# Serology and Immunoassays

اللهم إني أسألك فهمَ النبيّين وحفظَ المرسلين وإلهامَ الملائكة المقرّبين،
اللهم اجعل لساني عامرًا بذكرك، وقلبي بخشيتك، وسِرّي بطاعتك،
اللهم افتح عليّ فتوح العارفين، ووفّقني وبارك لي في وقتي وجهدي، واجعل علمي حُجّةً لي لا عليّ

## 1) Core ideas (Serology)

- Serology studies **in vitro** reactions between **antigens** and **antibodies**; it is essential for identifying infections.
- The system relies on **immune specificity**: an antibody reacts only with its inducing antigen.
- **Cross-reactions** can occur between related antigens, limiting a test's specific utility.
- Serology is used for **infectious diseases**, **autoimmune disorders**, and is critical for **blood and tissue typing**.

### Major uses of serologic tests

- Diagnose infections when organisms are hard or dangerous to culture (e.g., syphilis, hepatitis viruses, Rickettsia).
- Helpful when organisms grow too slowly; provides answers when cultures would take weeks.
- Detect autoimmune antibodies against body components (e.g., anti-DNA antibodies in lupus, rheumatoid factor).
- Use known antibodies to determine ABO and Rh blood types and identify HLA antigens before transplantation.

## 2) Titers (how to interpret antibody levels)

- A **titer** is the **highest dilution** of serum yielding a positive reaction; it quantifies antibody concentration.
- A higher denominator indicates more antibodies (e.g., 1/64 implies more antibodies than 1/4).
- A **fourfold IgG rise** between acute and convalescent samples is required.
- High early **IgM** titers suggest a current infection; **IgG** titers usually indicate past exposure or immunity.

## 3) Overview: diagnostic test families (from the slides)

| Test family | Antigen type / principle | Output / what you see | Key notes (as stated in slides) |
|---|---|---|---|
| Agglutination | Particulate antigens (e.g., bacteria) or inert particles (latex beads); multivalent antibodies cross-link particles (lattice). | Visible clumping on a slide. | Simple and rapid; minimal equipment. Used in ABO blood typing and latex agglutination; effective screening tool. |
| Precipitation | Soluble antigens; antibodies cross-link molecules into aggregates. Requires zone of equivalence; both Ag and Ab must be multivalent. | Precipitate (solution) or precipitin lines (agar). | Reaction fails if ratios are unbalanced: antibody excess (prozone) or antigen excess (postzone). |

| | | | |
|---|---|---|---|
| Nephelometry | Precipitation in solution used to measure immunoglobulins in plasma. | Optical density (light refraction) proportional to precipitate. | Value is compared with a standard curve. |
| Immunodiffusion | Precipitation in agar: single radial diffusion or double diffusion (Ouchterlony). | Rings (single radial) or precipitin lines (double diffusion). | Ring size indicates antigen concentration; patterns indicate antigen identity or non-identity. |
| Immunoelectrophoresis | Electrophoresis + immunodiffusion: serum proteins separated by charge, then antibody diffusion forms arcs. | Precipitin arcs. | Arc shapes reveal specific proteins; detects abnormal globulin production (e.g., myeloma proteins). |

| Test family | Principle | Readout | Key notes (as stated in slides) |
|---|---|---|---|
| RIA | Radio-labeled antigens compete with patient antigens for antibody binding sites. | Less radioactivity in complex = more patient antigen (inverse relationship). | Highly sensitive quantitative; measures hormones and drugs. RAST is a specialized RIA for IgE antibodies. |
| ELISA | Uses enzyme-linked conjugates (no radiation). Enzyme converts substrate to a colored product. | Color measured by spectrophotometer as optical density / absorbance. | As sensitive as RIA; no radioactive waste; standard screening tool; safe and widely available. |
| Immunofluorescence | Fluorescent dyes on antibodies detect antigens in tissues/cells. | Fluorescence under microscope. | Direct: one-step rapid antigen detection in biopsy specimens. Indirect: two-stage; more sensitive than direct. |
| Complement fixation | Ag-Ab complexes fix (consume) complement; second stage detects free complement using sensitized RBCs. | No hemolysis vs hemolysis. | Endogenous serum complement must be inactivated first by heating. No hemolysis = positive; hemolysis = negative. |
| Western blot | Viral proteins separated by electrophoresis then transferred to filter paper; patient serum binds specific protein bands. | Bands visualized using enzyme-labeled anti-IgG. | Confirms positive screening results; confirms specific antibodies are present. |

