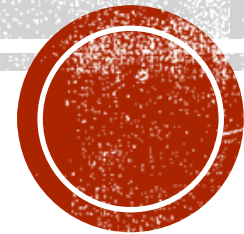


# Lab-2- Laboratory Diagnosis Immunological Tests

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## (Direct) test

### -Antigen Detection Tests

Antigens can be detected by wide range of Serological techniques utilizing Polyclonal or Monoclonal antibodies.

### -Antibody detection

The same techniques , utilizing purified antigens , can be used to detect specific antibodies to those Microorganisms in the Patients Serum.

There are many types of serological method for immunological  
Diagnosis

# Immunology/Serology Tests

## Classical methods

\*Precipitation

\*Agglutination

\*Complement Fixation test(CFT)

\*Immunofluorescence Test (IF)

# Immunology / Serology Tests

## Newer Technique

- \*Radioimmunoassay (RIA)
- \*Enzyme Linked Immunosorbent Assay (ELISA) &(EIA).
- \* Western blot Test(WB)
- \*Recombinant immunoblot assay( RIBA).
- \*Immunochemistry.

# Precipitation

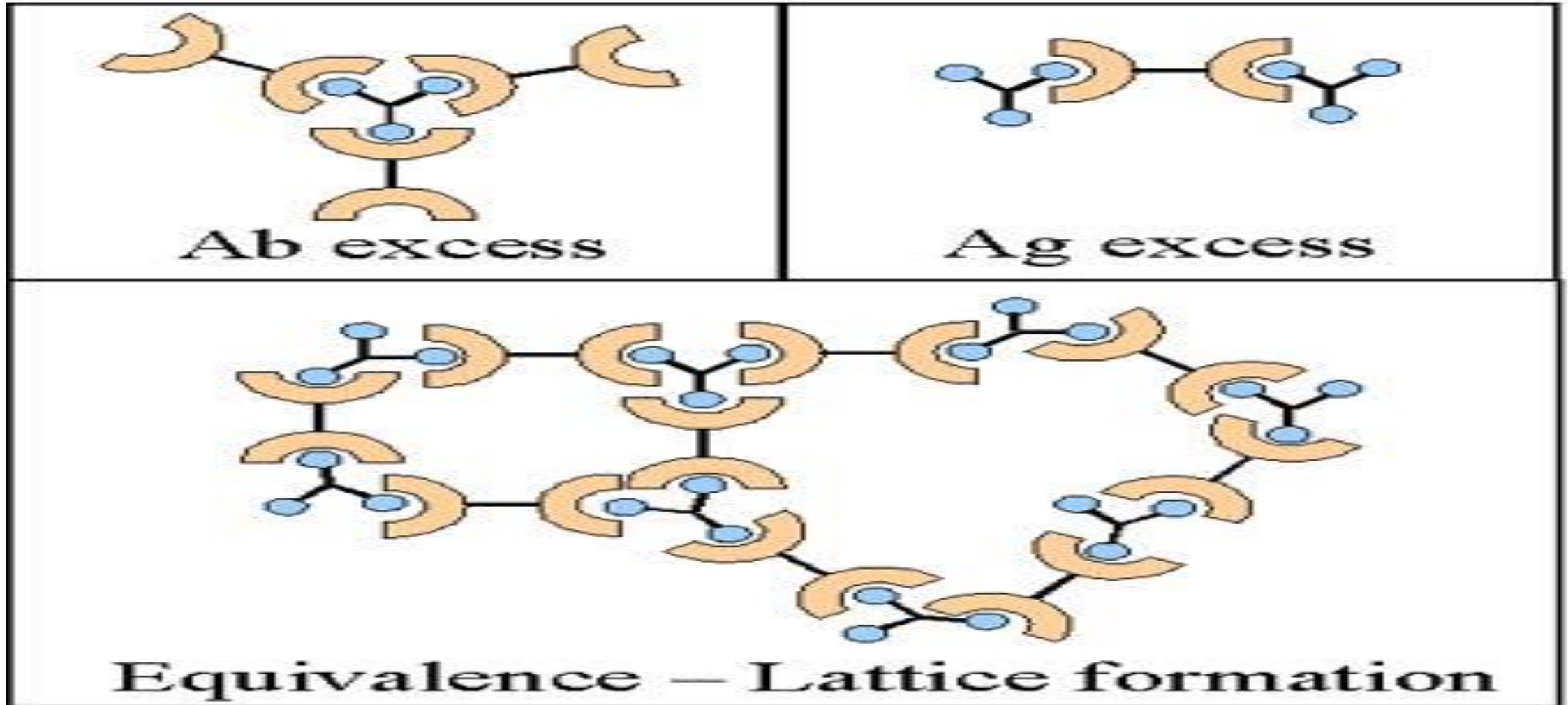
## Principle

- \*Soluble antigen combines with its Specific Antibody
- \*antigen-antibody complex is too Large to Stay in Solution and Precipitations.

