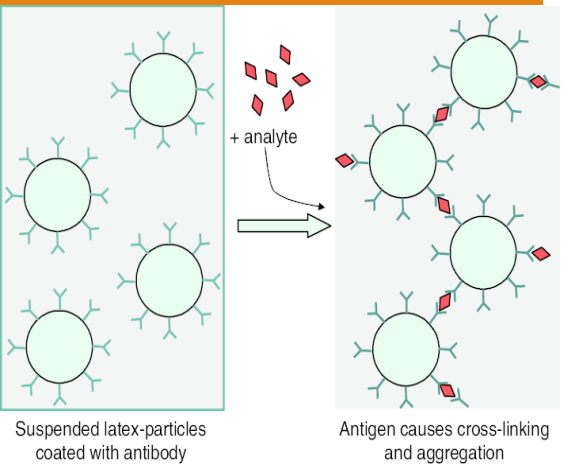
Agglutination Reactions

Department of Microbiology – College of Medicine

3rd Year Medical Students

Academic Year 2025-2026

Definition of Agglutination



- Agglutination is an immunological reaction characterized by the visible clumping of **particulate antigens** (such as cells, bacteria, or latex particles) when they interact with their specific antibodies under suitable conditions.
- Unlike precipitation, which involves soluble antigens, agglutination occurs with insoluble, particulate antigens, making the reaction **macr**oscopically visible.
- This reaction forms the basis of many rapid diagnostic tests used in clinical microbiology, immunology, and hematology.

Immunological Principle of Agglutination

The agglutination reaction depends on:

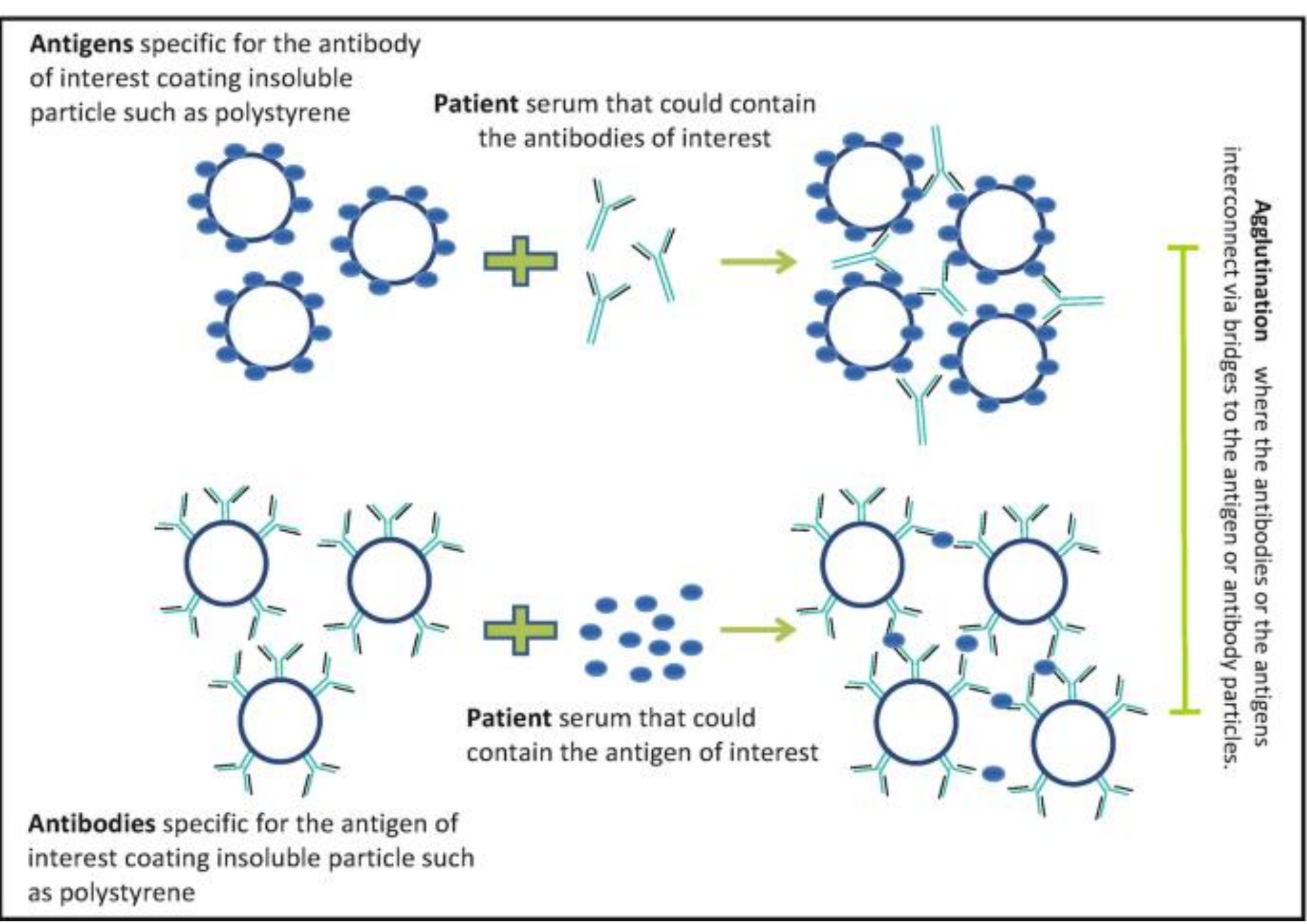
- **Specific antigen–antibody binding**
- **Multivalency of both antigen and antibody**
- **Formation of cross-linked lattices** between antigenic particles

