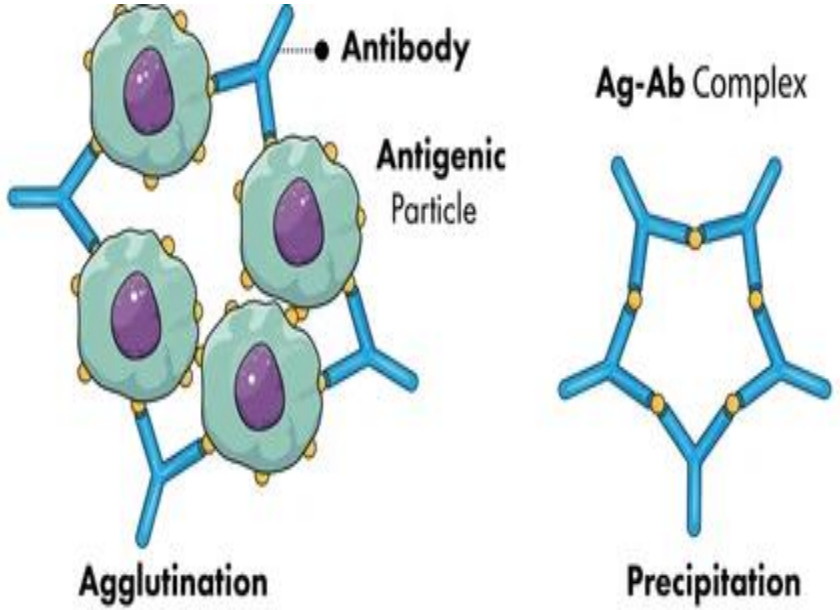
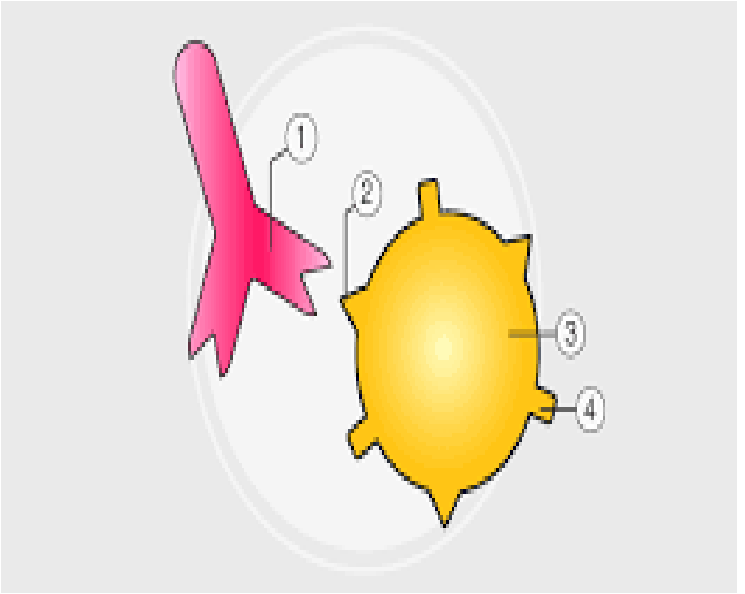
Antigen- Antibody Reaction

by
Prof. Dr. Rafal Khaleel Farhan

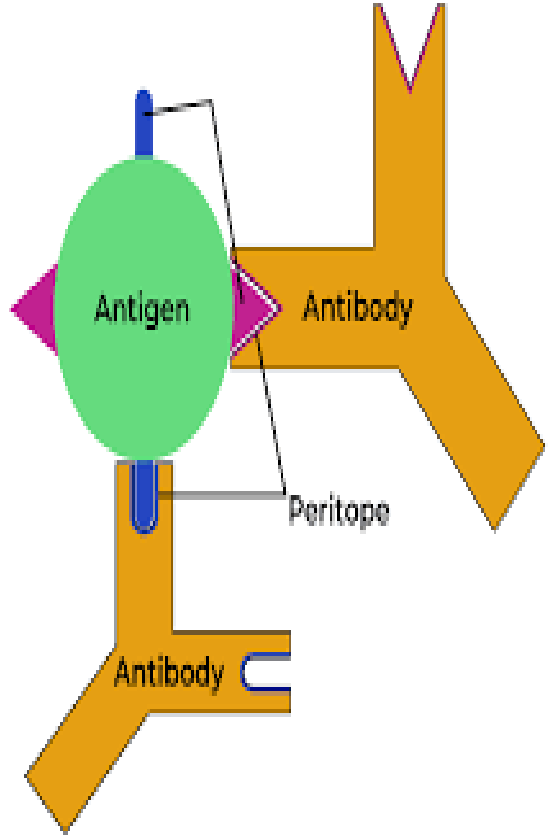


Antigen-antibody reaction

- **Antigen-antibody interaction, or antigen-antibody, is a specific chemical interaction between antibodies produced by B cells of the white blood cells and antigens during immune reaction.**
- **The antigens and antibodies combine by a process called agglutination.**
- **It is the body's fundamental reaction by which it is protected from complex foreign molecules, such as pathogens and their chemical toxins.**
- **In the blood, the antigens are specifically and with high affinity bound by antibodies to form an antigen-antibody complex.**
- **The immune complex is then transported to cellular systems where it can be destroyed or deactivated.**