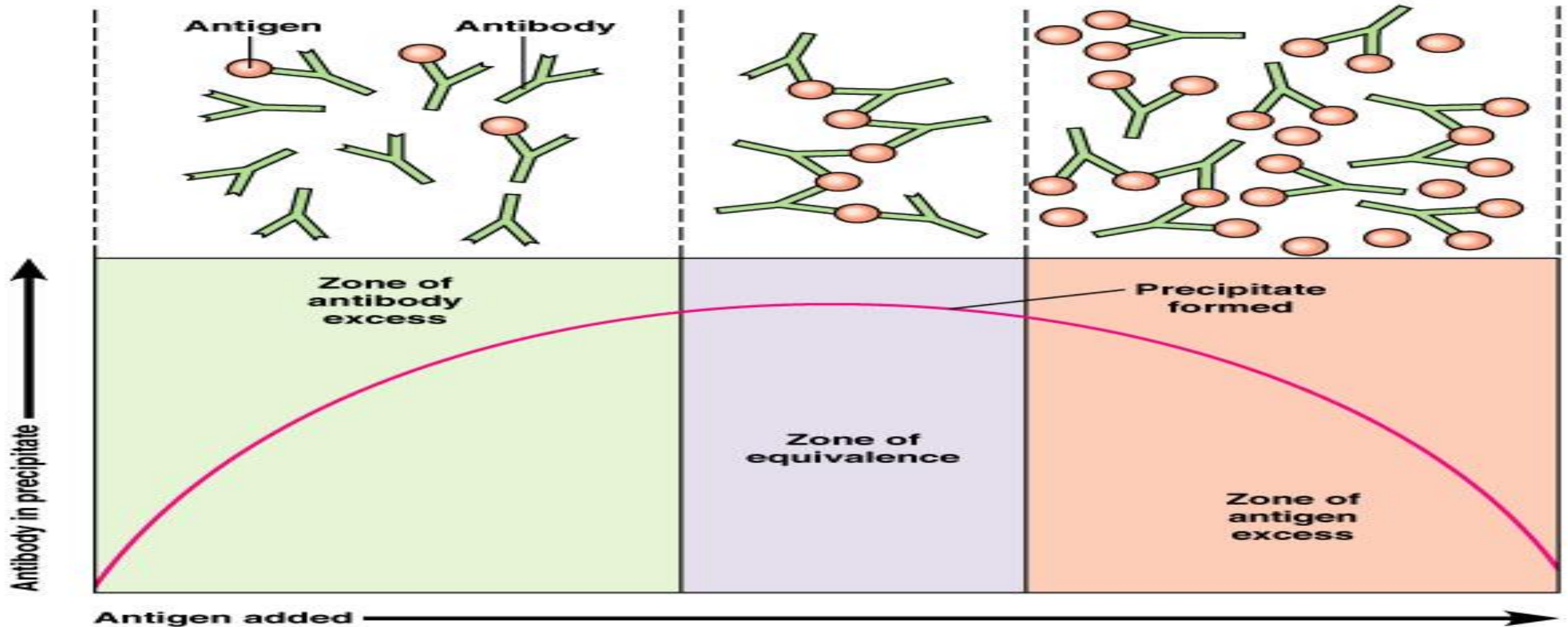
## Precipitation curve

- **Pro zone – antibody excess, many antibodies coat all antigen sites- results in false negative**
- **Post zone – antigen excess, antibody coats antigen but cannot get lattice formation, results in false negative**
- **Zone of Equivalence – antigen and antibody present in optimal proportions to bind and give visible reaction**

# Precipitation Curve



# Precipitation Curve



# Precipitation

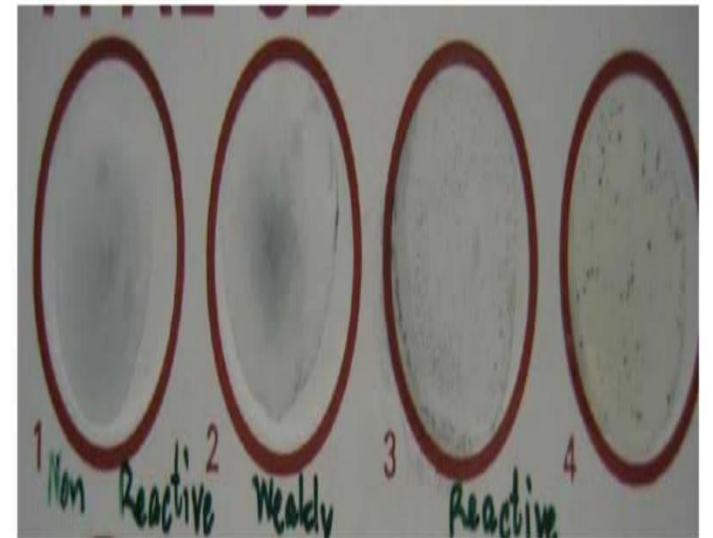
## Examples

\_Flocculation test.

\_immuno-diffusion test.

\_Counter-immuno-electrophoresis (CIEP)

## Flocculation test (A precipitation reaction)



(1) Non Reactive

(2) Weakly Reactive

(3,4) Reactive

RPR card test



# Precipitation Reaction(Flocculation test)

## **Advantages**

Sensitive for Antigen detection.

## **Limatations:**

Limited applications.

