DEPARTMENT OF BIOTECHNOLOGY

Agglutination Reactions

By
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In 1896, Gruber and Durham published the first report about the ability of antibody to clumps cells, based on observation of agglutination of bacterial cells

by serum

Typhoid fever
Brucellosis

ABO blood group

Precipitation Reaction & diagnostic technique based on these Reacn

we saw when soluble Ag aggregates by Ab's they form immune complex (IC)

↓

when these IC become large enough to settle out soln they become visible

Particulate Ag-

↳ when specific Ab's to these particulate Ag's are added cross linking occurs

↓

These crossling results in clumping or agglutination of particulate Ag's

↓

These clumping are visible enough to be seen by naked eye

precipitatn & Agglutinatn

to be seen by

The difference b/w precipitat^n & Agglutinat^n is that

Precipitation
↓
aggregation of soluble Ag molecules

Agglutinat^n involves
↓
clumping of insoluble or particulate Ag
↓
like

like precipit^n react^n, agglutinat^n also occurs when Ag & Ab's are in equivalent proportion

pplicat^n -> It is used for the detect^n of Ag & Ab's in serum & other body fluids

Agglut^n test are performed routinely by blood banks to determine ABO & Rh Blood Typing n & preparation of blood Transfusion

bind

Particulate Ag + Specific Ab

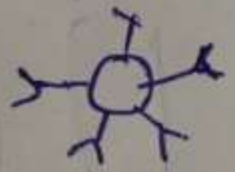
(insoluble Ag)

↓ → Electrolyte
→ Desired temp

Agglutination / clumping

(Agglutinate)

Ag + Ab ⟶ clumping

O Y ⟶ 

Ab → Agglutinins

Ag → Agglutinogen

React^n → Agglutination

Types

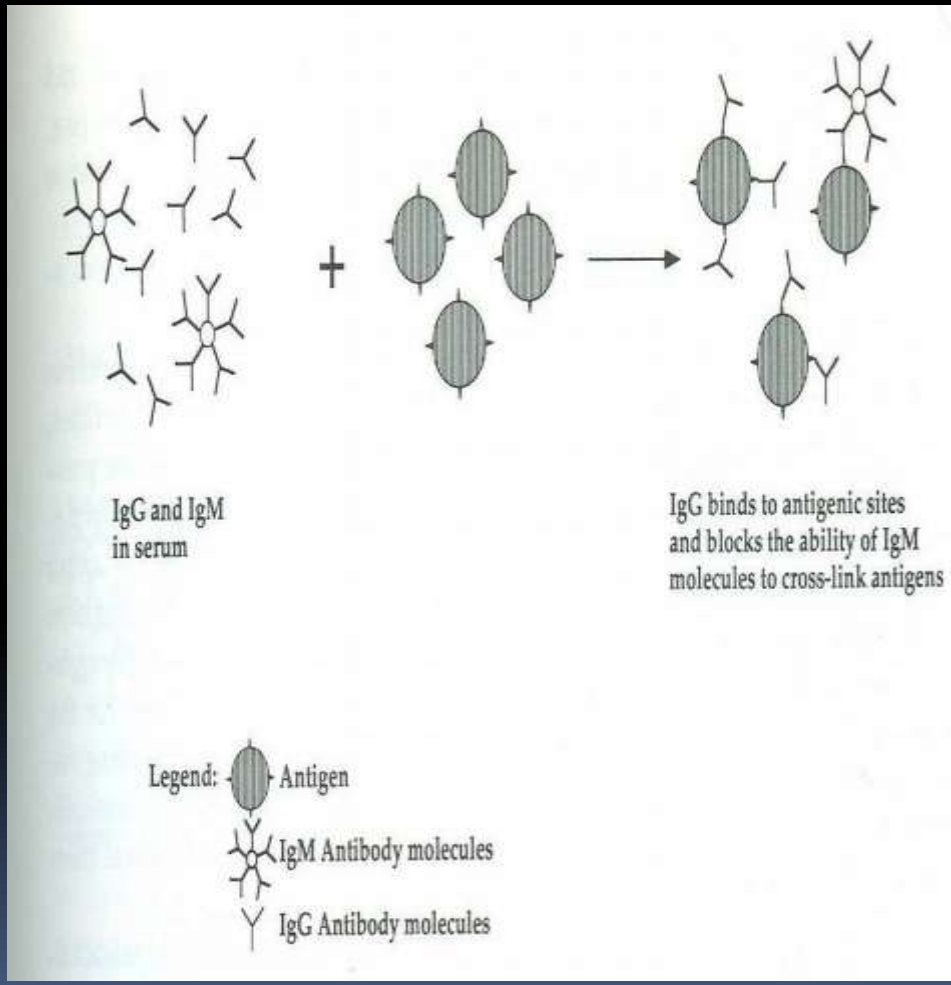
Agglutination test

- It is one of important laboratory method to detect antigen antibody reaction.
- It provides flexible and useful method for semi quantitating of either antigen or antibody concentration.
- The reaction occurs between insoluble antigen and appropriate antibody.
- The reaction will results in forming aggregate or agglutinate.
- Antibodies that produce such reactions are called agglutinins.

Stages of agglutination reaction

Primary phage- Sensitization

- Antibody reacts with single antigenic determinants on or close to particle surface.
- It is a rapid and reversible reaction.
- Based on Law of Mass Action

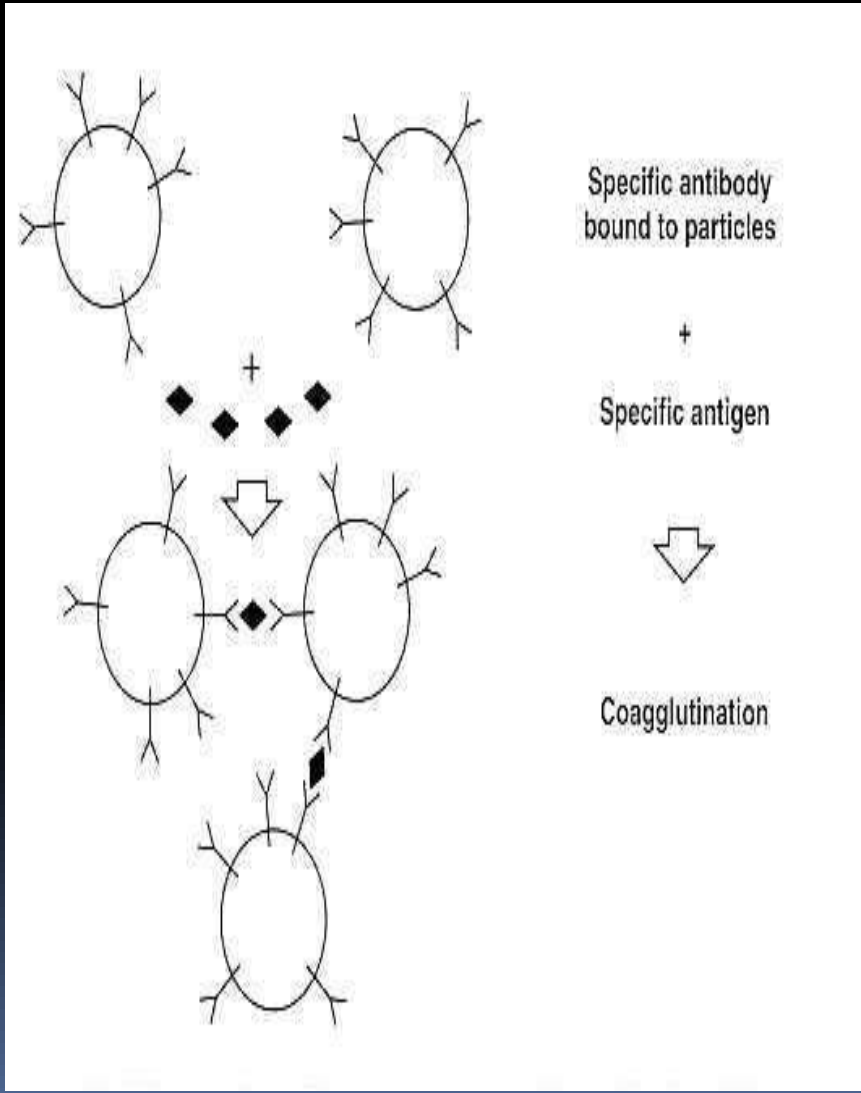


Secondary phase- Lattice formation

A single antibody molecule binds t antigenic determinants on adjacent particles.

- The visible reaction occur under appropriate conditions and over time, particles remain connected and interconnected by antibody bridge.

IgM is 700 times more efficient in agglutination than IgG (Restricted hinge region of IgG than IgM)



Enhancement of lattice formation

- Erythrocytes and bacterial cells have slightly negative charge in their surface
- Like charges tend to repel
- Use of low ionic strength saline (Use of 5 to 30% albumin neutralize the surface charge)
- Increase the viscosity (red cells)- add dextran or polyethylene glycol (PEG)
- Agitation and centrifugation
- Temperature- (IgG- 30-37/ IgM-4-27); ABO-IgM
- pH- best 6.5-7.5; IgM- best at low pH

Agglutination tests- type of particles involved

- Antibodies can agglutinate multivalent particulate antigens, such as Red Blood Cells (RBCs) or bacteria or latex particles
- Some viruses also have the ability to agglutinate with RBCs.
- This behavior is called agglutination.
- Serological tests based on agglutination are usually more sensitive than those based on precipitation

Types of agglutination Reaction

- Direct
- Passive
- Reverse Passive
- Agglutination Inhibition
- Coagglutination

Types of Agglutination Reactions

Active
Agglutination

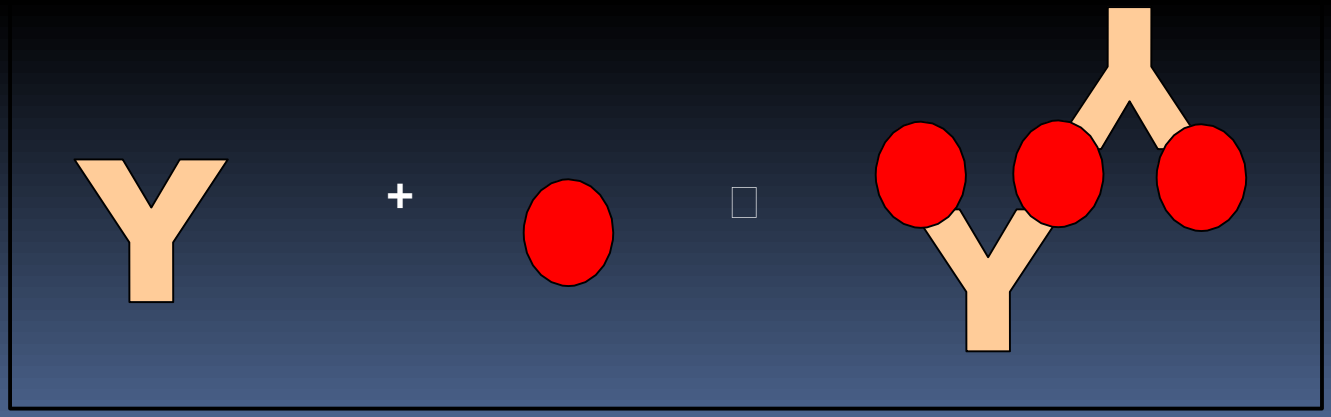
Passive
Agglutination

Basis of classification:

Antigens or epitopes are present naturally on the target cells/particles or not.