- **There are several types of antibodies and antigens, and each antibody can bind only to a specific antigen.**
- **The specificity of the binding is due to the specific chemical constitution of each antibody.**
- **The antigenic determinant or epitope is recognized by the paratope of the antibody, situated at the variable region of the polypeptide chain.**
- **The variable region in turn has hyper-variable regions which are unique amino acid sequences in each antibody.**
- **Antigens are bound to antibodies through weak and noncovalent interactions such as electrostatic interactions, hydrogen bonds, Van der Waals forces, and hydrophobic interactions**



General Properties Of Antigen-antibody Reactions

1-Specificity

The ag-Ab reaction involves specific interaction between the epitope of an antigen with the corresponding paratope of its homologous antibody

2-Strength

The strength or the firmness of the association is influenced by the affinity and avidity of the antigen–antibody interaction.

3-Affinity

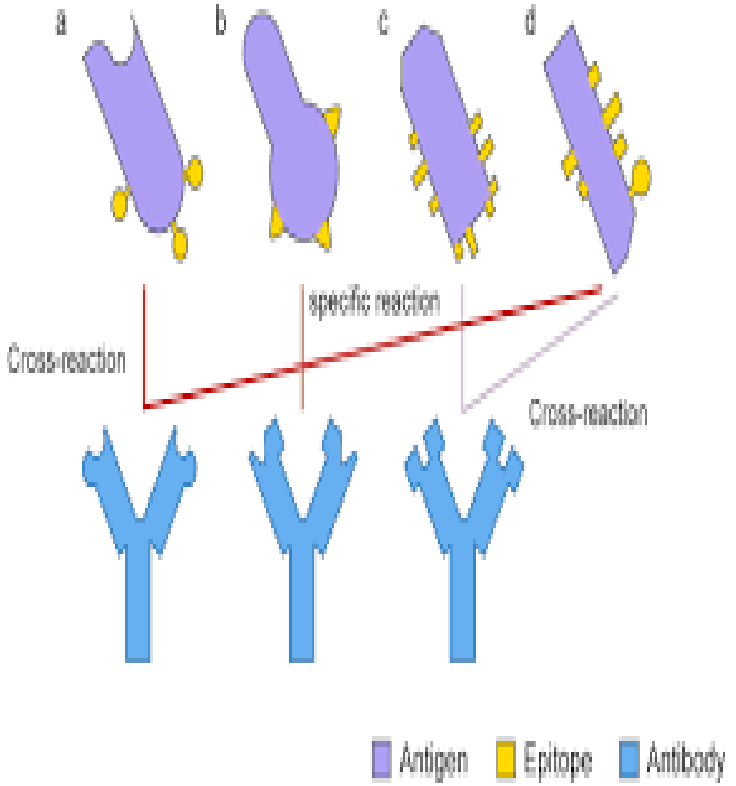
It refers to the sum total of noncovalent interactions between a single epitope of an antigen with its corresponding paratope present on antibody

4-Avidity

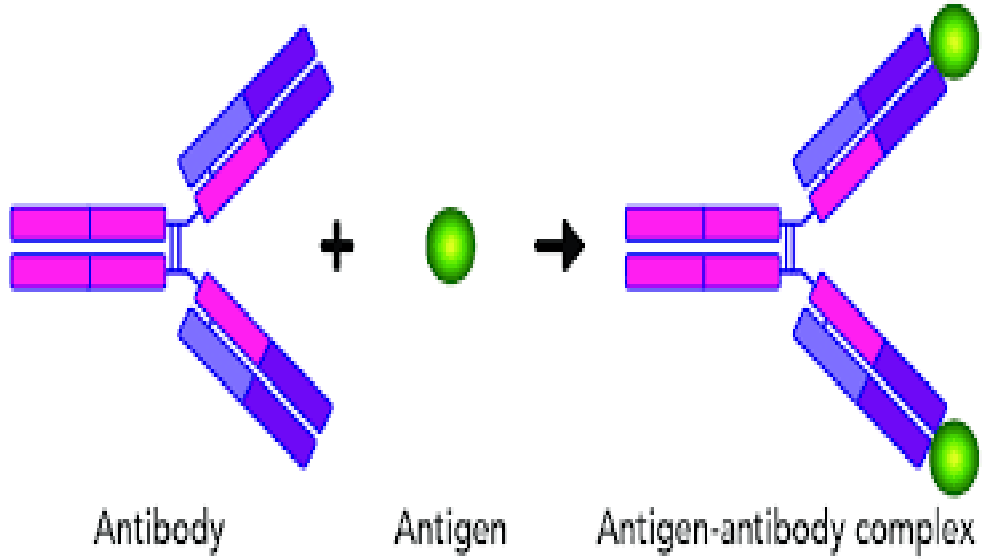
It is a term used to describe the affinities of all the binding sites when multivalent antibody reacts with a complex antigen carrying multiple epitopes.