## **Time taken**

10minutes.

# Agglutination

-The interaction between Antibody and a particulate (insoluble) Antigen in the presence of electrolytes at an optimal temperature and PH results in Visible Clumping of Particles.

\*Antigen may be:

On cell(Direct agglutination)

Attached to Latex spheres(Indirect or Passive agglutination).

Agglutination reactions is aided by elevated temperature (37-56C) and by movement which increase the contact between antibody and antigens.

\*Clear Supernatant.

\*Clumping aggregate and settle as large Visible Clumps.

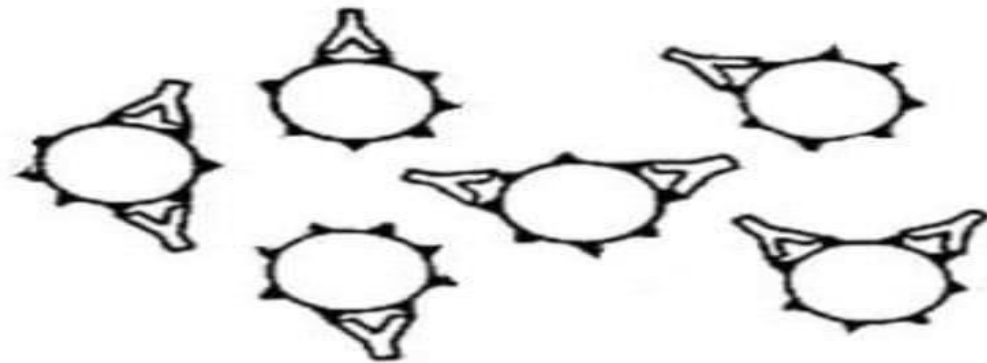
# Steps in agglutination

- primary phenomenon(sensitization)
- Lattice formation
- Tertiary phenomenon

# Steps in agglutination

## 1-primary phenomenon(sensitization)

Involve antigen-antibody combination through single antigenic Determination on the Particle.



