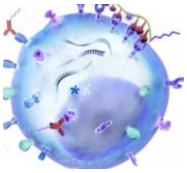


IMMUNOLOGY - SEROLOGY

COURSE CODE IS – LAB WEEK 1 – MIDTERMS

CHAPTER 1: AGGLUTINATION METHODS

- **Agglutination** - is an antigen-antibody reaction in which particulate antigens become visibly **clumped** after reacting with their corresponding antibodies.

Unlike **precipitation**, which involves soluble antigens, agglutination involves antigens that are naturally present on or artificially attached to particles such as:

- Red blood cells
- Bacteria
- Latex particles
- Charcoal particles
- Synthetic beads

The visible clumping makes agglutination particularly useful in routine clinical laboratories because the reaction can often be detected rapidly without sophisticated instrumentation.

- The basic reaction can be represented as:

Particulate antigen + Specific antibody → Antigen-antibody lattice → Visible agglutination

For visible agglutination to occur:

1. The antibody must recognize the antigen.
2. The antibody must have sufficient binding sites.
3. Antigenic sites must be appropriately positioned.
4. Antigen and antibody must be present in appropriate proportions.
5. The reaction must occur under suitable environmental conditions.

IMPORTANT CONCEPTS

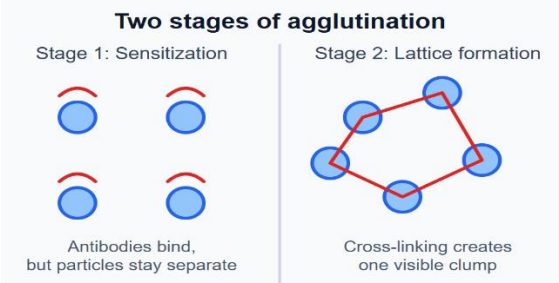
- **Agglutination does not simply mean that antigen and antibody have reacted.**

An antigen-antibody reaction may occur without visible clumping if the conditions do not permit lattice formation.

Why an Antigen–Antibody Reaction Does Not Always Cause Agglutination

- An antigen–antibody reaction is simply the binding of an antibody to its specific antigen.

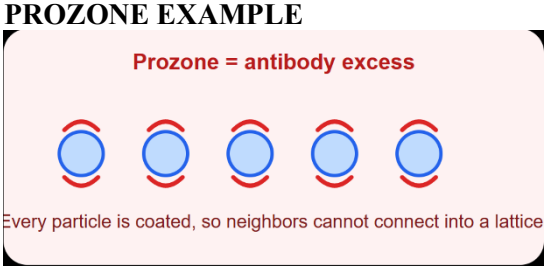
Agglutination is the visible clumping that occurs only when enough antigen–antibody complexes are cross-linked together to form a large lattice.



Why binding may occur without agglutination?
Several situations prevent lattice formation.

SITUATION	WHAT HAPPENS?
Prozone	Too much antibody; particles cannot cross-link effectively.
Postzone	Too much antigen; antibodies become saturated.
Low-affinity antibody	Binding occurs but bridges are weak.
IgG antibodies	Often coat RBCs without producing direct agglutination.
Poor reaction conditions	Incorrect pH, temperature, or ionic strength prevents lattice formation.

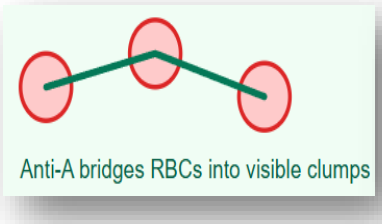
PROZONE EXAMPLE

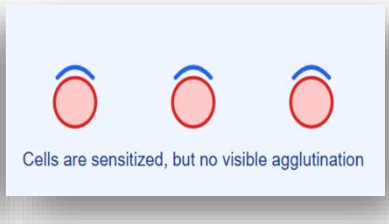


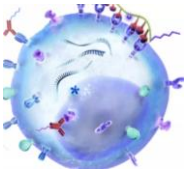
CLINICAL LABORATORY EXAMPLE

ABO Blood Typing
If anti-A reagent is

Rh Testing
If IgG anti-D binds Rh-positive RBCs:







IMMUNOLOGY - SEROLOGY

COURSE CODE | IS – LAB WEEK 1 – MIDTERMS

BOARD EXAM TAKEAWAY
Agglutination requires two events:

1. **Sensitization** – antibody binds antigen (microscopic, invisible)
2. **Lattice formation** – antibodies cross-link multiple particles (macroscopic, visible)

Remember this sentence:
All agglutination requires an antigen–antibody reaction, but not all antigen–antibody reactions produce agglutination.