Cross Reactivity

- An antiserum raised against an Ag, can also react with a similar Ag of another type.
- This is called cross reaction and the Ag which produces the cross reaction is called Cross-reactive Ag.
- But the strength of Ab raised against its own Ag is strong.

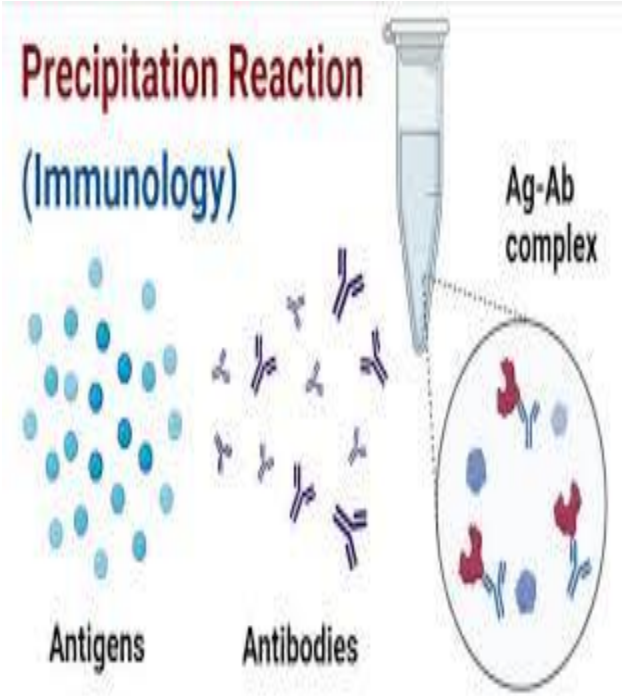


- **The principles of specificity and cross-reactivity of the antigen-antibody interaction are useful in clinical laboratories for diagnostic purposes.**
- **One basic application is the determination of the ABO blood group.**
- **It is also used as a molecular technique for infection with different pathogens, such as HIV, microbes, and helminth parasites.**