Agglutination reactions

- Active/Direct agglutination test
- The antigen part of a particulate matter naturally
- examples
  - Salmonella, vibrio, hemagglutination(ABO group)



Active Agglutination

- Epitopes of interest are naturally found on a test particle.
- Antigens found on RBCs, bacterial and fungal cells

Types

Direct / Active

↳ Slide test (Qualitative)

Eg. Blood grouping
Cross matching

↓

Ag +ve or not

↳ Tube test (Ag amount)

Qualitative →

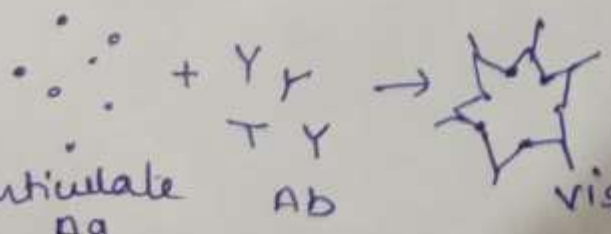
Bacterial colony + 1 drop of normal saline ↓ milky white suspension

↓

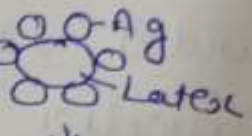
Result +ve visible clumping

↓

clear



Indirect / Passive



↳ Ag's are naturally +ve in soluble or non particulate form

↳ such Ag are coated or attach to carrier molecule to make them insoluble

↳ Earlier RBC were used but synthetic carrier particle provide uniformity, stability & consistency

+ 1 drop of Antiserum / Ab

2) Tube Agglutination test

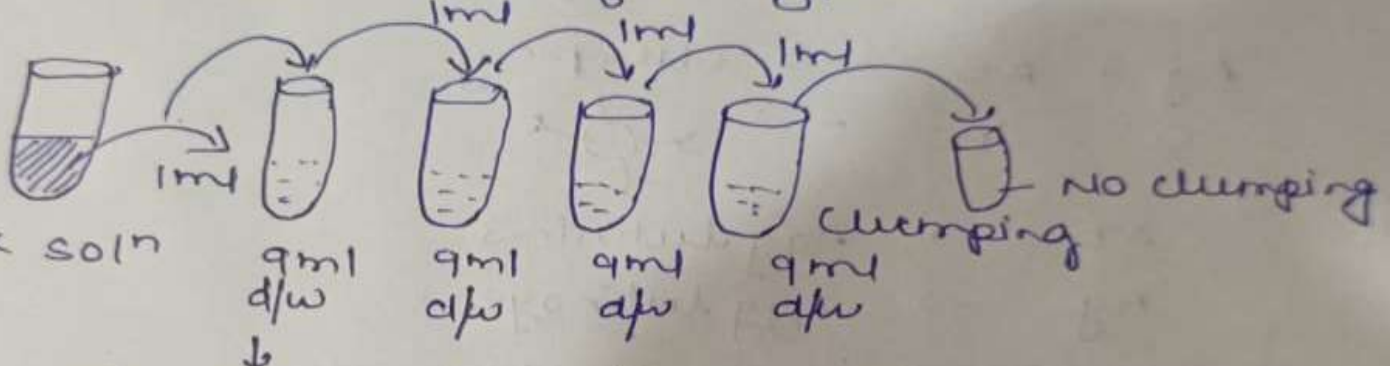
↓

Quantitative

+

Qualitative

Ex - widal Test for Typhoid



↓

Dilution 1/10 1/100 1/1000 1/10000

10^{-2} 10^{-3} 10^{-4}

↓

clumping → Ag + Ab

↳ widal test for diagnosis of typhoid fever

↳ Brucella agglutinatn test for Brucellosis

↳ Weil felix test for Rickettsiosis

Slide Agglutination Test

- Used for serotyping (e.g. Salmonella)
- Antigen: isolated Salmonella in suspension
- Antibody: specific antisera against Salmonella
- Place test Salmonella in a drop of saline on a slide
- Add a drop of antiserum, mix and rock slide for approx 1 minute
- Examine for agglutination

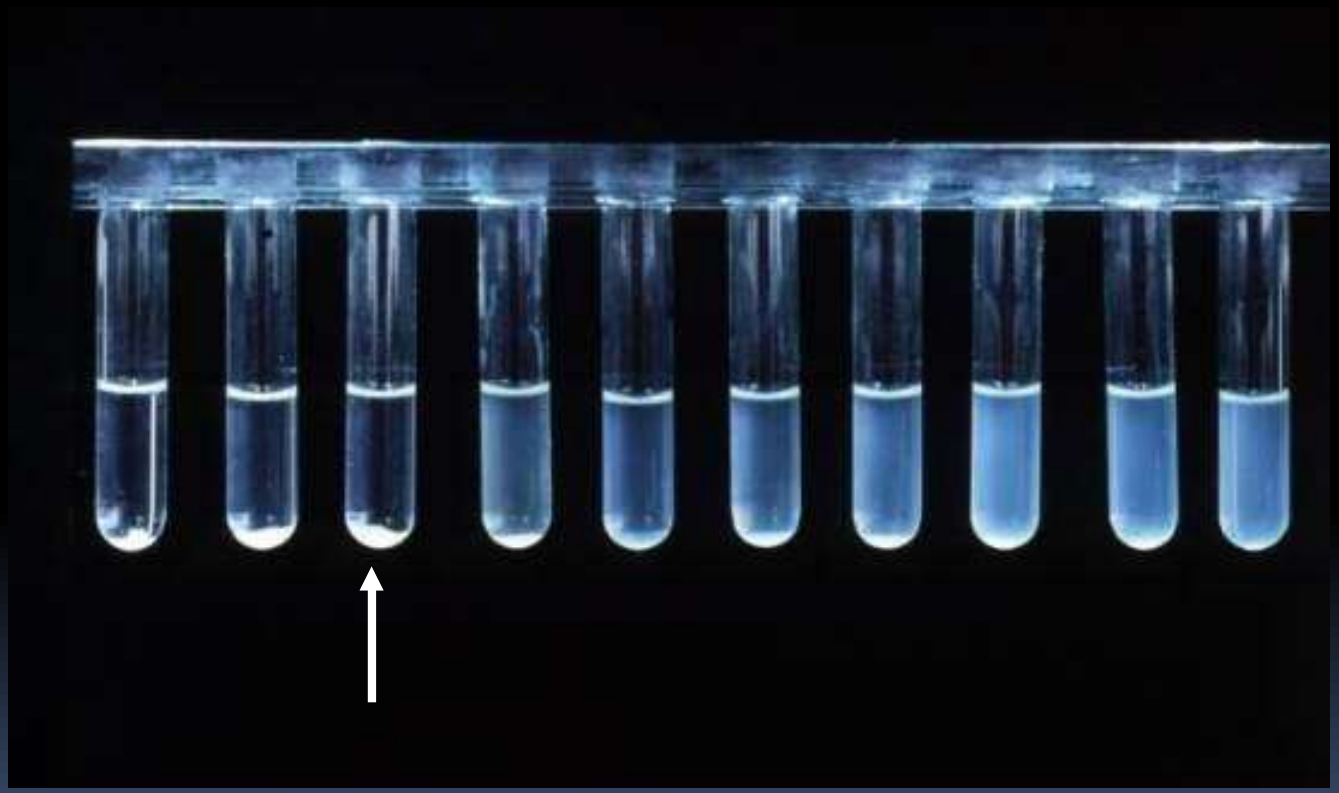
Slide Agglutination Test

Tube Agglutination Test

⑩ Also known as the standard agglutination test or serum agglutination test (SAT)

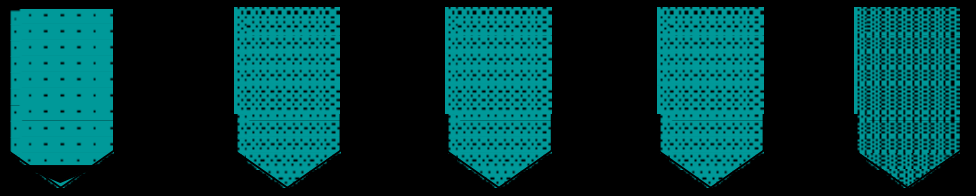
- Test serum is diluted in a series of tubes (doubling dilutions)
- Constant defined amount of antigen is then added to each tube and tubes incubated for ~20h @37°C Particular antigen clumps at the bottom of the test tube
- Test is read at 50% agglutination Quantitative
- Confirmatory test for ELISA reactors Example: Brucellosis screening

Tube Agglutination Test



Tube Agglutination Test

Agglutination No agglutination



1/10 1/20 1/40 1/80 1/160 1/320 Neg. ctrl

In this case, the titre is 40

Passive Agglutination

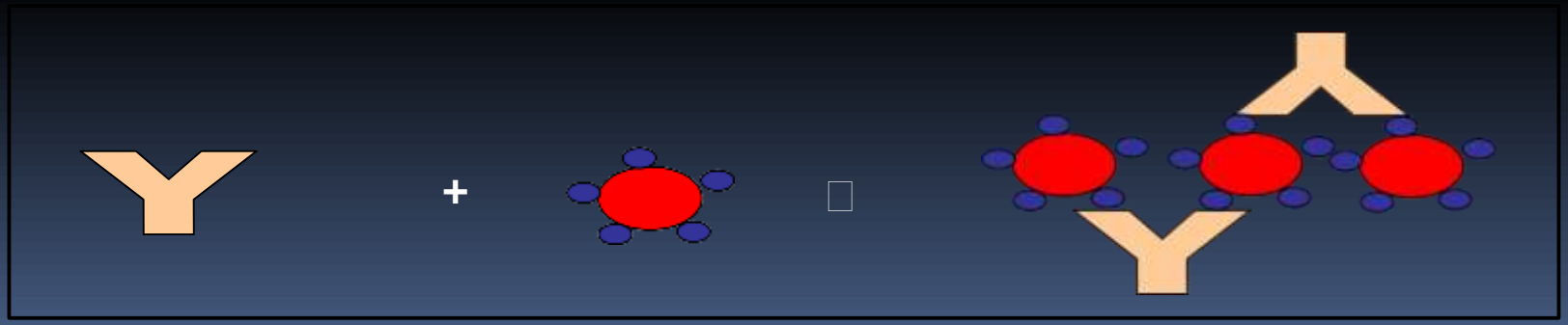
- The epitope of interest does not occur naturally on the cells or particles to be agglutinated
- Epitopes or soluble antigens are chemically fixed to carrier particles
- Carrier particles: Latex, Polystyrene, Bentonite

