 - **IgG** titers usually indicate **past exposure or immunity**.

II. Major Diagnostic Test Types

1. Agglutination & Precipitation

- **Agglutination:** Involves **particulate antigens** (e.g., bacteria, red cells, or latex beads). Multivalent antibodies cross-link these into a visible **lattice structure** (clumping).
- **Precipitation:** Involves **soluble antigens**.
 - **Zone of Equivalence:** The state of optimal proportions where **maximal lattice formation** occurs.
 - **Prozone Effect: Antibody excess** inhibits lattice formation, potentially causing **false-negative** results.
 - **Postzone: Antigen excess** prevents visible precipitation.
- **Nephelometry:** Measures immunoglobulins in plasma by light refraction (**optical density**).

2. Labeled Immunoassays

- **Radioimmunoassay (RIA):** Highly sensitive; **radio-labeled antigens** compete with patient antigens. Radioactivity is **inversely proportional** to the patient's antigen concentration.
- **ELISA:** Uses **enzyme-linked** conjugates instead of radiation. The resulting **color intensity** (measured by a spectrophotometer) correlates with the amount of patient antibody.
- **Western Blot (Immunoblot):** Used to **confirm** positive screening results. Proteins are separated by **electrophoresis** before reacting with patient serum.

- **Immunofluorescence:**

- **Direct:** Labeled antibodies are applied directly to **tissue/biopsy** specimens for rapid diagnosis.
 - **Indirect:** A **two-stage** test; more sensitive than the direct method.

III. Specialized Clinical Tests

- **Complement Fixation:** Ag-Ab complexes **fix (consume)** complement.
 - **Positive Result: No hemolysis** (complement was consumed).
 - **Negative Result: Hemolysis** (complement remained free to lyse indicator RBCs).
- **Coombs Test (Antiglobulin):** Uses **Coombs reagent** (anti-human Ig) to detect antibodies on RBCs.
 - **Direct Coombs:** Detects antibodies bound to RBCs **in vivo** (e.g., HDN).
 - **Indirect Coombs:** Detects antibodies in the **serum**; essential for **blood transfusion cross-matching**.
- **Flow Cytometry:** Uses **lasers** to count single cells labeled with **fluorescent monoclonal antibodies** (e.g., CD4 counts).

IV. Blood Groups & Compatibility

ABO and Rh Systems

- **Universal Donor: Group O** (lacks A and B antigens).
- **Universal Recipient: Group AB** (lacks Anti-A and Anti-B antibodies).
- **Rh System:** Defined by the **D antigen**. Rh antibodies are **IgG** and are **not** naturally occurring.
- **Hemolytic Disease of the Newborn (HDN):** An **Rh-negative mother** produces **IgG** antibodies that **cross the placenta** and attack Rh-positive fetal RBCs.
 - **Prevention: RhoGAM** injection at 28 weeks and delivery to prevent maternal sensitization.

Summary Table: Diagnostic Immunoassays

Test Type	Antigen State	Key Principle/Detection	Major Clinical Use
Agglutination	Particulate	Visible clumping (lattice)	ABO Typing, Latex tests
Precipitation	Soluble	Zone of Equivalence	Nephelometry (Ig levels)
RIA	Soluble	Radioactive competition	Hormones, Drugs, RAST
ELISA	Fixed/Known	Enzyme-linked color change	HIV/Pathogen Screening
Western Blot	Electrophoresed	Protein band visualization	Confirmation of HIV
Direct IF	Tissue Biopsy	One-step fluorescent labeling	Rapid biopsy diagnosis
Complement Fixation	Variable	Positive = No Hemolysis	Fungal/Viral serology
Direct Coombs	Bound to RBC	Anti-human Ig (Coombs reagent)	Autoimmune Hemolytic Anemia
Indirect Coombs	Serum	Mixing serum with normal RBCs	Cross-matching blood
Flow Cytometry	Single Cells	Laser & Fluorescent markers	CD4+ counts in HIV