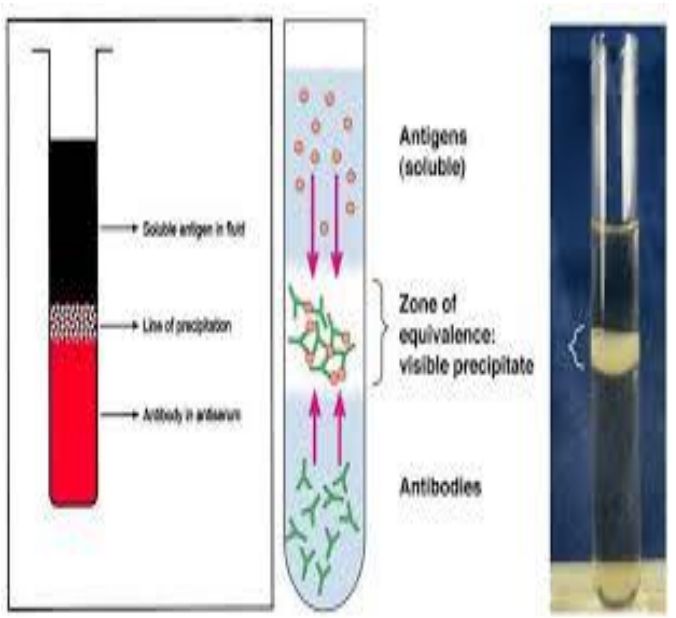
Precipitation Reaction

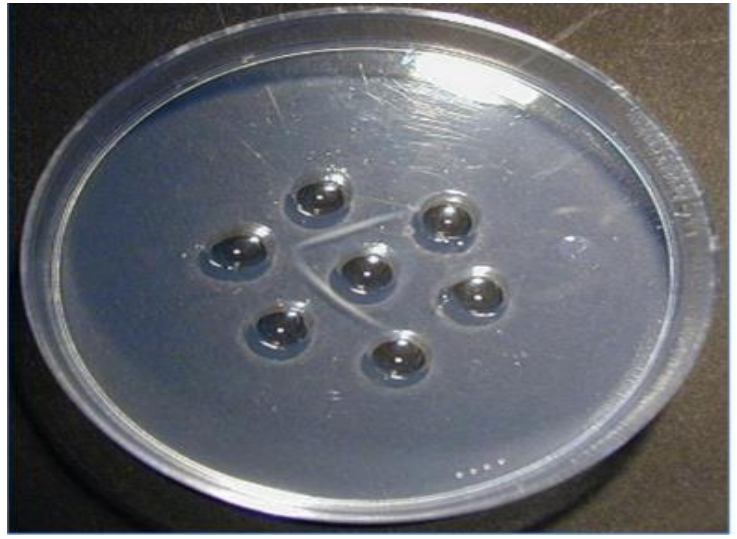
- **Soluble antigens combine with soluble antibodies in presence of an electrolyte at suitable temperature and pH to form insoluble visible complex. This is called a precipitation reaction.**
- **It is used for qualitative and quantitative determination of both antigen and antibody.**
- **It involves the reaction of soluble antigen with soluble antibodies to form large inter locking aggravates called lattice**
- **the antigen and antibody rapidly form antigen-antibody complexes within few seconds and this is followed by a slower reaction in which the antibody-antigen complexes forms lattices that precipitate from the solution**



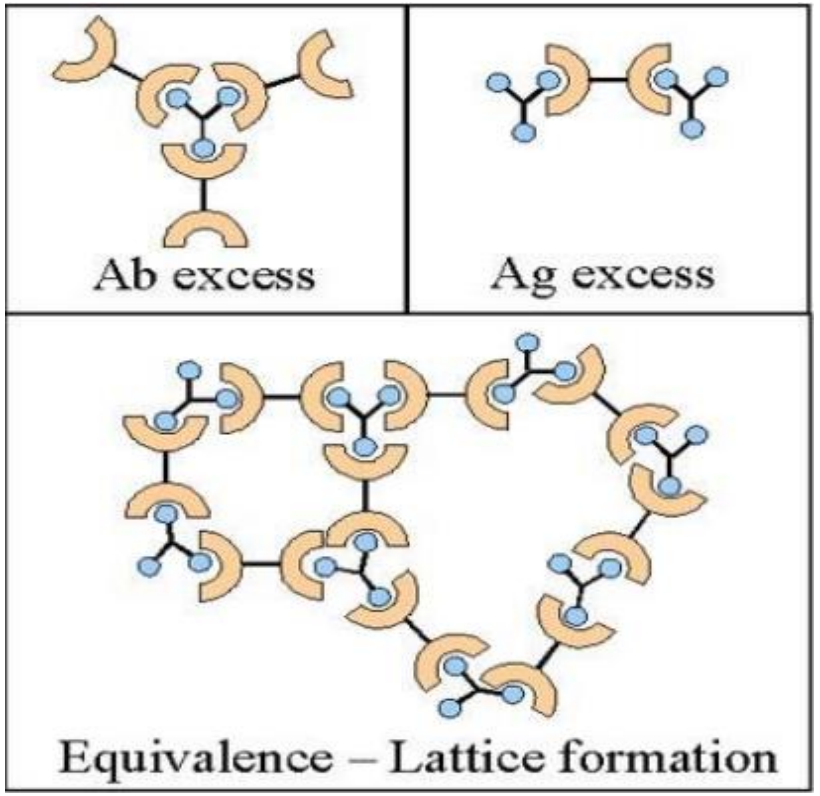
Precipitation Reaction

- The precipitation reaction occurs in both liquid and gel media.
- **Liquid Precipitation:** An antigen-antibody reaction is carried out by adding increasing amounts of antigen to tubes containing a constant amount of antibody.
- Precipitation results from the combined reaction of the antigen and antibody.
- **Gel Precipitation:** Petri plates or plates with agar gel or a similar gel are used in these methods.
- In the gel system, both Ag and Ab rapidly diffuse in all directions.
- A zone of equivalency, observed as visible precipitation, will form at a specific point depending on the diffusion rate and concentration of reactants.