## 4) Agglutination vs precipitation (key differences)

| Feature | Agglutination | Precipitation |
|---|---|---|
| Antigen type | Particulate (bacteria, RBCs) or inert latex beads. | Soluble antigens. |
| Mechanism | Multivalent antibodies cross-link particles -> lattice -> visible clumping. | Antibodies cross-link soluble molecules -> aggregates -> fall out of solution. |
| Readout | Immediate visible clumping (often on a slide). | Visible precipitate or precipitin lines; depends on zone of equivalence. |
| Key caveats | Used as screening tools with immediate visual results. | False negatives can occur with prozone (Ab excess) or postzone (Ag excess). |
| Examples in slides | ABO typing; latex agglutination; hemagglutination. | Immunodiffusion; nephelometry; precipitin curve concepts. |

# 5) Precipitation-based tests and diffusion methods

## Precipitin curve and the prozone/postzone effects

- The precipitin curve plots reaction efficiency; the peak is the **zone of equivalence**.
- **Prozone**: antibody excess inhibits lattice formation -> can cause **false-negative** results; labs **dilute serum** to fix.
- **Postzone**: antigen excess prevents visible precipitation; complexes are too small to settle.

## Precipitation in solution: nephelometry

- Used to measure immunoglobulins in plasma.
- Nephelometry measures precipitate amount by optical density (light refraction) and compares with a standard curve.

## Immunodiffusion (in agar)

- Single radial diffusion: antibody is mixed into agar; antigen diffuses from a well to form a ring; ring size indicates antigen concentration (e.g., IgG, IgM, complement).
- Double diffusion (Ouchterlony): antigen and antibody diffuse toward each other; precipitin lines form where concentrations meet; patterns indicate antigen identity or non-identity.
- Ouchterlony patterns shown in the slides include: identity, nonidentity, and partial identity.

## Immunoelectrophoresis and counter-immunoelectrophoresis

- Immunoelectrophoresis combines electrophoresis with immunodiffusion: serum proteins are separated by charge, then antibody diffusion forms precipitin arcs.
- Arc shapes reveal specific proteins and can detect abnormal globulin production (e.g., myeloma proteins).
- Counter-immunoelectrophoresis uses electricity to drive antigen and antibody together rapidly.

## 6) RIA vs ELISA (high-sensitivity immunoassays)

| Feature | RIA | ELISA |
|---|---|---|
| Label | Radio-labeled antigen. | Enzyme-linked conjugates (no radiation). |
| Principle | Competition: radio-labeled Ag competes with patient Ag for Ab binding sites. | Enzyme converts substrate to colored product. |
| Relationship | Less radioactivity in complex means more patient antigen (inverse). | Color intensity (optical density/absorbance) indicates antibody level; intensity correlates with amount of patient antibody (as described in ELISA methodology slide). |
| Uses in slides | Measures hormones and drugs; RAST is a specialized RIA for IgE antibodies. | Standard screening tool; safe and widely available. |

### ELISA methodology (as shown in the slides)

- Known antigen is attached to a surface; patient serum is added.
- Patient antibodies bind the fixed antigen; an enzyme-linked anti-human IgG is added as secondary antibody.
- Substrate is added to react with the enzyme; resulting color indicates antibody level; intensity correlates with amount of patient antibody.

## 7) Immunofluorescence and Western blot

| Feature | Direct immunofluorescence | Indirect immunofluorescence |
|---|---|---|
| Steps | One-step: labeled antibodies applied directly to tissue. | Two-stage: patient serum reacts with fixed antigen first, then labeled anti-human IgG is added. |
| Target | Detects antigens in biopsy specimens. | Detects patient antibodies (through labeled anti-human IgG). |
| Sensitivity | Rapid diagnosis. | More sensitive than the direct test. |
| Readout | Fluorescence under UV using a fluorescence microscope. | Fluorescence under UV using a fluorescence microscope. |

### Western blot (immunoblot) - confirmation test

- Used to confirm positive screening results.
- Viral proteins are separated by electrophoresis and transferred to filter paper.
- Patient serum is applied; antibodies bind specific protein bands.
- Enzyme-labeled anti-IgG visualizes the bands, confirming specific antibodies are present.

## 8) Complement fixation test (CFT): principle and interpretation

- Ag-Ab complexes fix (consume) complement.
- Patient serum is mixed with known antigen and complement: if antibodies are present, complement is fixed; if absent, complement remains free.