AGGLUTINATION VS PRECIPITATION

Feature	Precipitation	Agglutination
Antigen	Soluble	Particulate
Visible reaction	Precipitate	Clumping
Antigen size	Soluble molecules	Cells/particles
Common applications	Immunodiffusion	Blood grouping, bacterial serology, latex tests
Detection	Often requires longer reaction	Frequently rapid
Example	Radial immunodiffusion	ABO blood typing

Precipitation = soluble antigen
Agglutination = particulate antigen

MECHANISM OF AGGLUTINATION

Agglutination occurs through two major stages.

Stage 1 — Sensitization
The antibody binds specifically to antigenic determinants on the particle.
Antigen + antibody → sensitized particle
This stage may not yet produce visible clumping.

Stage 2 — Lattice formation
Antibodies cross-link adjacent antigen-bearing particles.
Particle — antibody — particle — antibody — particle
As the lattice becomes sufficiently large, visible agglutination occurs.

ROLE OF ANTIBODY
Different antibody classes have different abilities to produce visible agglutination.
IgM - is particularly effective in agglutination because it has multiple antigen-binding sites and exists primarily as a pentamer.
It can efficiently bridge particulate antigens.
IgG - may bind particulate antigens but is often less effective at directly producing visible agglutination because of its smaller size and structural configuration. This is particularly important in red blood cell agglutination.

CHAPTER 2: TYPES OF AGGLUTINATION

I. I. DIRECT AGGLUTINATION

In direct agglutination, the antigen is naturally present on the particle.
Examples include:

- Bacterial agglutination
- ABO blood grouping
- Widal test
- Weil-Felix test

Basic principle
Naturally particulate antigen + specific antibody → visible agglutination

1. BACTERIAL AGGLUTINATION

Bacterial agglutination occurs when **antibodies bind to antigens naturally present on the surface of bacteria**, causing the bacterial cells to **clump together**.

- **Basic principle**

Bacterial cell + specific antibody → cross-linking → visible clumping

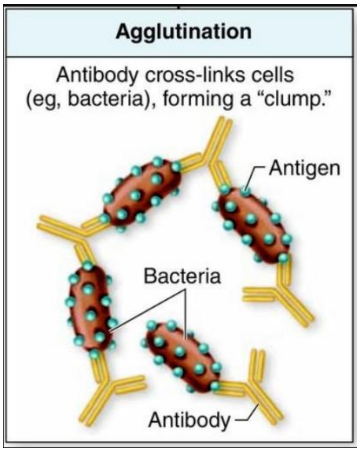
- **Why does clumping happen?**

A single antibody can bind antigenic structures on different bacterial cells.
So the antibody acts like a **bridge**:

Bacterium — Antibody — Bacterium — Antibody — Bacterium
Eventually, a large lattice forms and becomes visible as **clumps**.

- **Laboratory application**

Bacterial agglutination can be used to help **identify bacteria based on their antigenic characteristics**.



2. ABO BLOOD GROUPING

This is another excellent example of **direct agglutination**, specifically **hemagglutination**. Here, the particulate antigen is the **red blood cell (RBC)**.
RBC antigens

Blood group	RBC antigen
A	A antigen
B	B antigen
AB	A and B antigens
O	Neither A nor B

Interpretation

Anti-A	Anti-B	Blood group
+	–	A
–	+	B
+	+	AB
–	–	O

3. WIDAL TEST

The Widal test is a serologic agglutination test traditionally used to detect antibodies associated with infection by Salmonella enterica serovar Typhi and certain other Salmonella serovars.

- What is being detected?

The patient's serum antibodies are tested against standardized Salmonella antigen preparations.

- So unlike ABO typing, we're mainly asking:

Does the patient's serum contain antibodies that react with these bacterial antigens?

- Basic principle

Patient serum containing antibody + standardized Salmonella antigen → agglutination

- **AND H ANTIGEN**
- The traditional Widal test evaluates antibodies against:
- **O antigen** – somatic antigen
- **H antigen** – flagellar antigen
- Depending on the test system, other *Salmonella* antigen preparations may also be evaluated.
- **Why do we perform dilutions?**
- The patient's serum can be serially diluted:
- **1:20 → 1:40 → 1:80 → 1:160 → 1:320**
- The highest dilution that still produces the defined positive reaction is the **titer**.