Passive Agglutination

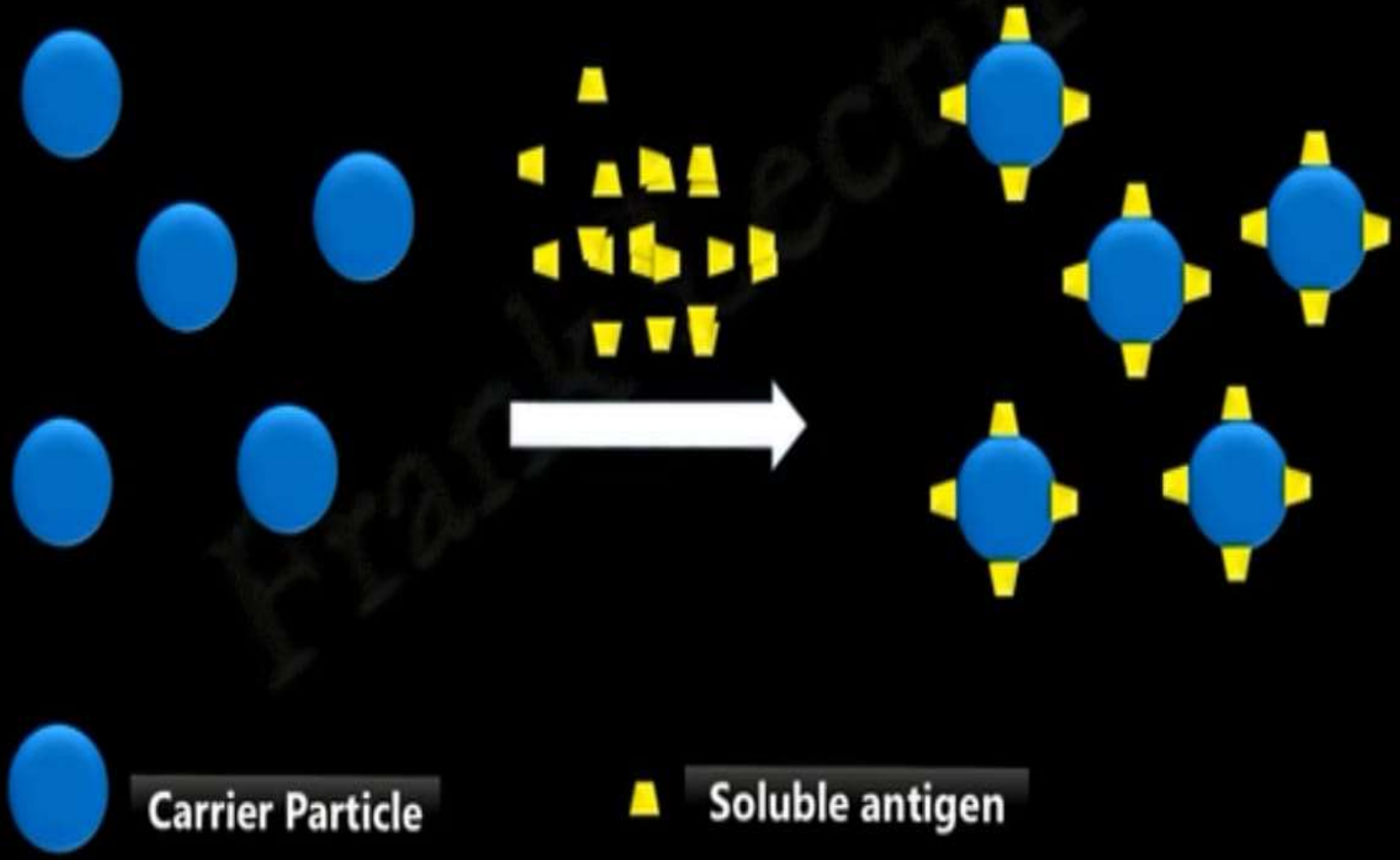
- When pathogen culture is not feasible
- When only soluble antigens are available
- Viral Diseases

Agglutination reactions

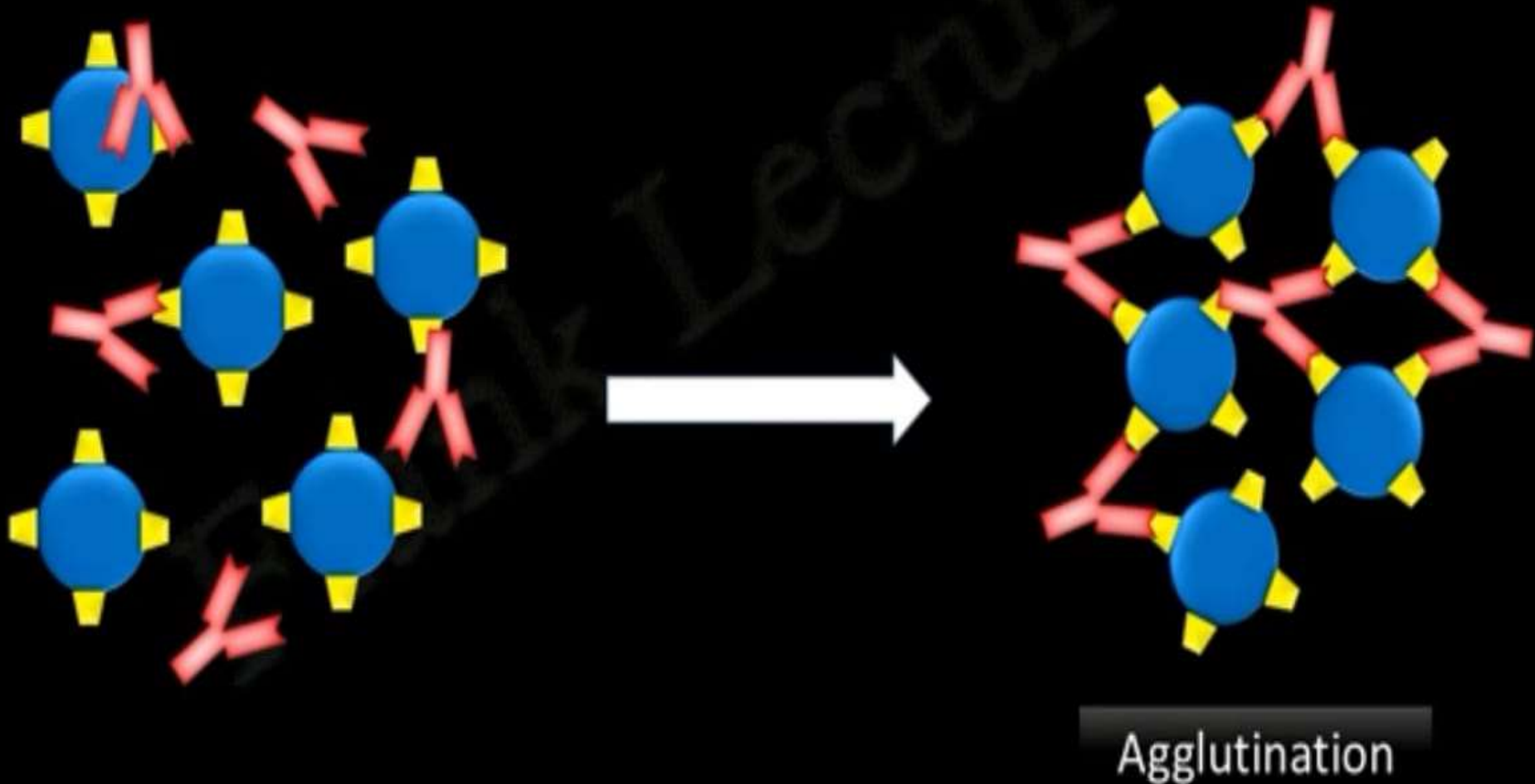
- Passive/Indirect agglutination test
- Antigen or antibody are not part of particulate matter but are attached (rided on inert particles like latex, carbon, gelatin, silicates)- consistancy, uniformity, stability
- Particle size- 7um to 0.8um



Passive Agglutination



Passive Agglutination



Passive Agglutination Test

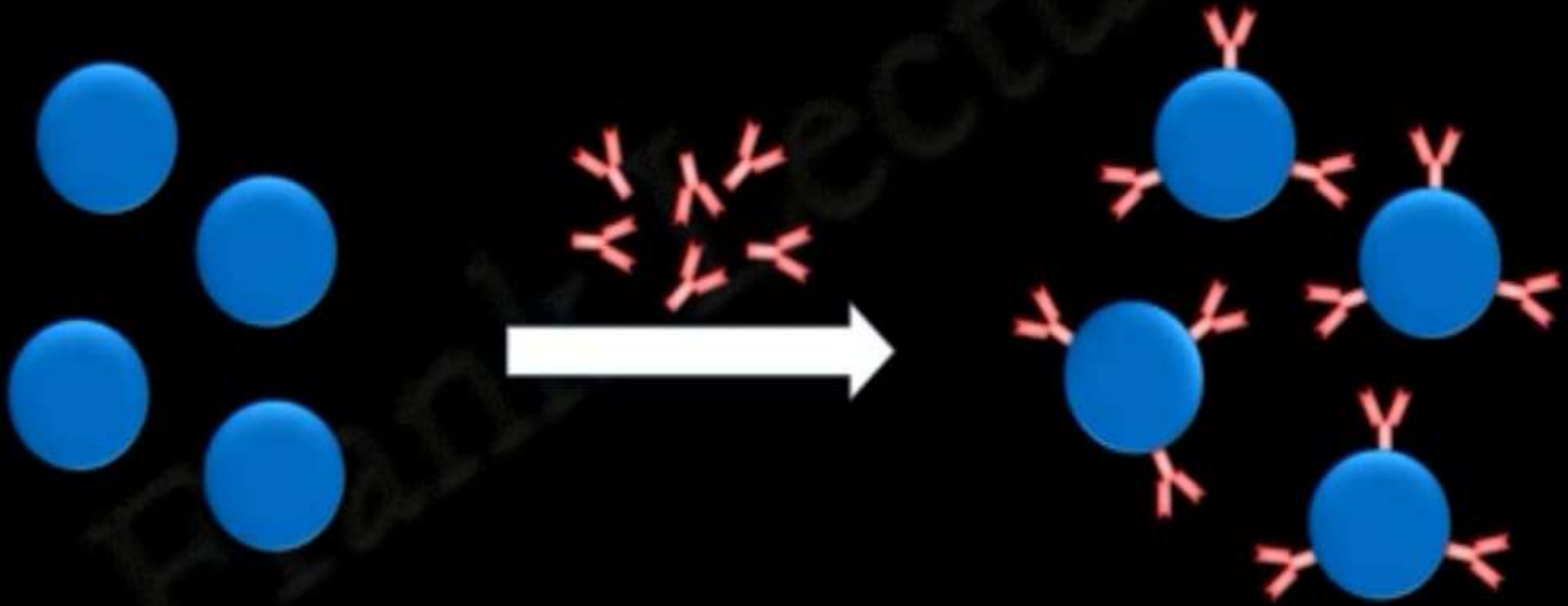
- Converting a precipitating test to an agglutinating test
- Chemically link soluble antigen to inert particles such as LATEX or RBC
- Addition of specific antibody will cause the particles to agglutinate
- Examples: RA factor, ANA, ASO, Spirochete antibody

Reverse Passive Agglutination test

- Antibody is attached to the particulate carrier. One of the most commonly used laboratory tests that involves latex particle agglutination is the procedure for C-Reactive Protein (CRP).
- Reverse PAT: antibody linked to LATEX

e.g. Lancefield grouping in Streptococci.