- Endogenous serum complement must be inactivated first by heating the serum.

| Indicator step (sensitized RBCs) | What happens? | Meaning |
|---|---|---|
| Free complement present | RBCs lyse (hemolysis). | Negative: complement was left free to attack RBCs. |
| Complement consumed (fixed) | No hemolysis. | Positive: complement was consumed by patient antibodies. |

## 9) Neutralization and hemagglutination tests

| Neutralization tests (slides) | What they measure |
|---|---|
| Principle | Antibodies block toxin or viral effects; biological activity is measured. |
| Cell culture example | Antibodies prevent viral damage in cell culture (inhibition of cytopathic effects). |
| Host animal example | Antibodies protect mice from lethal toxins by neutralizing them. |
| Specificity | Highly specific. |
| Named example | Plaque Reduction Neutralization Test (PRNT). |

| Hemagglutination tests | Key points from the slides |
|---|---|
| Active hemagglutination | Some viruses naturally clump RBCs (e.g., influenza viruses). |
| Hemagglutination inhibition | Patient antibodies can inhibit this clumping (basis of inhibition tests). |
| Passive hemagglutination | Uses antigen-coated RBCs as carriers for soluble antigens; antibodies cross-link cells to cause clumping, making precipitation reactions visible. |

## 10) Antiglobulin (Coombs) test

- In some diseases (e.g., hemolytic disease of the newborn [Rh incompatibility] and drug-related hemolytic anemias), patients become sensitized but do not exhibit symptoms.
- Antibodies against RBCs form and bind to the RBC surface but do not cause hemolysis.
- Coombs reagent is an anti-human immunoglobulin: it binds to these human antibodies on RBCs causing agglutination.
- The test diagnoses hemolytic anemias by detecting antibodies on RBCs (direct) or in serum (indirect).

| Direct vs Indirect Coombs | Direct Coombs | Indirect Coombs |
|---|---|---|
| What it detects | Bound antibodies **in vivo** on washed patient RBCs. | Antibodies in the patient's serum (serum + normal RBCs + anti-human immunoglobulins). |
| Slide examples | Autoimmune hemolytic anemia; hemolytic disease of the newborn. | Crucial for blood transfusion cross-matching to prevent transfusion reactions. |
| Positive result | Agglutination after adding Coombs reagent. | Agglutination occurs if antibodies are present in serum. |

## 11) Flow cytometry (slides)

- Counts cells in a fluid stream using laser analysis of single cells.
- Cells are labeled with fluorescent monoclonal antibodies targeting surface markers (e.g., CD4).
- Machine analyzes cell size and fluorescence to quantify specific immune cell populations.
- FACS (fluorescence-activated cell sorter) physically sorts cells based on markers.

## 12) ABO, Rh, transfusion compatibility, and HDN

| ABO antigens (surface sugars) | Key points |
|---|---|
| Base structure | H antigen is the base structure (precursor for A and B). |
| Group A | Adds N-acetylgalactosamine to H antigen. |
| Group B | Adds galactose to H antigen. |
| Group O | Lacks modifying enzymes; displays only H antigen. |

| ABO antibodies | Key points from the slides |
|---|---|
| Rule | People have antibodies against missing antigens (Landsteiner's Law). |
| Examples | Type A has anti-B; type O has both anti-A and anti-B. |
| Type | These are "natural" IgM antibodies; detectable in the first 3-6 months; develop against cross-reacting bacterial antigens. |
| Effect | They activate complement and cause hemolysis; detectable early in life. |

| Transfusion compatibility (slides) | Key points |
|---|---|
| Why match? | Incompatibility causes immediate cell lysis; mismatches trigger shock and hemolysis due to complement activation. |
| Universal donor | Group O is the universal red cell donor (cells lack A and B antigens). |
| Universal recipient | Group AB is the universal recipient (plasma lacks A and B antibodies). |

| Rh blood group system | Key points from the slides |
|---|---|
| Definition | Defined by the D antigen; presence of D means Rh-positive (about 85% of humans). |
| Antibody type | Rh antibodies are not naturally occurring; they are IgG antibodies. |
| How they form | Form after exposure to Rh-positive RBCs via transfusion or pregnancy. |
| Clinical note | Rh incompatibility is a major clinical concern and differs from ABO in antibody type. |

### Hemolytic disease of the newborn (HDN) - slides

- HDN involves Rh incompatibility: an Rh-negative mother carries an Rh-positive fetus.
- Maternal IgG antibodies cross the placenta and attack Rh-positive fetal RBCs.
- This causes hemolysis in the newborn; the Direct Coombs test is positive.
- Prevention: RhoGAM (anti-Rh immunoglobulins) injection at 28 weeks of gestation and upon delivery to prevent maternal sensitization.

## 13) Immune complexes in diagnosis

- Immune complexes are antigen-antibody lattices; they can deposit in tissues and be detected by fluorescent labeled complement.
- Serum complexes bind to complement as C1q or specific cells in culture.
- Detection aids diagnosing inflammatory diseases, identifying pathology such as glomerulonephritis.