For example:

- Dilution I Result
- 1:20+
- 1:40+
- 1:80+
- 1:160+
- 1:320−
-

4. WEIL-FELIX TEST

The **Weil-Felix test** is also an agglutination test, but it is unusual because it uses **Proteus** bacterial antigens to detect antibodies associated with certain **rickettsial infections**.

- **Why can Proteus be used?**

Certain **Rickettsia** and **Proteus** antigens share antigenic determinants.
Therefore, antibodies produced during some rickettsial infections may **cross-react** with specific *Proteus* strains.
This is an example of **cross-reactivity**.
The important Proteus strains
The traditional test uses:

- **OX19**
- **OX2**
- **OXK**

The patient's serum is mixed with these antigen preparations.
If the patient's antibodies cross-react with the corresponding Proteus antigen:
→ **agglutination occurs**

Basic concept

Rickettsial infection
↓
Patient produces antibodies
↓
Some antibodies cross-react with **Proteus OX antigens**
↓
Agglutination

Why isn't it highly specific?
Because the reaction is based on **cross-reactivity**, the test can have limited sensitivity and specificity.
Therefore, Weil-Felix is considered an **older/limited serologic method**, and more specific methods are preferred when available.

EASIEST WAY TO REMEMBER THEM ☺

- **ABO**

Known antibody + patient's RBC antigen
"What antigen is on the patient's RBC?"

- **Widal**

Known Salmonella antigen + patient's antibody
"Does the patient have antibodies reacting with Salmonella antigens?"

- **Weil-Felix**

Known Proteus antigen + patient's cross-reacting antibody
"Does the patient's serum contain antibodies that cross-react with Proteus OX antigens?"

- **Bacterial agglutination**

Bacterial particle + specific antibody
"Can this antibody recognize the bacterial antigen?"

II. PASSIVE AGGLUTINATION

In passive agglutination, a **soluble antigen is artificially attached to a particulate carrier**.
Common carrier particles include:

- Latex
- Red blood cells
- Synthetic particles

The carrier makes an otherwise invisible antigen-antibody reaction visually detectable.

- **Example**

Latex particles coated with antigen are mixed with patient serum containing the corresponding antibody.
Antibody present → particles cross-link → agglutination

III. REVERSE PASSIVE AGGLUTINATION

Reverse passive agglutination detects **antigen rather than antibody**.

- In this method:

Antibody is attached to a particulate carrier.
When the corresponding antigen is present in the patient's specimen, it cross-links the particles.

- **Basic reaction**

Antibody-coated particles + patient antigen → agglutination

- **Clinical significance**

This approach can be used for rapid detection of certain microbial antigens.
Explanation ni sir:

Patient specimen
↓
Add antibody-coated particles
↓

Mix
↓
Allow specified reaction time
↓
Observe
↓
Agglutination?
YES → POSITIVE → Target antigen detected
NO → NEGATIVE → Target antigen not detected
↓
Check controls
↓
Report only if controls are valid

IV. COAGGLUTINATION

- Coaglutination uses bacterial cells, particularly **Staphylococcus aureus**, as carrier particles.

- The bacterial cell surface contains **Protein A**, which binds the Fc portion of IgG.

This allows antibodies to become oriented with their antigen-binding Fab regions exposed.

- **Simplified Mechanism**

Staphylococcus Protein A → binds Fc of IgG
↓
Fab regions remain available
↓
Specific antigen binds
↓
Cross-linking
↓
Visible agglutination

V. LATEX AGGLUTINATION

Latex agglutination uses microscopic synthetic particles coated with either:

- Antigen, or
- Antibody

The reaction is usually performed on a card, slide, or reaction surface.