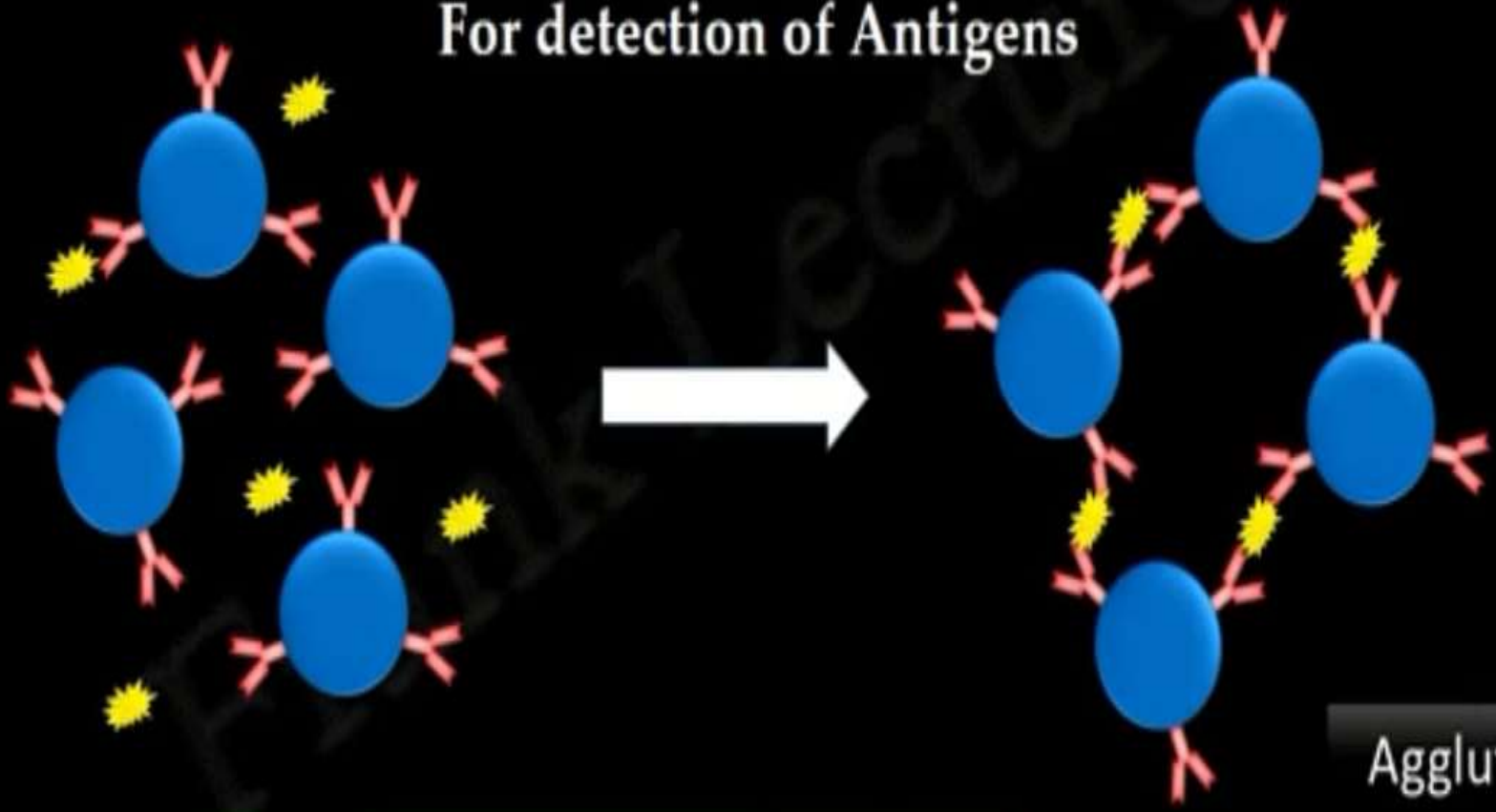
Passive Agglutination



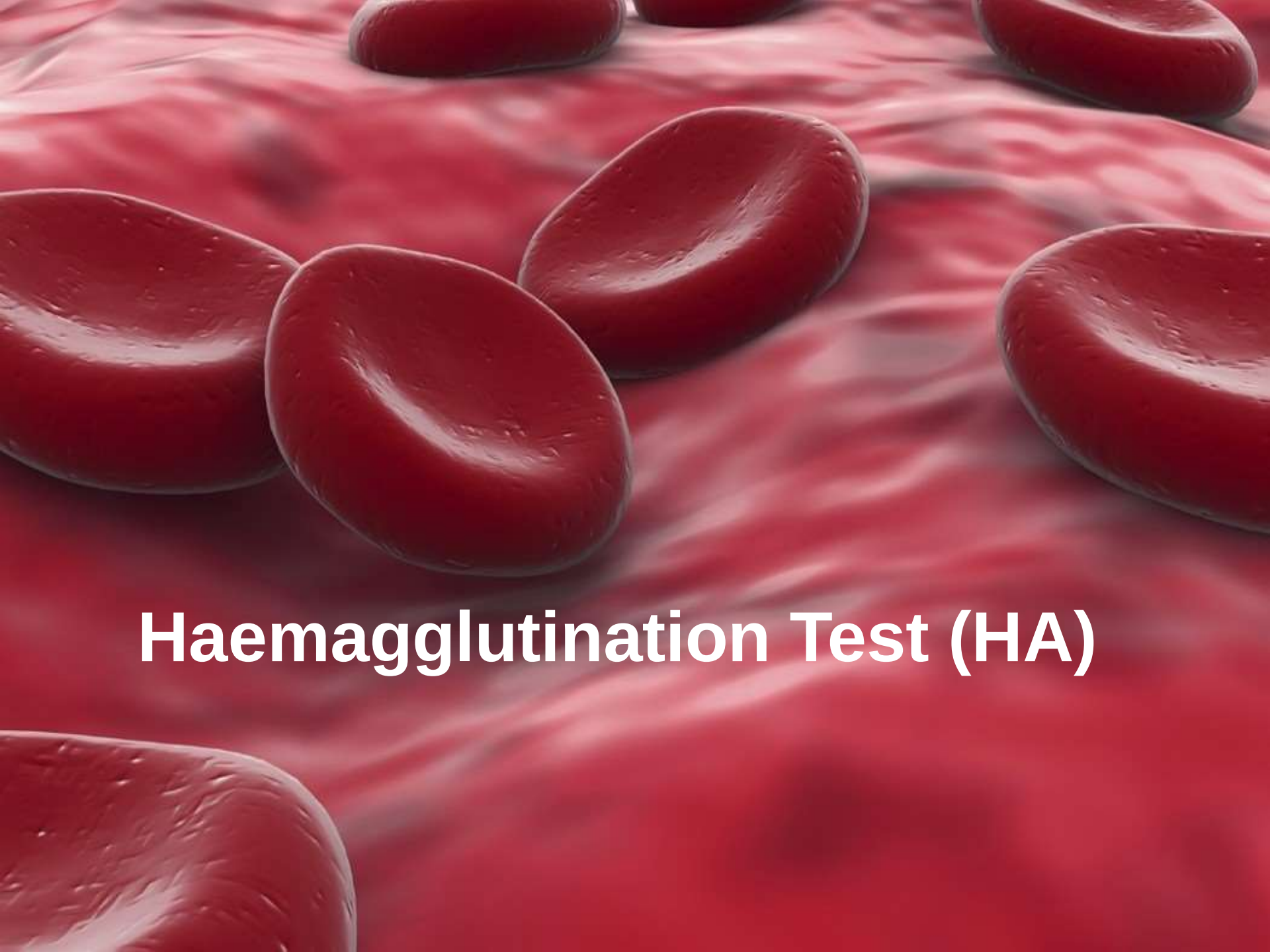
For detection of Antigens

Passive Agglutination

For detection of Antigens

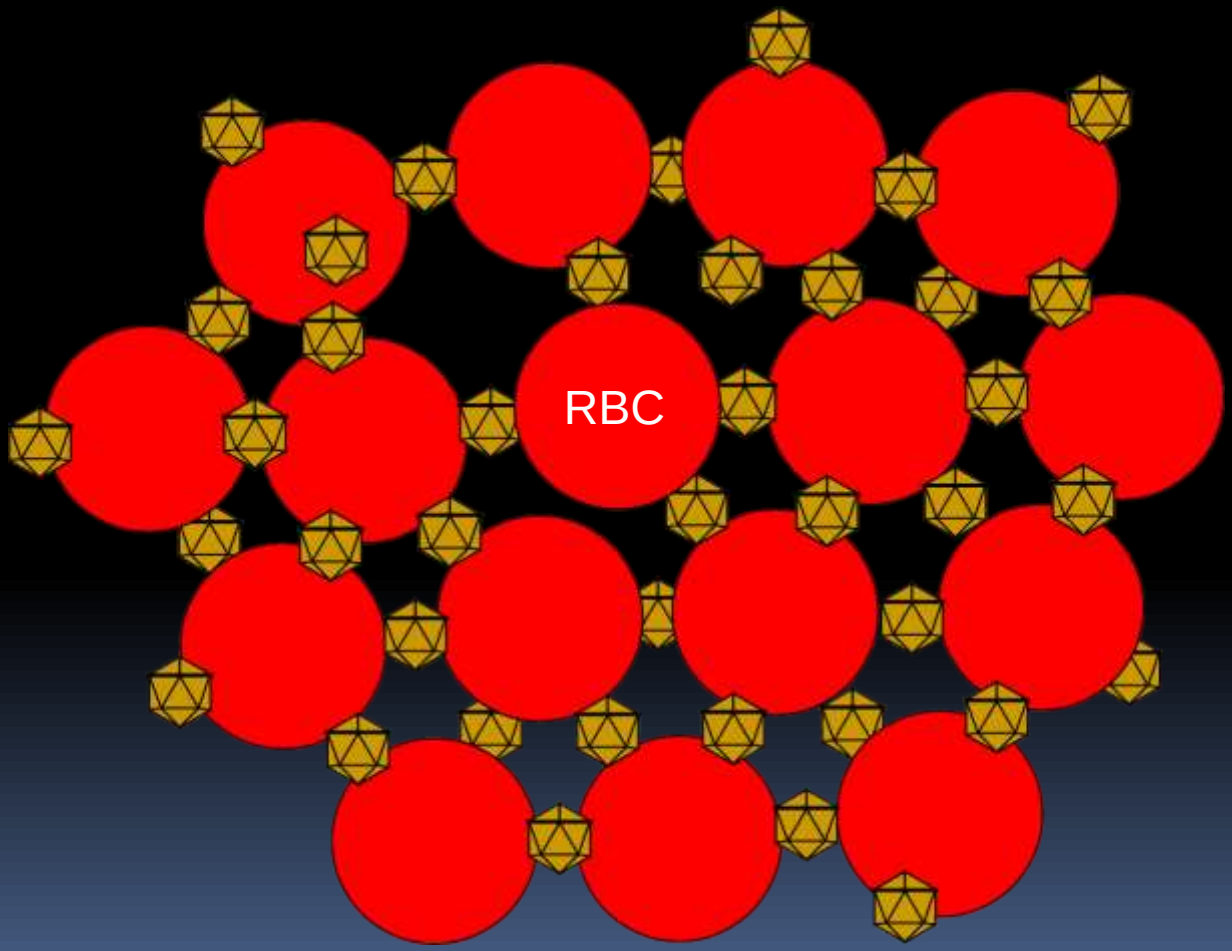


Reverse Passive Agglutination



Haemagglutination Test (HA)

Haemagglutination



Hemagglutination

Principle :: Haemoagglutination based on the principle that the nucleic acid of virus code for Heamagglutinin or other reactive protein exposed on virus envelope interact with sialic acid receptor on the surface of RBCs and create a network or combinat" of interacted RBCs and virus.