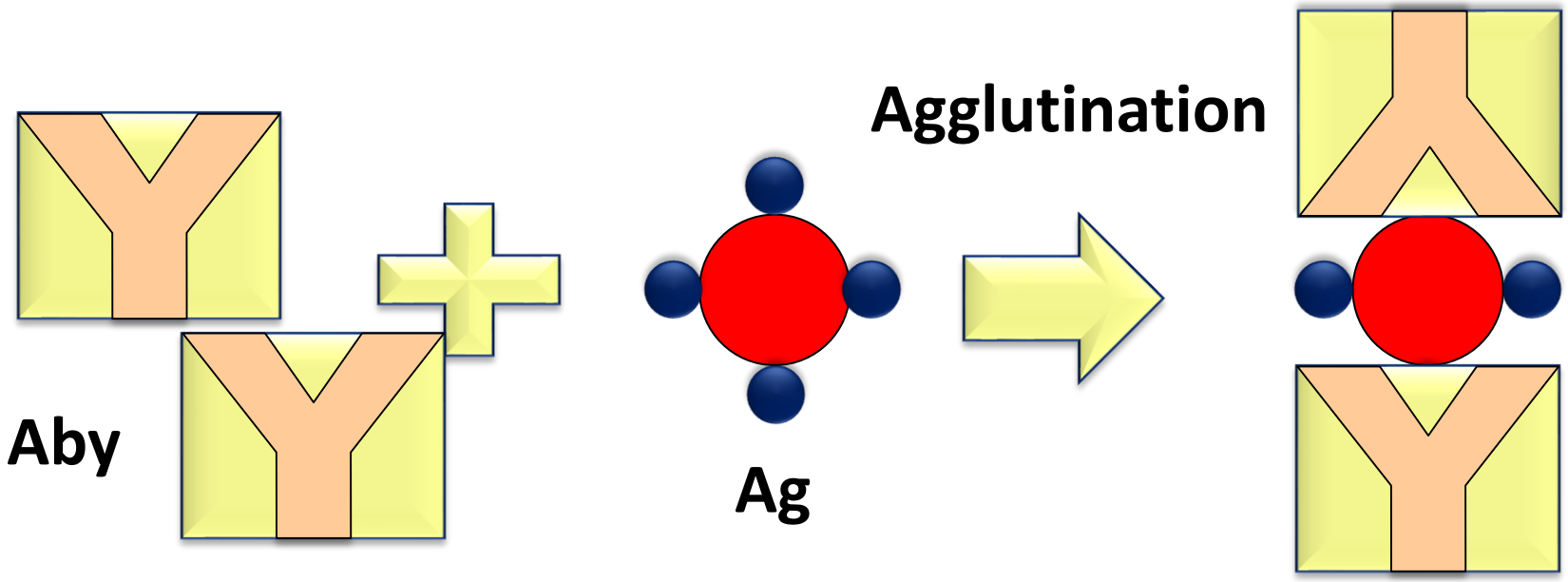


In the titration of precipitating antigen, three general zones of reaction may be observed, three are
1- the zone of antibody excess
2- the zone of equivalence
3- the zone of antigen excess



Agglutination

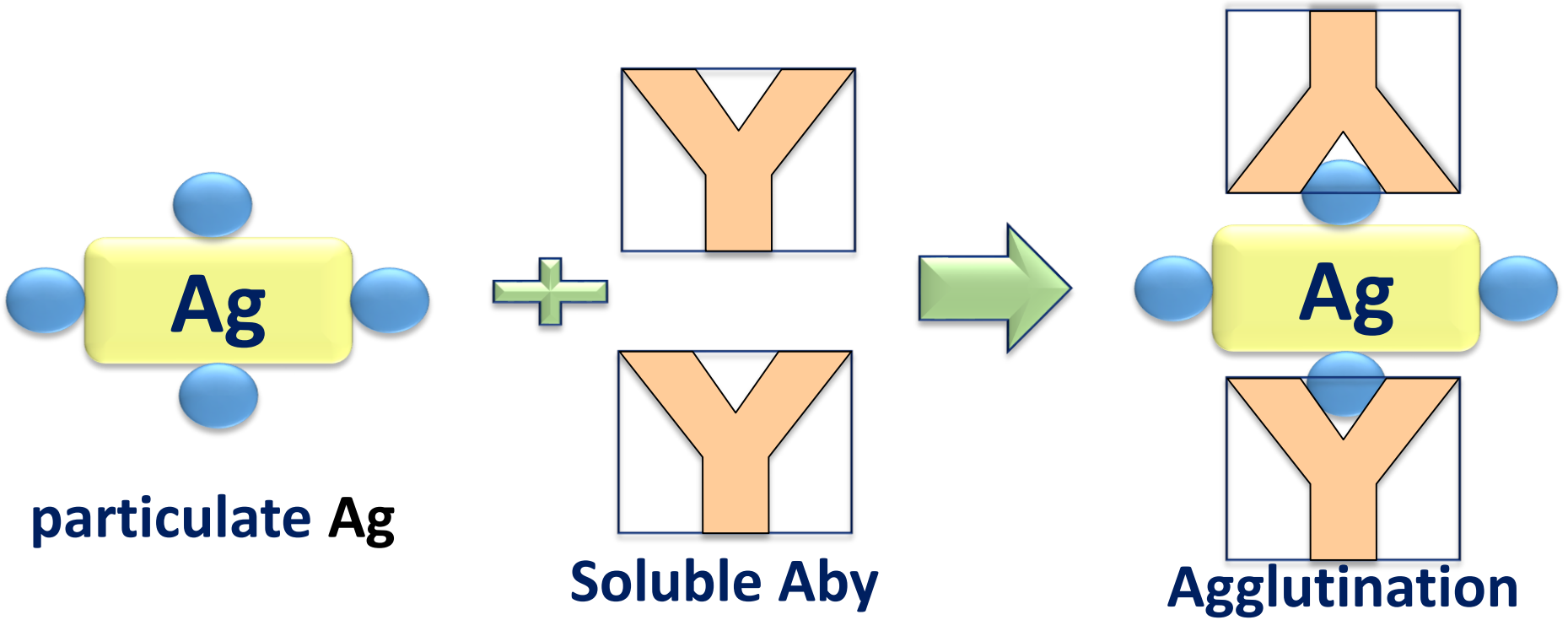
An antibody that forms clumps or aggregates when reacts with cells or particular antigen