Antibody molecules attach to their corresponding Antigenic site (epitope) on the red blood cell membrane. There is no .visible clumping

# Steps in agglutination

## 2- Lattice formation (aggregation stage)

Represent the sum of interaction between antibody and multiple antigenic determinants on a particle

dependent on environmental conditions as well as the concentration of antigen and antibody.



Antibody molecules crosslink RBCs forming a lattice that results in visible clumping or agglutination

## -Tertiary phenomenon

- Reaction not visible, detected by affect of reaction on tissues or cells.

# Uses of agglutination Reaction

1- Aid in the identification, by means of known antisera (serum containing antibodies specific for a given antigen), microorganisms cultured from clinical specimens.

2- Help estimate the titer of antibacterial agglutination in the serum of Patients with unknown disease.

# Types of agglutination reaction

- 1-Direct agglutination(Active)
- 2-Indirect agglutination(Passive)
- 3-Reverse agglutination
- 4-Hemagglutination
- 5-Hemagglutination inhibition
- 6-Coagglutination

•

# 1-Direct agglutination(Active)

## Principle

Combination of an insoluble (Particulate) antigen with its Soluble antibody.

Large complex Antigen (e.g Virus, Bacteria, Fungal and M ammial cells) can be agglutinated by specific Antibodies

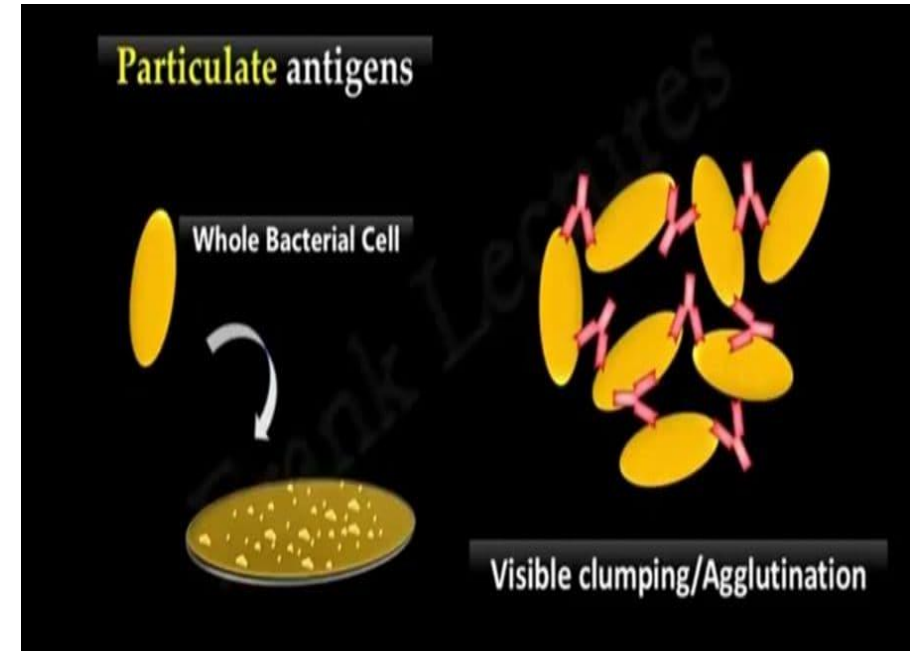
-Form antigen-antibody complex

-Particles clump / agglutinate

Used for antigen detection

Examples:

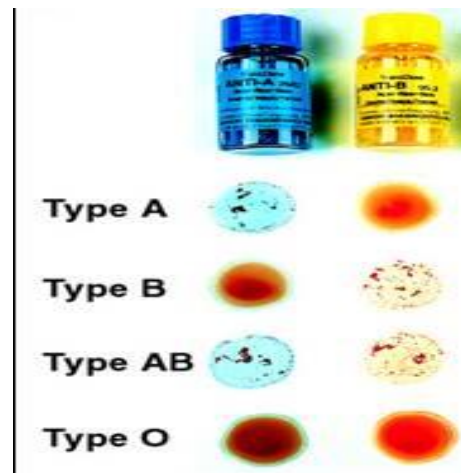
Bacterial agglutination tests for Sero-typing and Serogrouping..e.g Vibrio cholera , Salmonella spp ABO group.



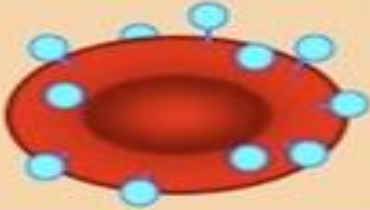



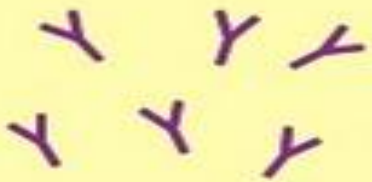




# Blood typing

- Blood type can be determined by using antibodies that bind to the A or B blood group antigens in a sample of blood.
- For example, if antibodies that bind the A blood group are added and agglutination occurs, the blood is either type A or type AB.
- To determine between type A or type AB, antibodies that bind the B group are added and if agglutination does not occur, the blood is type A.
- If agglutination does not occur with either antibodies that bind to type A or type B antigens, then neither antigen is present on the blood cells, which means the blood is type O.



# Blood typing

<b>ABO Blood Groups</b>				
<b>Antigen</b> (on RBC)	Antigen A 	Antigen B 	Antigens A + B 	Neither A or B 
<b>Antibody</b> (in plasma)	Anti-B Antibody 	Anti-A Antibody 	Neither Antibody	Both Antibodies 
<b>Blood Type</b>	<b>Type A</b> Cannot have B or AB blood Can have A or O blood	<b>Type B</b> Cannot have A or AB blood Can have B or O blood	<b>Type AB</b> Can have any type of blood Is the universal recipient	<b>Type O</b> Can only have O blood Is the universal donor

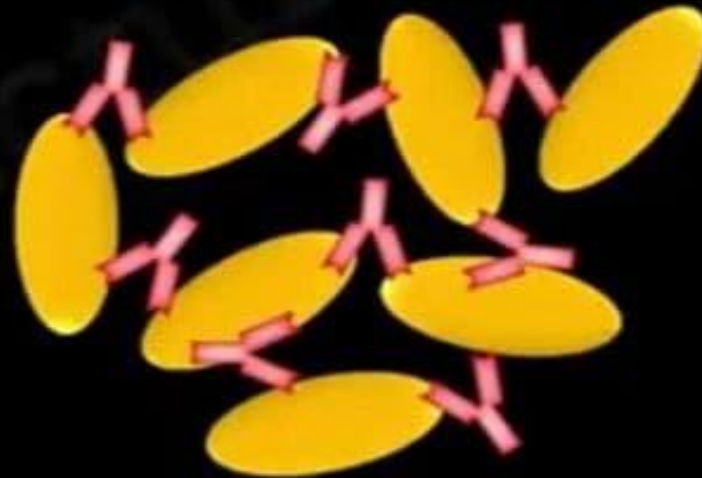
# Precipitation & Agglutination Reaction

## Precipitation Reactions



**Soluble** antigens

## Agglutination Reactions



**Particulate** antigens

## 2-Indirect agglutination(Passive)

### Principle

Precipitation reaction converted into agglutination coating antigen onto the surface of carrier particles Like red blood cell , latex, gelatin, Bentonite.