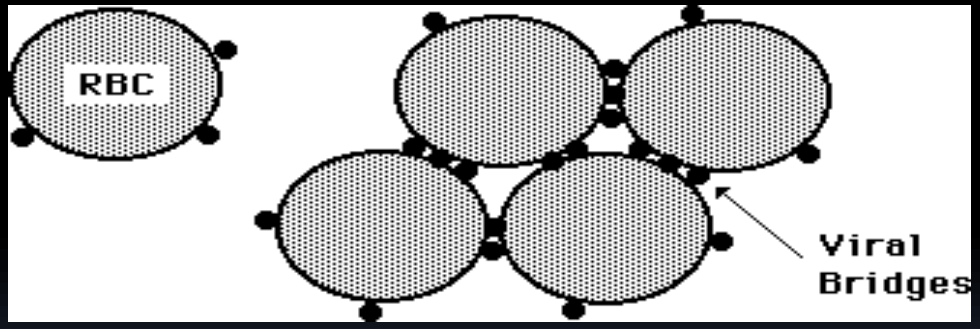
→ In some bacterial species eg staphylo. coccus Vibrio etc also shows hemagglutin" reaction which is similar to virus heamagglutination reaction

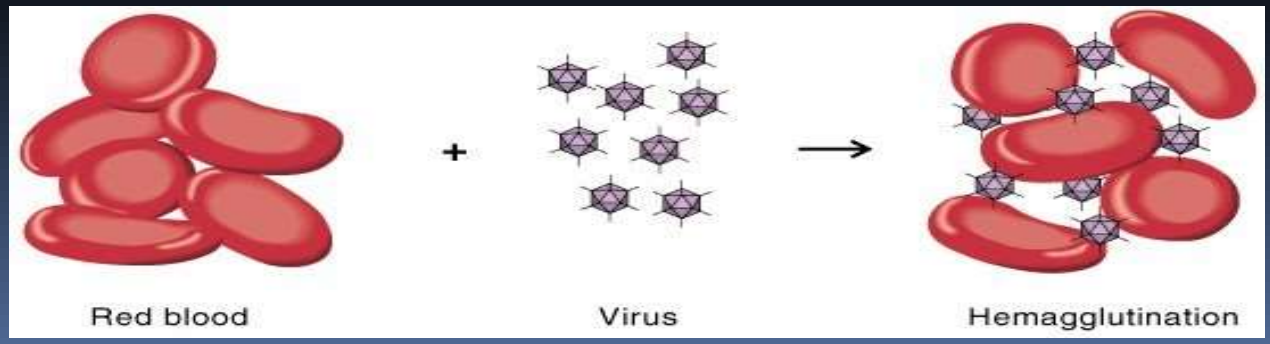
→ Heamoagglutination is a reaction that causes clumping of red blood cells in +nce of some enveloped virus such as the influenza virus.

Viral Haemagglutination

Some viruses and microbes contain proteins which bind to erythrocytes (red blood cells) causing them to clump together

MMR
Adenovirus III
Influenza
RSV
Mycoplasma



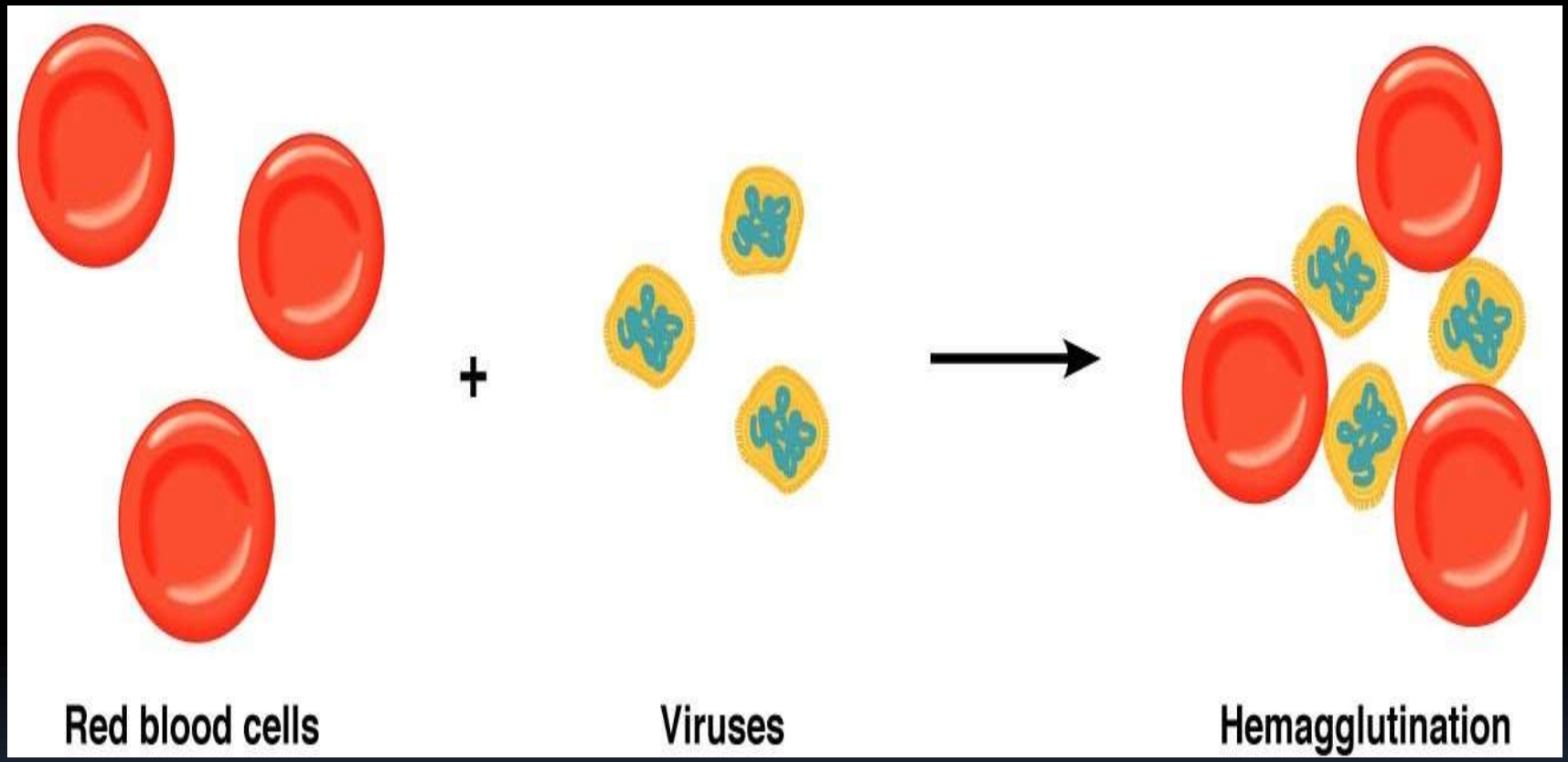


→ A glycoprotein on the viral surface, namely hemagglutinin, interacts with red blood cells, leading to clumping of red blood cells and the formation of a lattice.

→ In absence of an enveloped virus, red blood cell precipitate at the bottom of a container, forming a red coloured dot.

→ However, in presence of a virus, red blood cells clumps are ~~disappeared~~ dispersed, forming no red colour dot. This is a basic principle of hemagglutination assay.