Definition of Direct agglutination

When a particulate antigen (e.g. bacteria, fungi, RBC or WBC) is mixed with is specific antibody, agglutination (clumping) occurs directly.

Principle of Direct agglutination

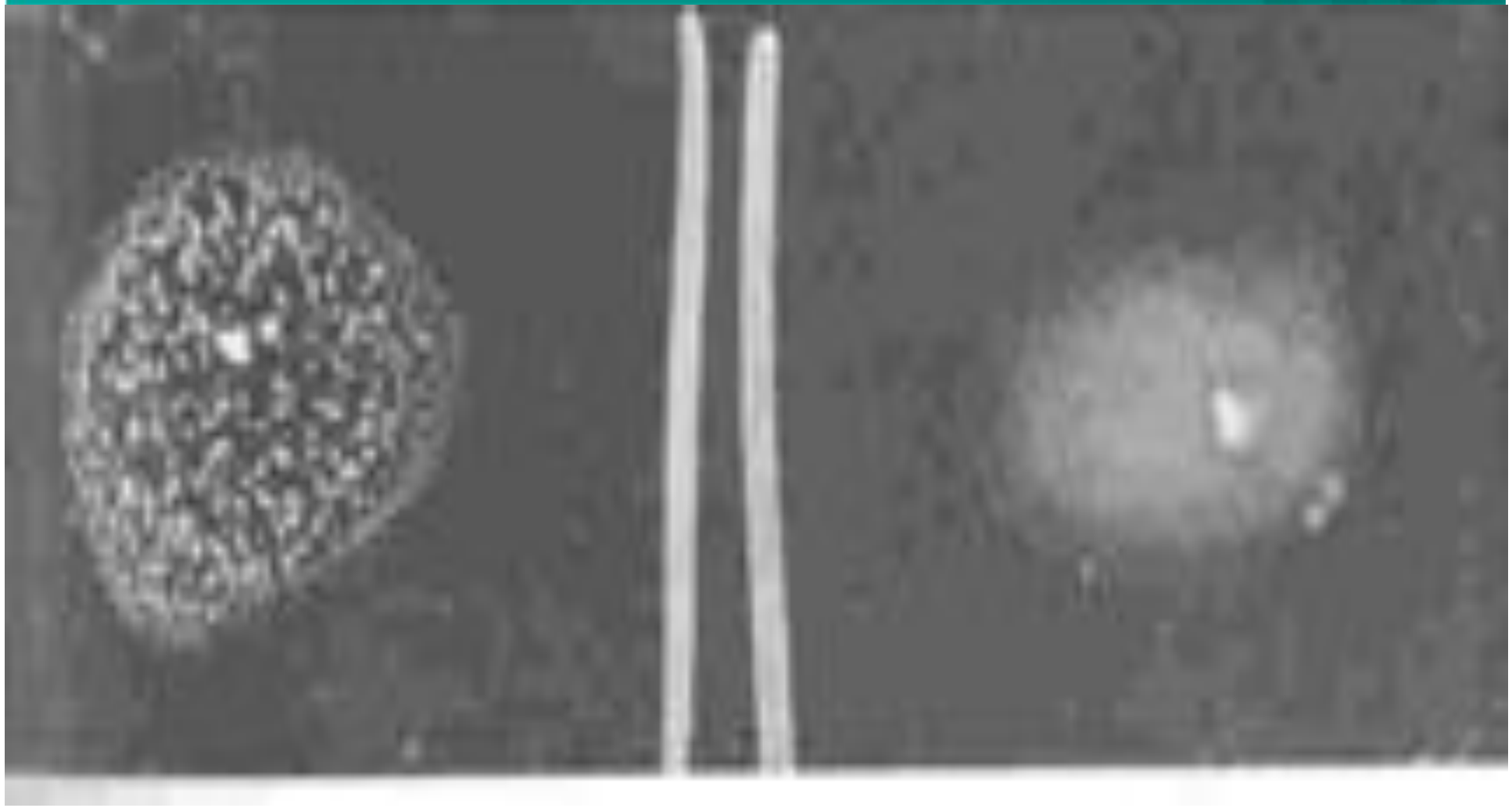


particulate Ag

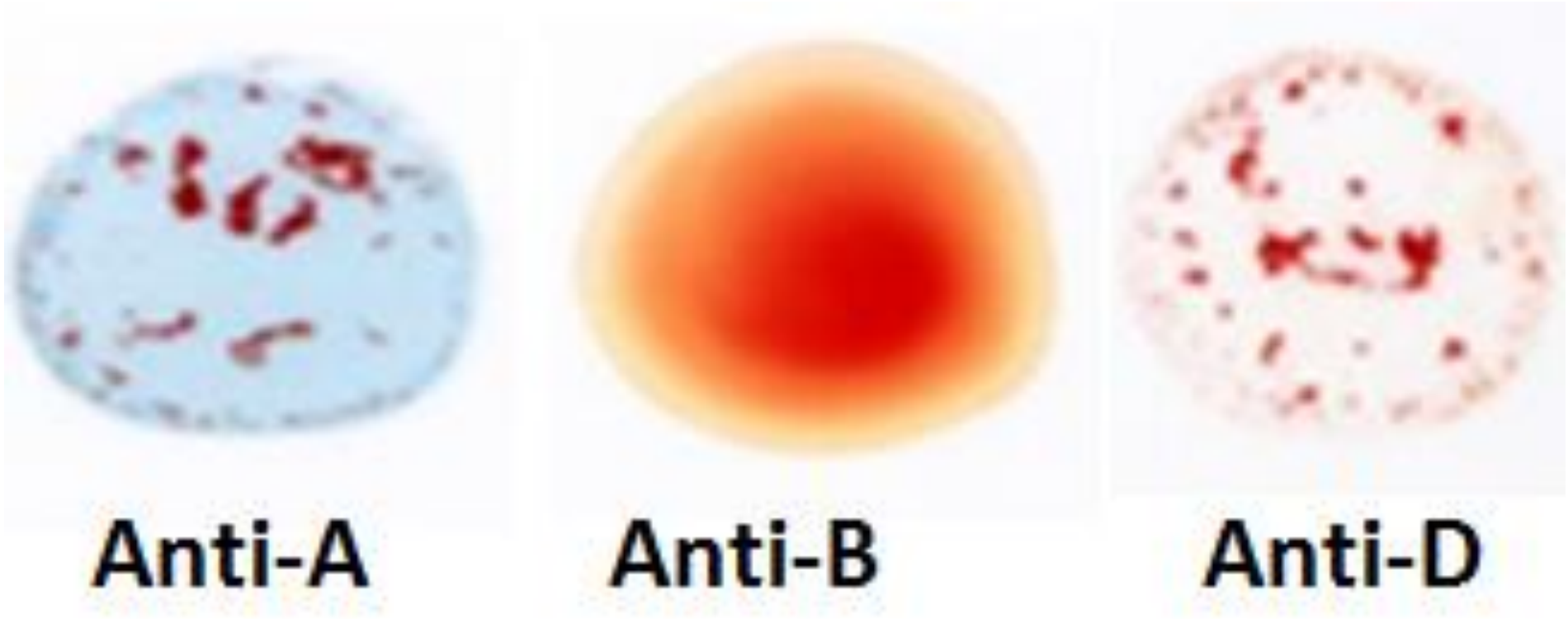
Soluble Aby

Agglutination

Principle of Direct agglutination



1) Applications of Direct agglutination