Mechanism

1. Antibodies bind to epitopes on separate antigenic particles.
2. Each antibody bridges between multiple particles.
3. A three-dimensional lattice network forms.
4. This results in visible clumps (agglutinates).

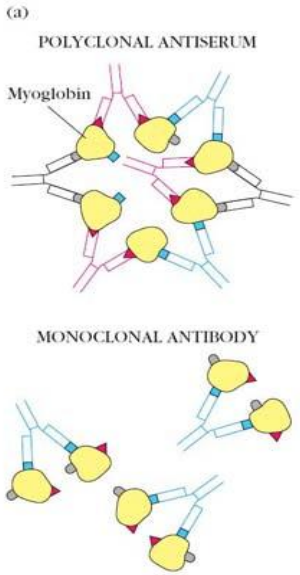
Antibody Classes Involved

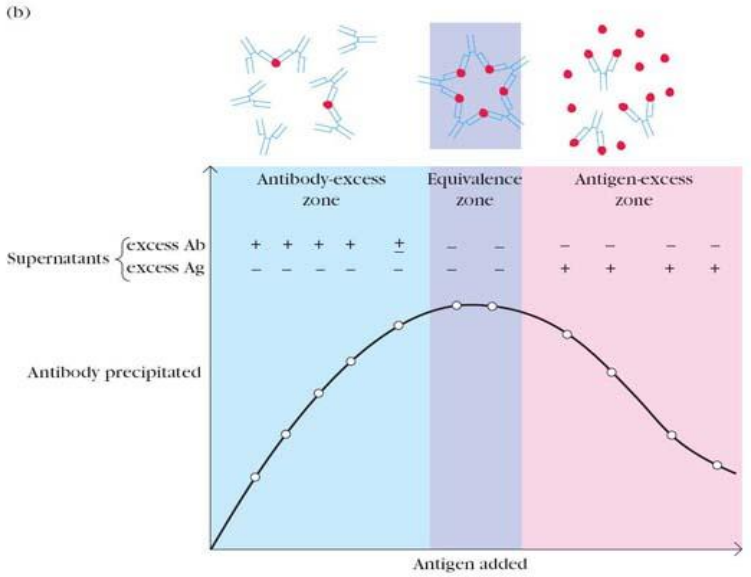
- **IgM** → most efficient agglutinating antibody (Pentameric structure with 10 binding sites)
- **IgG** → weaker but functional in some tests