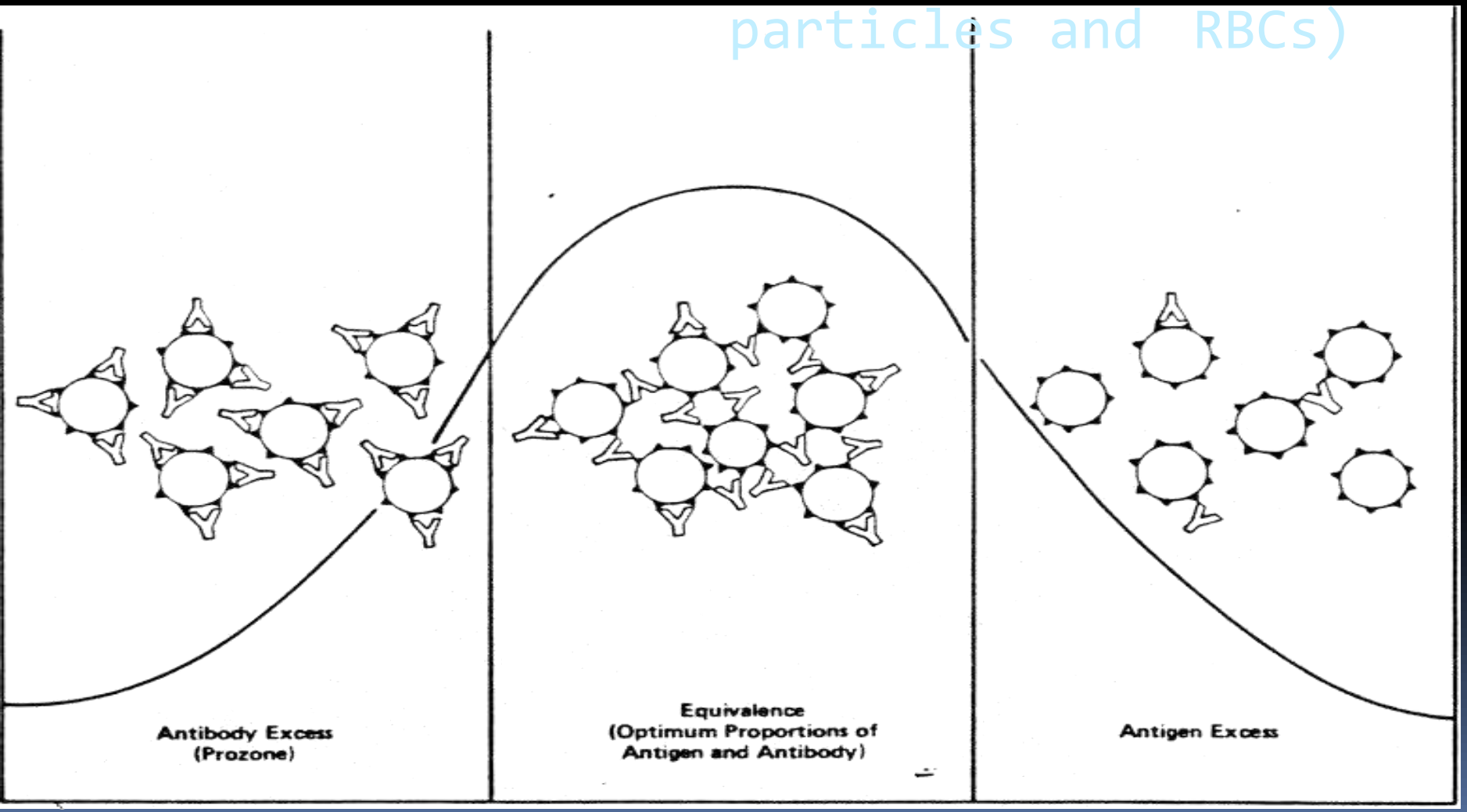
Viral Hemagglutination

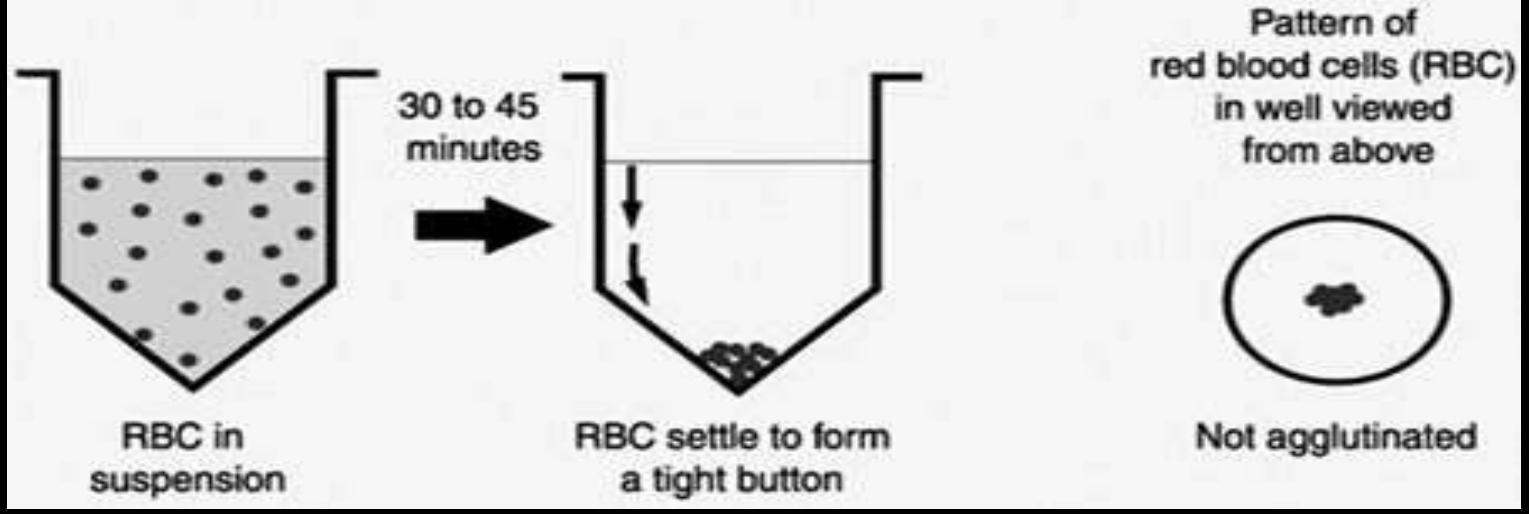


- the attachment of viral particles by their receptor sites to more than 1 cell.
- As more and more cells become attached in this manner agglutination becomes visible

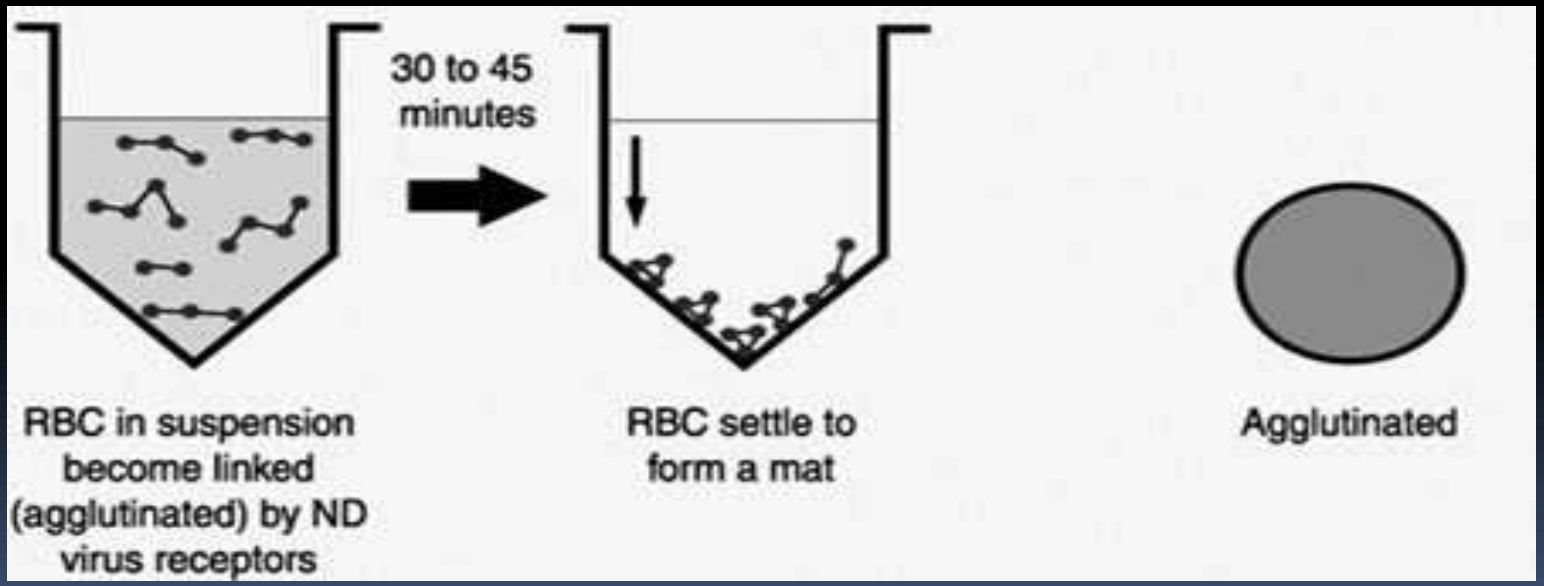
Equivalence point:

(suitable proportion between the virus particles and RBCs)





Negative control well (only RBCs+ buffer) (no haemagglutinin)



Positive control well (contains haemagglutinin)

Readings The results

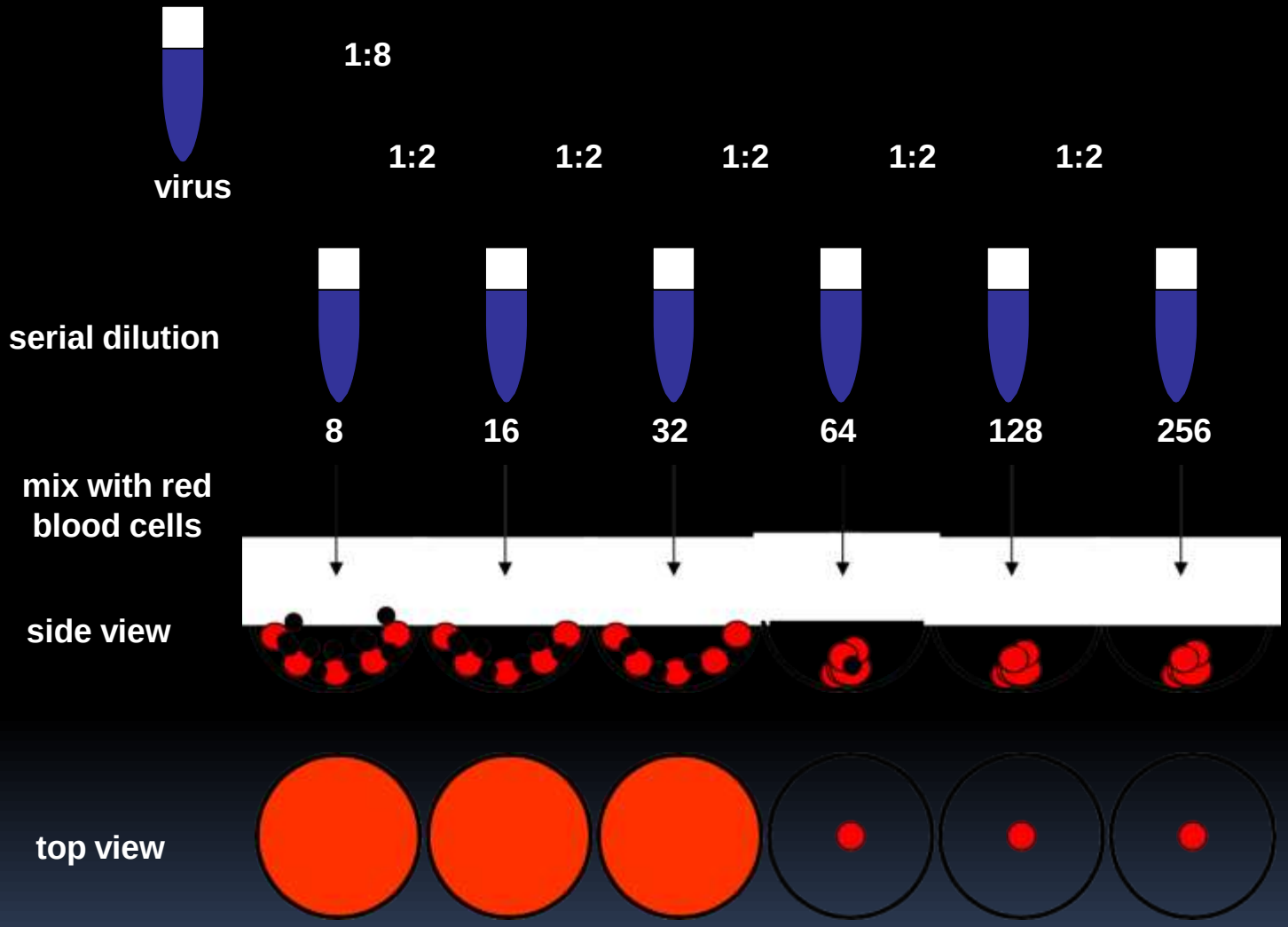
- **Titer:** The maximum dilution that gives visible agglutination.
- **The end point:** is the well with the lowest concentration of the virus where there is haemagglutination

2 4 8 16 32 64 128 256 512 1024 2048 4096

The HA titer of this virus in this row is 256 or 2^8

(1:256 dilution contains (**1 HA unit**) (one haemagglutinating unit)