1) Blood grouping

A +

1) Applications of Direct agglutination

Anti-A

Anti-B

Anti-D

1) Blood grouping

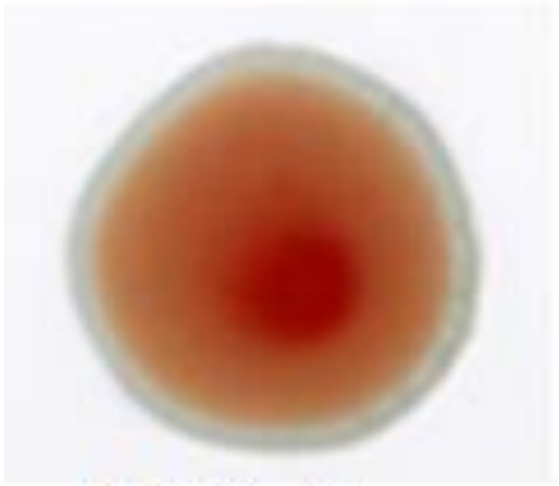
B +

1) Applications of Direct agglutination

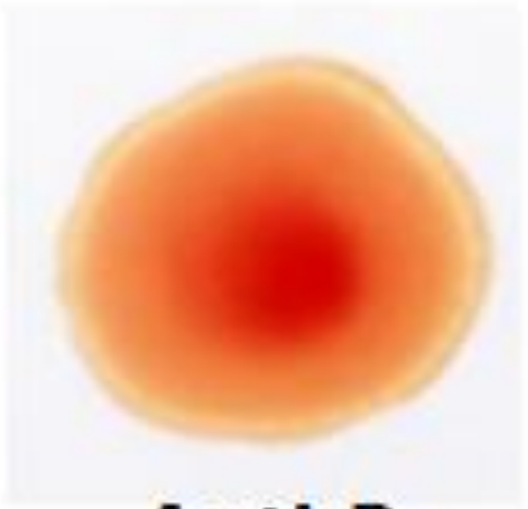
1) Blood grouping

AB +

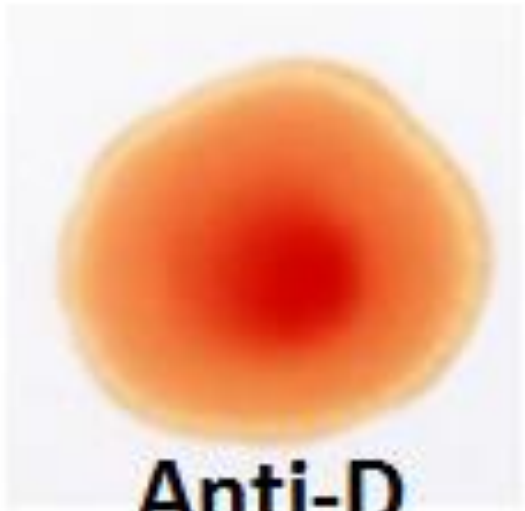
1) Applications of Direct agglutination



Anti-A



Anti-B



Anti-D