\*Back ground clears

Examples of types

\*Latex agglutination

\*Co-agglutination

\*Passive agglutination tests have been used to detect Rheumatoid Factor and antinuclear antibody.

\*Passive hemagglutination (treated blood cells made resistant).

## 3-Reverse Passive agglutination.

### Principle

-Antigen binds to Soluble antibody Coated on Carrier Particles and results in agglutination.

-Detect Antigens

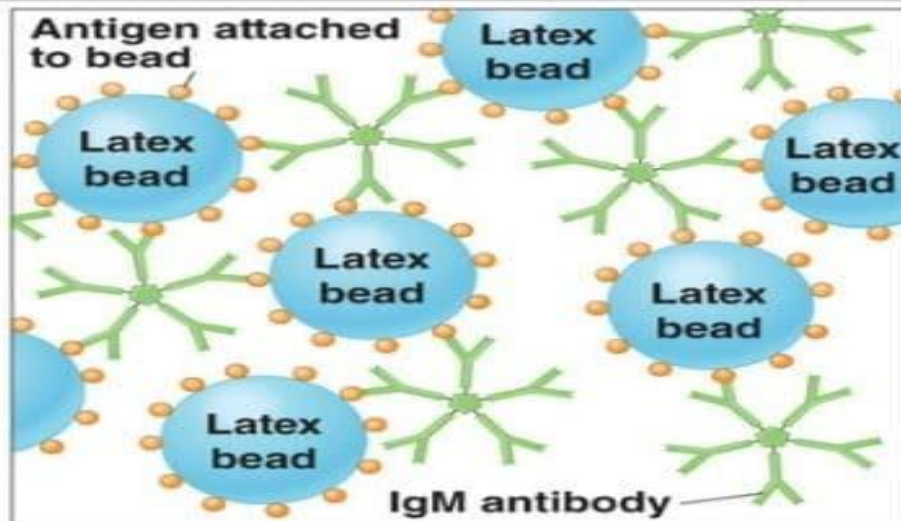
### Example

\*Detecting of Cholera toxin.

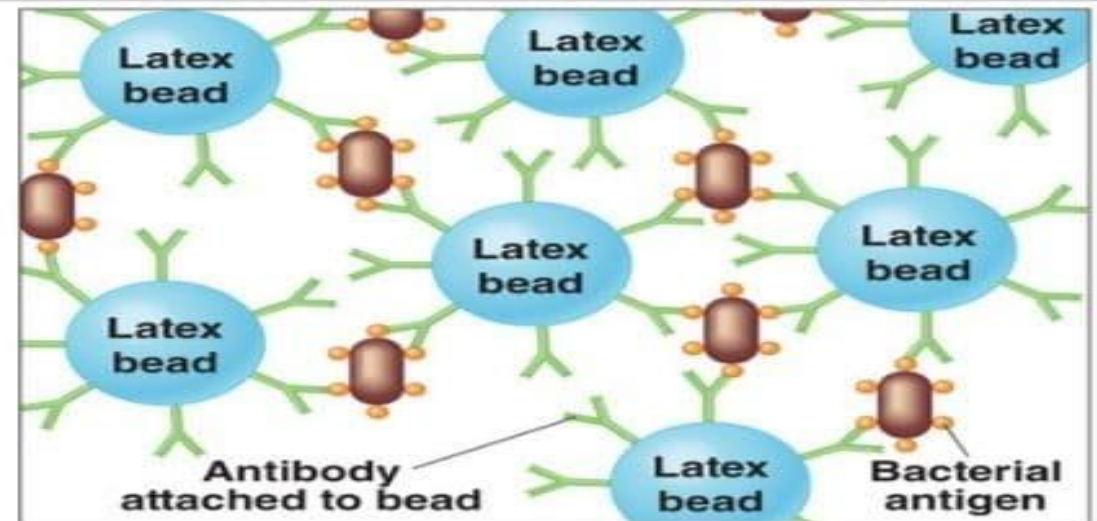
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### 3-Passive and Reverse Passive agglutination.