Hemagglutination test: method



Titer = 32 HA units/ml

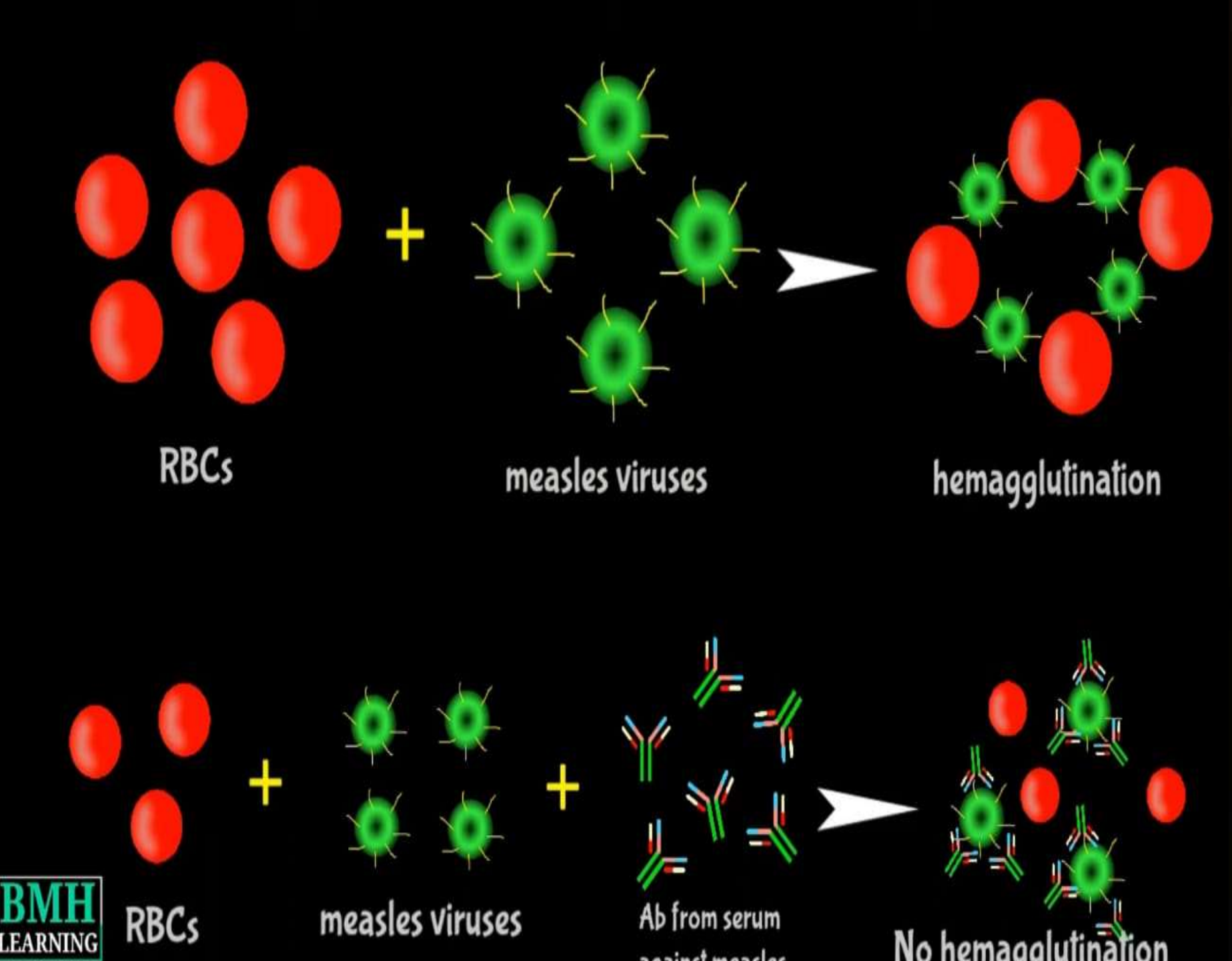
One HA unit :minimum amount of virus that causes complete agglutination of RBCs

HEMAGGLUTINATION INHIBITION TEST (HI)

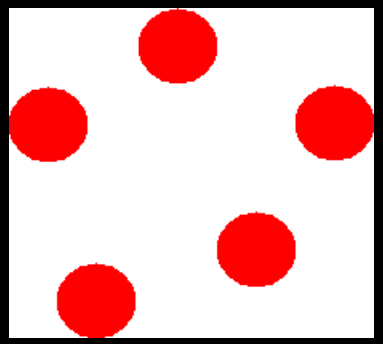
VIRUSE

Red Blood Cell

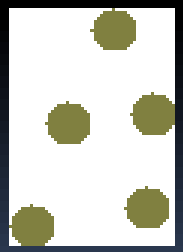
SERUM


RBCs
measles viruses
hemagglutination
RBCs
measles viruses
Ab from serum
No hemagglutination
BMH
LEARNING

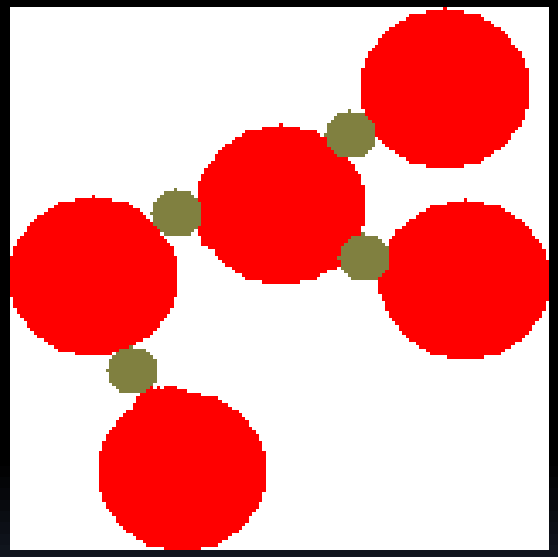
In the absence of anti-virus antibodies



Erythrocytes

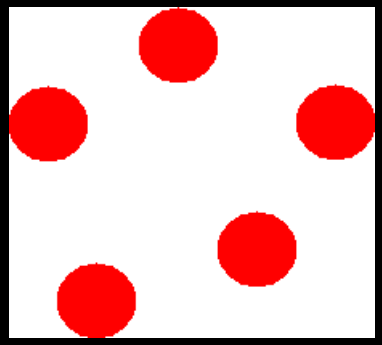


Virus

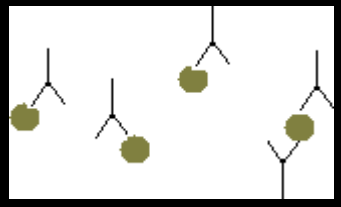


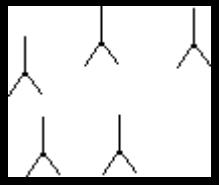
Virus agglutination of erythrocytes

In the presence of anti-virus antibodies



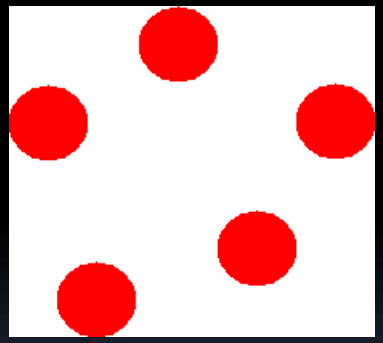
Erythrocytes



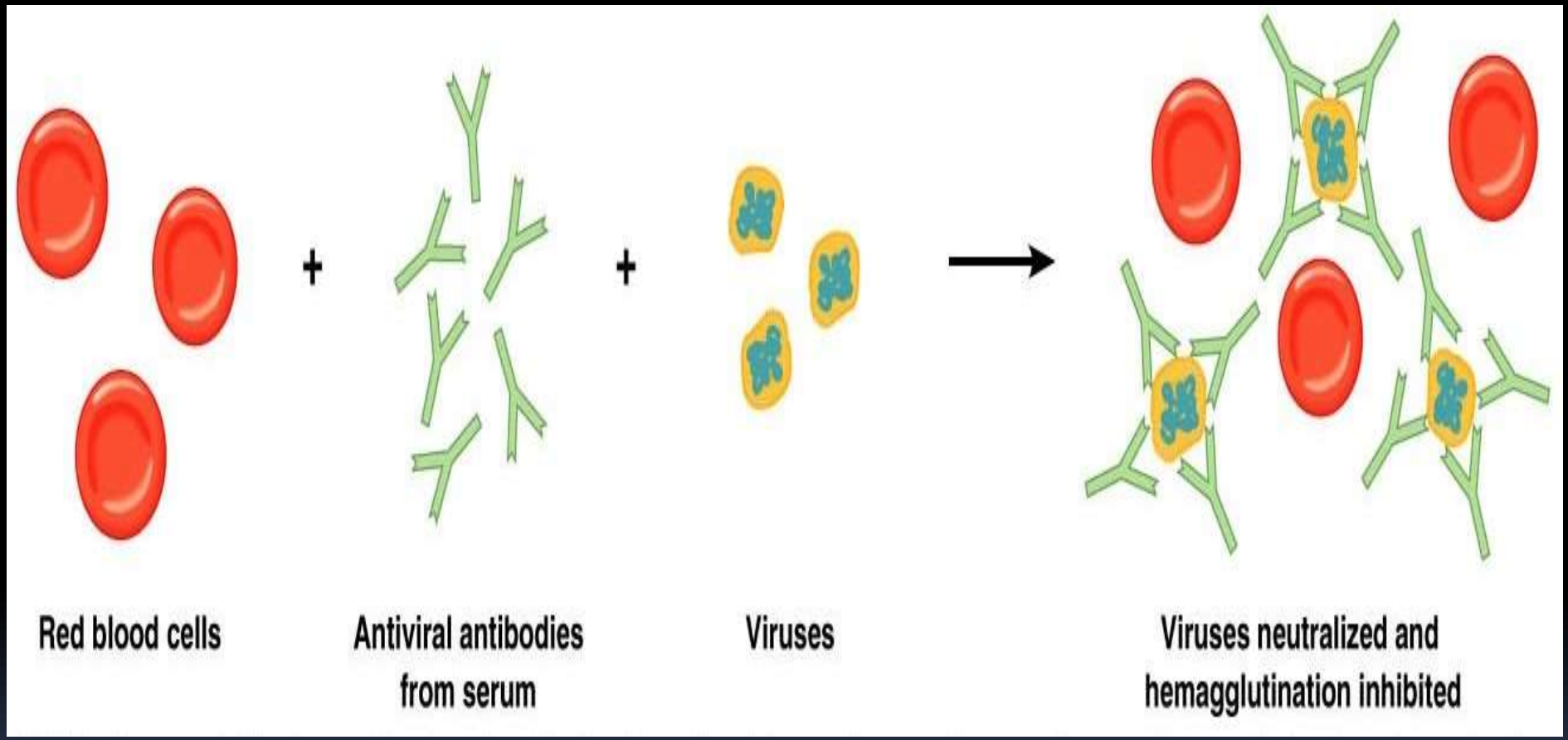


Virus

Anti-virus antibodies



Viruses unable to bind to the erythrocytes


Red blood cells
Antiviral antibodies from serum
Viruses
Viruses neutralized and hemagglutination inhibited

- Purpose: To quantitate serum antibody to a specific avian antigen

Procedure:

1. A constant amount of haemagglutinating (HA) antigen is added to each well in a microtiter plate.
2. The test serum is then placed in the first well and serially diluted.
3. The plates are incubated for one hour and then chicken RBCs are added to each well. If antibody is present in the test serum the RBCs will not agglutinate with the HA antigen.
    1. HI NEGATIVE wells will have a diffuse sheet of agglutinated RBCs covering the bottom.
    2. HI POSITIVE wells will have a well circumscribed button of unagglutinated RBCs

Antibody

- Is the lowest concentration of antibodies against a particular antigen.