Antigens specific for the antibody of interest coating insoluble particle such as polystyrene
Patient serum that could contain the antibodies of interest
Agglutination where the antibodies or the antigens interconnect via bridges to the antigen or antibody particles.
Patient serum that could contain the antigen of interest
Antibodies specific for the antigen of interest coating insoluble particle such as polystyrene

Factors Affecting Agglutination

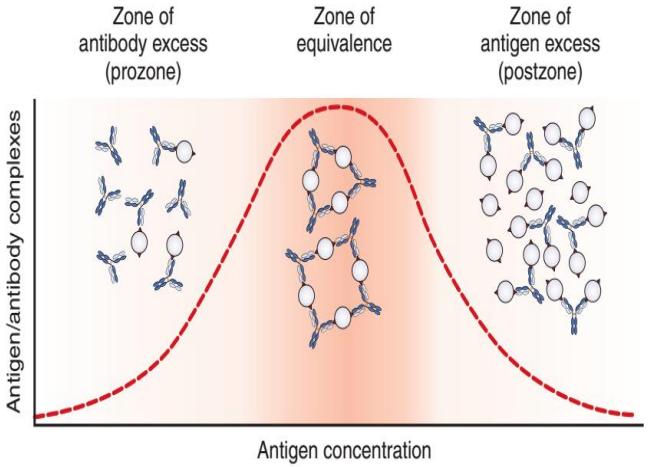
Factor	Effect
Antibody concentration	High levels may cause prozone
Antigen concentration	Excess causes postzone
Temperature	Optimal at room temperature or 37°C
pH	Optimal near physiological pH
Incubation time	Insufficient time → false negatives





Zones of Agglutination

- Like precipitation, agglutination occurs optimal in the **zone of equivalence**.
- **Prozone** phenomenon is clinically important in diseases with very high antibody levels such as syphilis, brucellosis, and autoimmune diseases.

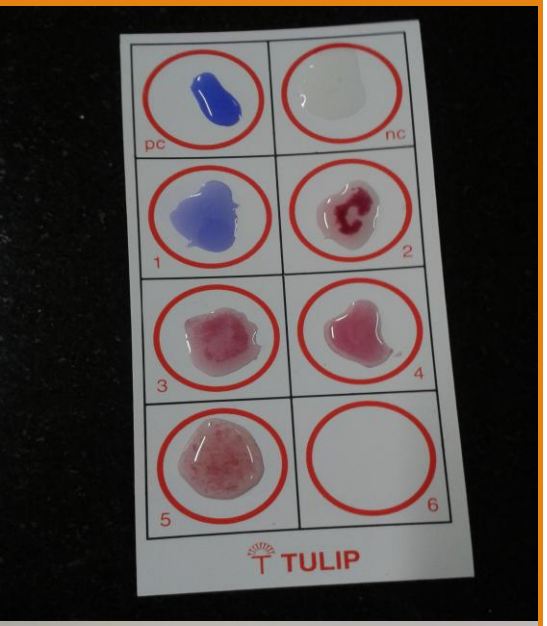


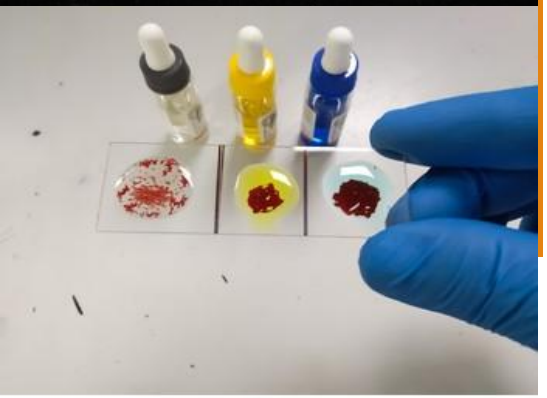
Zone	Description	Result
Prozone	**Excess antibody**	**False negative**
Equivalence	**Balanced Ag–Ab**	**Strong visible agglutination**
Postzone	**Excess antigen**	**Weak or absent agglutination**