- **Advantages**

Rapid
Simple
Requires minimal equipment
Easily adapted to qualitative testing
Can sometimes be used for semiquantitative testing

- **Examples of applications**

Latex agglutination may be used in testing for selected:
Microbial antigens
Antibodies
Rheumatoid factor
Certain proteins
Other clinically significant analytes

- Explanation

For antigen detection
Patient specimen
↓
Add antibody-coated latex particles
↓
Mix
↓
Rotate/rock according to instructions
↓
Observe within specified time
↓
Agglutination?
YES
→ Positive
→ Target antigen detected
NO
→ Negative
→ Target antigen not detected under test conditions
↓
Verify controls
↓
Report valid result

VI. HEMAGGLUTINATION

- Hemagglutination is agglutination involving **red blood cells**.
- It can occur when antibodies directly bind antigens on RBC surfaces.
- **Example**

ABO blood grouping
Anti-A reagent + A antigen on RBCs→ **Agglutination**

VII. PASSIVE HEMAGGLUTINATION

- In passive hemagglutination, soluble antigen is attached to red blood cells.
- The RBCs act as carrier particles.

- Patient antibodies can then cross-link the sensitized RBCs.

Important distinction

- **Hemagglutination:** RBCs themselves contain the relevant antigen.
- **Passive hemagglutination:** antigen is artificially attached to RBCs.

- Explanation

Passive hemagglutination
Patient serum
↓
Serial dilution if required
↓
Add antigen-coated RBCs
↓
Allow reaction
↓
Observe RBC pattern
↓
Diffuse mat → Positive
Compact button → Negative

HEMAGGLUTINATION INHIBITION

- Hemagglutination inhibition is conceptually different from ordinary hemagglutination.
- Instead of looking for clumping directly, the test determines whether an antibody or other component **prevents agglutination**.

Interpretation

- **No agglutination = inhibition occurred**
- **Agglutination = inhibition did not occur**

This principle has historically been important in certain viral serologic procedures.

AGGLUTINATION PATTERNS

Students should learn to distinguish:

- **Positive reaction**

-Visible granular or clumped appearance.

- **Negative reaction**

-Uniform suspension with no visible clumping.

- **Weak reaction**

-Small or barely visible aggregates.

- **Strong reaction**

-Large, obvious aggregates with clearing of the surrounding suspension.

THE ZONE OF PHENOMENON

PROZONE = too much antibody (**False-negative or weak reaction**)
EQUIVALENCE = optimal reaction (**Maximum visible agglutination**)
POSTZONE = too much antigen (**Weak or false-negative reaction**)

FACTORS AFFECTING AGGLUTINATION

1. Antigen concentration
Too little or excessive antigen can affect lattice formation.
2. Antibody concentration
Excess antibody can produce the prozone effect.
3. Temperature
Some antigen-antibody reactions have specific temperature requirements.
4. pH
Extreme pH can interfere with antigen-antibody binding.
5. Ionic strength
The ionic environment influences antibody-antigen interactions.
6. Incubation time
Insufficient or excessive reaction time can influence interpretation.
7. Mixing
Poor mixing may prevent adequate interaction.
8. Particle concentration
Improper particle concentration can produce misleading results.

QUALITATIVE AGGLUTINATION

A qualitative test determines whether agglutination is:
Positive or negative
Example:

Reaction	Interpretation
Visible clumping	Positive
No clumping	Negative

SEMIQUANTITATIVE AGGLUTINATION

A semiquantitative procedure estimates the amount of antibody by serially diluting the patient's specimen.
The **highest dilution that still produces a visible reaction** is reported as the titer.
Example:

Dilution	Result
1:2	+
1:4	+
1:8	+
1:16	+
1:32	–

Titer: 1:16

TITRATION

- Titration involves sequential dilution of a specimen.

For example:
1:2 → 1:4 → 1:8 → 1:16 → 1:32 → 1:64
The endpoint is the **highest dilution that produces a defined positive reaction**.

- **Important**

A higher titer does not automatically mean that the patient has a more severe disease.

SLIDE AGGLUTINATION

- Slide agglutination is commonly used for rapid qualitative testing.

procedure

1. Label the reaction area.
2. Add the required specimen.
3. Add the appropriate reagent.
4. Mix using the prescribed technique.
5. Rotate or rock the slide according to the procedure.
6. Observe for agglutination within the specified reaction time.
7. Compare with appropriate controls.
8. Record the result.

- **Critical point**

Do not interpret a reaction after the manufacturer's specified reading time.
Drying can produce artifacts that resemble agglutination.

TUBE AGGLUTINATION

- Tube agglutination provides a controlled environment for antigen-antibody interaction.

General steps include:

1. Prepare appropriate dilutions.
2. Add antigen or antibody reagent.
3. Incubate under specified conditions.
4. Observe the reaction.
5. Determine the endpoint.
6. Calculate/report the titer when applicable.