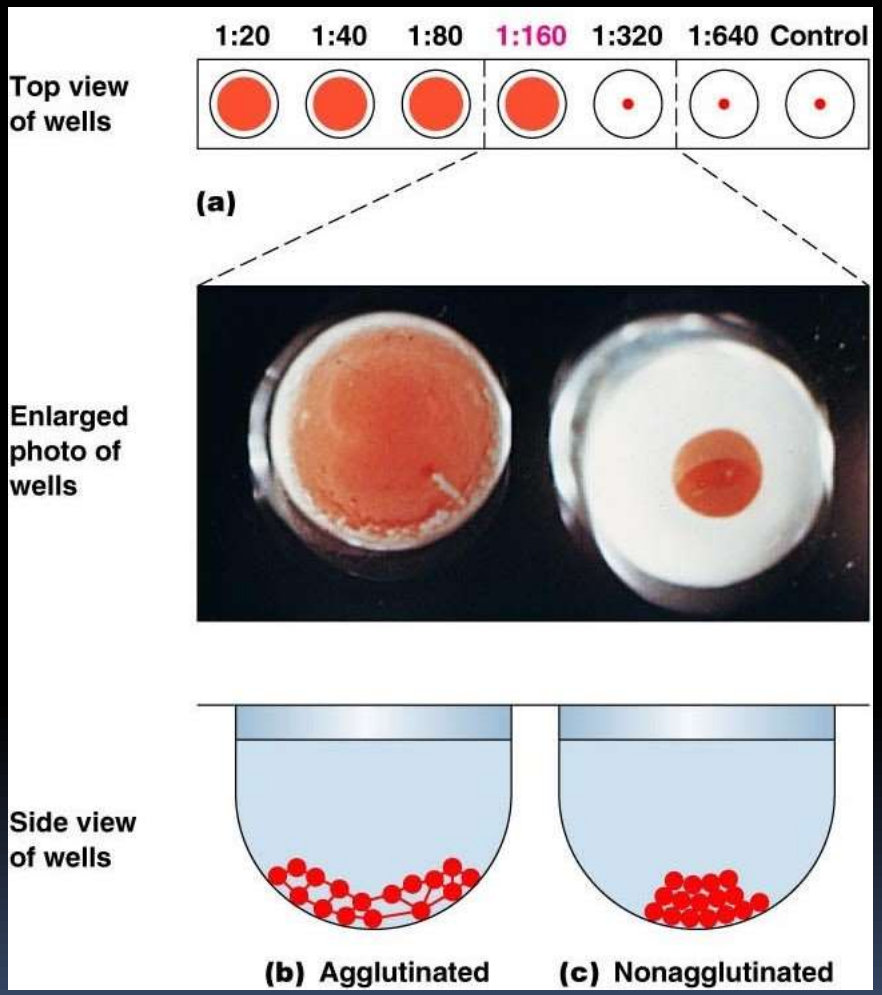
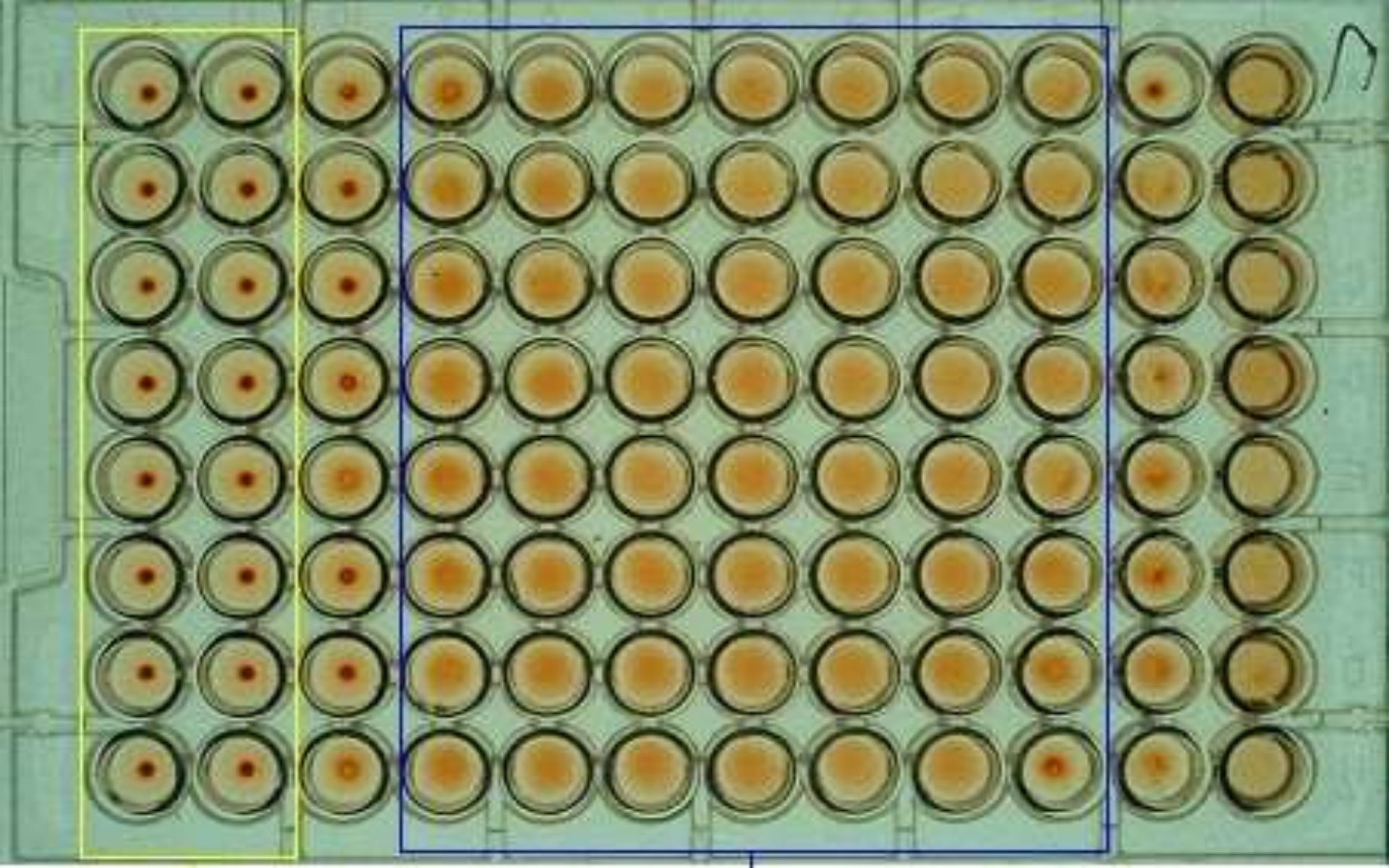
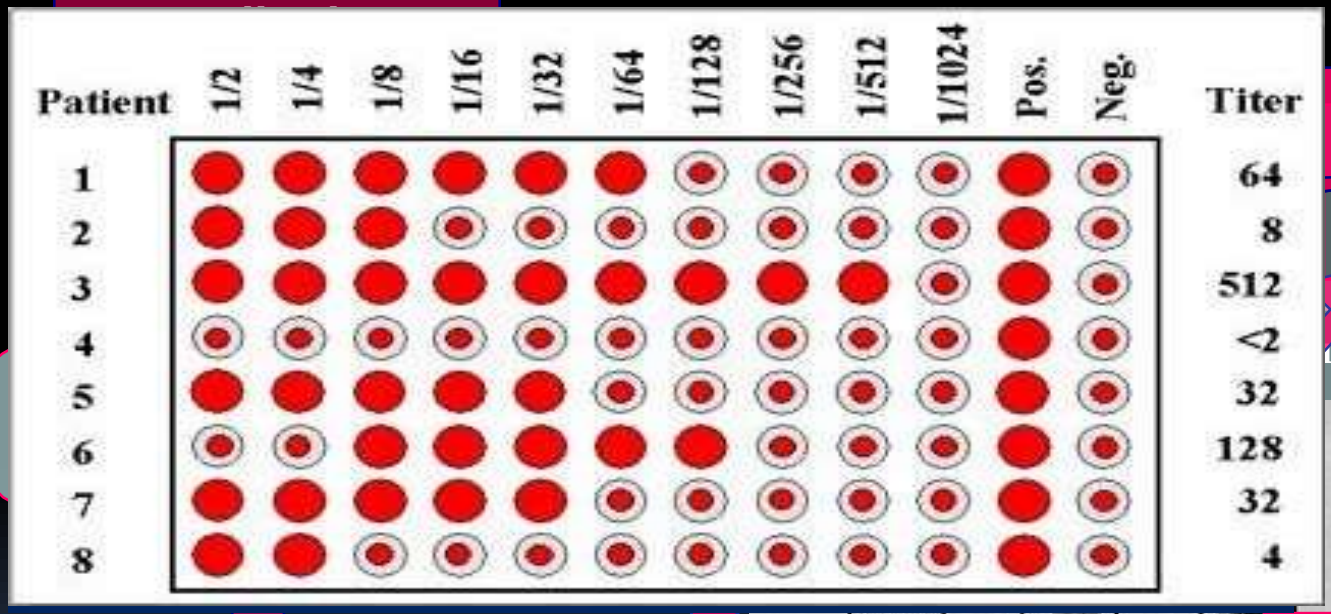


Figure 18.6


Antibodies
in the serum
No antibodies
in the serum

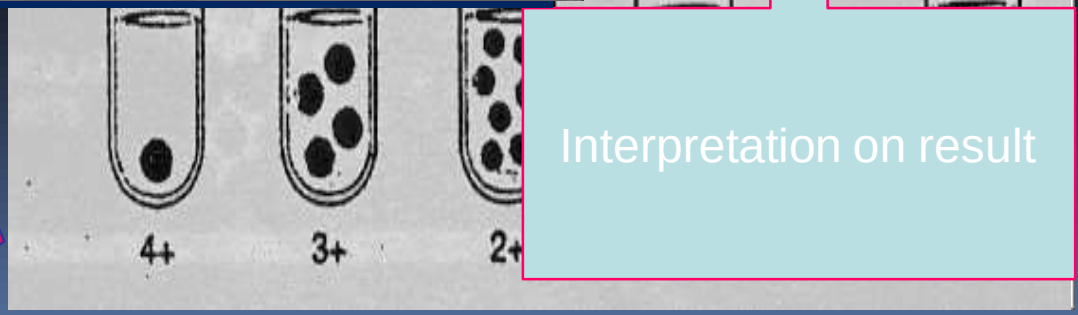
Agglutination Procedure



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

Quantitative

Interpretation on result



Interpretation on result

Advantages of agglutination methods

- ease of performance.
- speed of performance, usually requiring few minutes.
- high degree of sensitivity.

Disadvantages of agglutination methods

- the reaction are only semiquantitative.
- the occurrence of the prozone phenomenon, in which agglutination is inhibited by extreme antibody excess as a result of poor lattic formation.

Application of agglutination test

- several antibodies can be detected by this method such as Rheumatoid factor.

THANK YOU