## Passive and reverse passive



**(a)** Reaction in a positive indirect test for antibodies. When particles (latex beads here) are coated with antigens, agglutination indicates the presence of antibodies, such as the IgM shown here.



**(b)** Reaction in a positive indirect test for antigens. When particles are coated with monoclonal antibodies, agglutination indicates the presence of antigens.

## Qualitative agglutination test.

- . Agglutination tests can be used in a qualitative manner to assay for the presence of an antigen or an antibody.
- The antibody is mixed with the particulate antigen and a positive test is indicated by the agglutination of the particulate antigen.

# Quantitative agglutination test.

- Agglutination tests can also be used to quantitate the level of antibodies to particulate antigens.
- In this test
  - one makes serial dilutions of a sample to be tested for antibody
  - and then adds a fixed number of red blood cells or bacteria or other such particulate antigen
  - and determines the maximum dilution, which gives agglutination.
  - The maximum dilution that gives visible agglutination is called the titer.
  - The results are reported as the reciprocal of the maximal dilution that gives visible agglutination. This can be done using a micro titer plate.

Patient	1/2	1/4	1/8	1/16	1/32	1/64	1/128	1/256	1/512	1/1024	Pos.	Neg.	Titer
1	●	●	●	●	●	●	○	○	○	○	●	○	64
2	●	●	●	○	○	○	○	○	○	○	●	○	8
3	●	●	●	●	●	●	●	●	●	○	●	○	512
4	○	○	○	○	○	○	○	○	○	○	●	○	<2
5	●	●	●	●	●	○	○	○	○	○	●	○	32
6	○	○	●	●	●	●	●	○	○	○	●	○	128
7	●	●	●	●	●	○	○	○	○	○	●	○	32
8	●	●	○	○	○	○	○	○	○	○	●	○	4



# Determining Antibody titer

- **Titer** is the quantity of a substance required to produce a reaction with a given volume of another substance.
- **Antibody titer** is the highest dilution of the biological sample that still results in agglutination, with no agglutination being observed at any higher dilution.
- The term is used in serological reactions and is determined by preparing serial dilutions of antibody to which a constant amount of antigen is added.

# Determining Antibody titer

	Prozone					Equivalence Zone				Post Zone
<b>Serum Dilution</b>	<b>1:10</b>	<b>1:20</b>	<b>1:40</b>	<b>1:80</b>	<b>1:160</b>	<b>1:320</b>	<b>1:640</b>	<b>1:1280</b>	<b>1:2560</b>	<b>1:5120</b>
<b>Antigen Conc.</b>	<b>X</b>	<b>X</b>	<b>X</b>	<b>X</b>	<b>X</b>	<b>X</b>	<b>X</b>	<b>X</b>	<b>X</b>	<b>X</b>
<b>Agglutination</b>	-	-	+	++	+++	++++	+++	++	+	-

# Methods of agglutination

**1-slide agglutination(rapid):** Add a drop of antiserum , mix with antigen and rock slide for approx. 1minute.



**2-Tube Agglutination(slow) test**

Standard quantitative method for determination of Antibodies.

Routinely employed in diagnosis of different types of viruses & bacteria.