Types of Agglutination Reactions

- Agglutination is classified into:
  1. **Direct Agglutination**
  2. **Indirect Agglutination**

Direct Agglutination





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- **Definition**
- The antigen is **naturally** present **on the surface of particles** such as:
  - Red blood cells
  - Bacteria
- **No carrier is needed**
- **Examples**
  - Blood grouping (ABO, Rh)
  - Widal test for *Salmonella*
  - Rose Bengal test for *Brucella agglutination test*
- **Procedure (General)**
  1. Patient serum is diluted.
  2. Antigen suspension is added.
  3. Incubation is performed.
  4. Visible clumps indicate a positive result.

Indirect Agglutination Test

- Used when the antigen is:
  - **Soluble**
  - **Not naturally particulate**
- **Principle**
  - **Soluble antigens** are artificially adsorbed onto inert carrier particles such as:
    - Latex beads
    - RBCs (hemagglutination)
- This transforms soluble antigens into <u>particulate form.</u>
- **Applications**
  - **Rheumatoid factor (RF) test (**Antibody)
  - **CRP latex test (**Antigen).
  - Detection of viral antigens
- Highly sensitive
- Rapid screening tests

Soluble → attached to latex → visible agglutination

Agglutination Titer

- The **agglutination titer** is defined as:
  - The **highest dilution of serum that still gives visible agglutination**.
- **Clinical Importance**
  - Reflects **antibody concentration**
- Used for:
  - Widal test
  - Brucella test
  - *ASO titer
- A **four-fold rise in titer** between acute and convalescent serum is diagnostically significant.

* **ASO (Antistreptolysin O) titer is a blood test that measures antibodies against streptolysin O toxin of *Streptococcus pyogenes* and indicates a recent streptococcal infection. ASO titer detects antibodies against *Streptococcus* toxin and helps confirm recent infection.**

Interpretation of Agglutination Results

How to Read Agglutination Results?

- **Positive Agglutination:**
  - Visible clumps (granules or aggregates) appear in the reaction mixture
  - Indicates the **presence** of a specific antigen–antibody reaction
  - Example:
    - Positive Widal → antibodies against *Salmonella*
    - Positive CRP latex → presence of CRP antigen
- **Negative Agglutination:**
  - The suspension remains smooth and uniform
  - Indicates the **absence** of the specific antigen or antibody being tested
- **Weak Agglutination:**
  - Small or fine granules
  - May indicate:
    - **Low antibody level**
    - Early infection
    - Improper test conditions
- **False results may occur due to:**
  - Prozone phenomenon (excess antibody)
  - Old reagents
  - Improper temperature or technique

Comparison Between Agglutination and Precipitation

Feature	Agglutination	Precipitation
Type of antigen	Particulate (cells, bacteria, latex)	Soluble (proteins, toxins)
Visibility	Seen directly with naked eye	Seen as a precipitate in gel or solution
Speed	Rapid reaction	Slower reaction
Principle	Clumping of particles by antibodies	Formation of insoluble Ag–Ab complexes
Common examples	Widal, Blood grouping, CRP latex	Ouchterlony, Radial immunodiffusion
Clinical use	Rapid diagnosis, screening, blood bank	Protein analysis, immune profiling

Summary

- Agglutination is a visible antigen–antibody clumping reaction.
- IgM is the most effective agglutinating antibody.
- Two types:
- Direct → antigen is naturally particulate (blood grouping, Widal)
- Indirect (latex) → antigen is soluble and attached to latex (CRP, RF)
- Agglutination titer reflects antibody level.
- It is widely used for rapid clinical diagnosis.