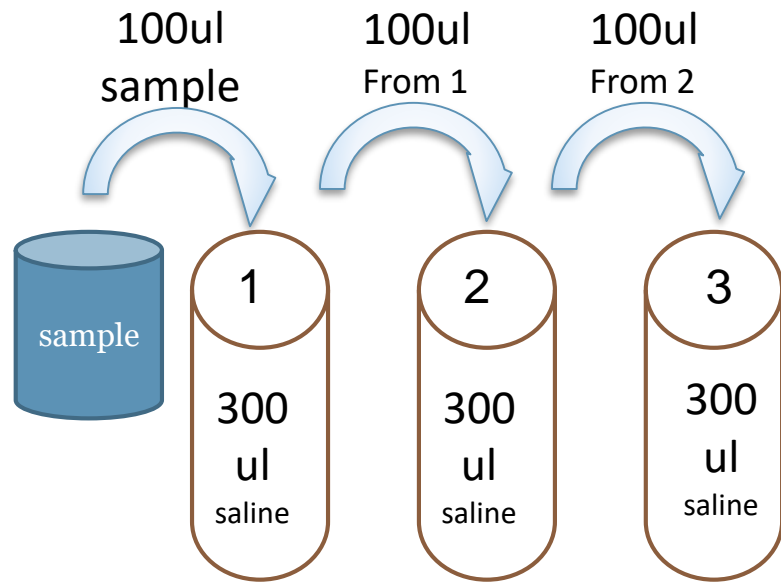




# Dilutions

- Dilution is decreasing the concentration of a solution by a calculated factor using an approved diluent.
- As well, dilution is used to prepare samples, buffers, and controls.
- In serology tests it is Used to detect the titer of a specific Ab.
- When a strong positive reaction is encountered, dilution should be made to detect the titer.

- for example: (1:4)
  - 1 express the volume of sample
  - 4 express the total volume( sample + reagent)
  - This is done by mixing 100 ul of sample with 300 ul of reagent.
- Serial dilution :



**Total  
Dillution**

→ **1:4**

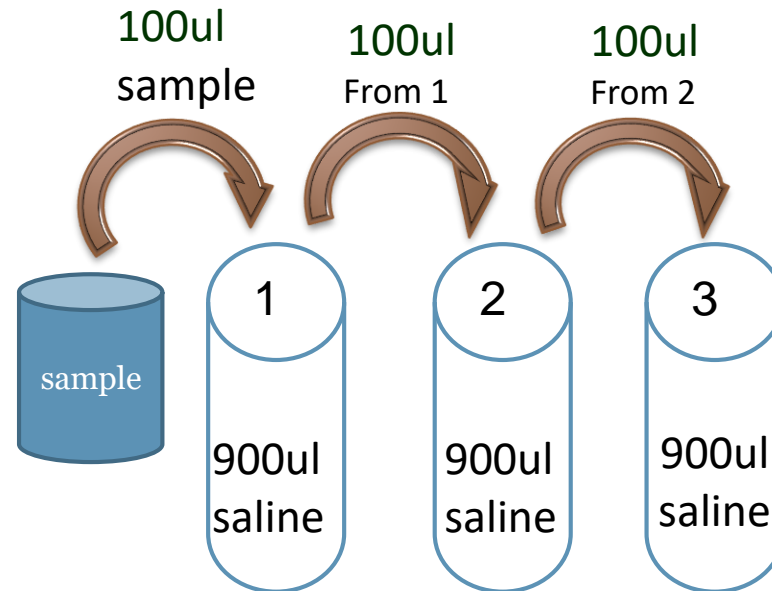
**1:16**  
=  $1/4 * 1/4$

**1:64**  
=  $1/4 * 1/16$

Dillution of tube 1

Dillution of tube 2

👉 Another example on serial dilution :



**Total  
Dilution**



**1:10**

**1:100**

**1:1000**

$= 1/10 * 1/10$

$= 1/10 * 1/100$

Dilution of tube 1

Dilution of tube 2

# Agglutination

## \*Advantages

### . Advantages of agglutination tests:

1. Low individual test cost.
2. Ability to obtain **semi quantitative results**.
3. Short time to obtain result.
4. Don't need expensive instrument.
5. Agglutination of insoluble native antigens or antigen-coated particles simple to read with or without the aid of a microscope
6. Increased degree of sensitivity
7. Great variety of detectable substances
8. If the sample contain micro-organisms, it does not need to be viable



# Agglutination

## Limitations

\*Prozone Phenomenon:

- Requires the right combination of quantities of antigen and antibody.
- handled through dilution to improve the match.
- May give false positive or negative results

## Time taken

\*10-30minutes



**THANK YOU**