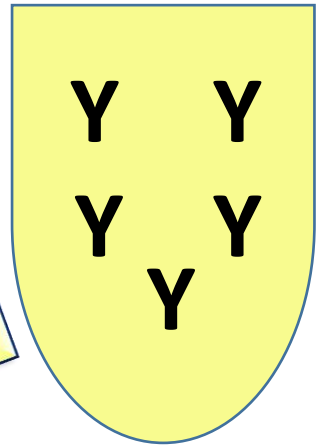
1) Blood grouping

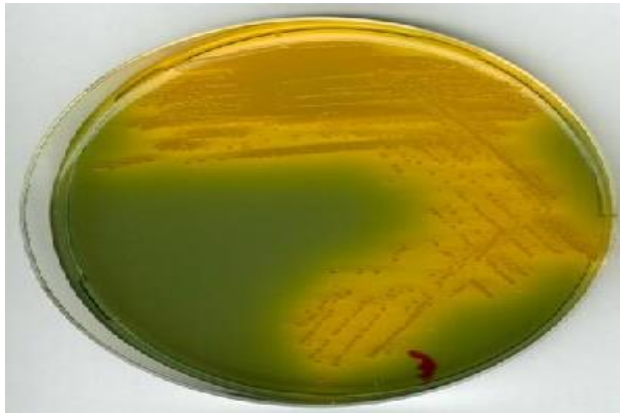
O –

1) Applications of Direct agglutination

2)Identification of an unknown organism.

Known antibodies

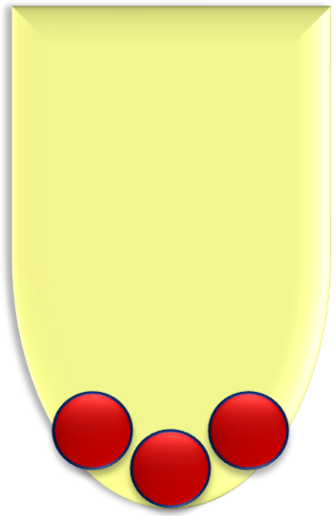




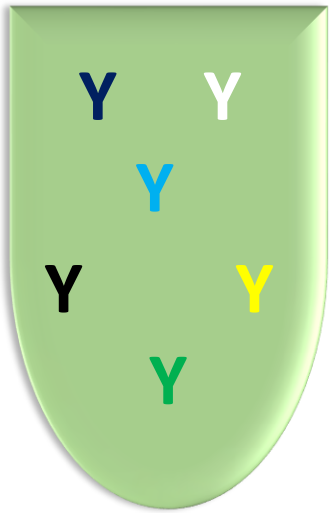
Unknown organism

1) Applications of Direct agglutination

3)Identification of an unknown antibody.



Known organism Salmonella



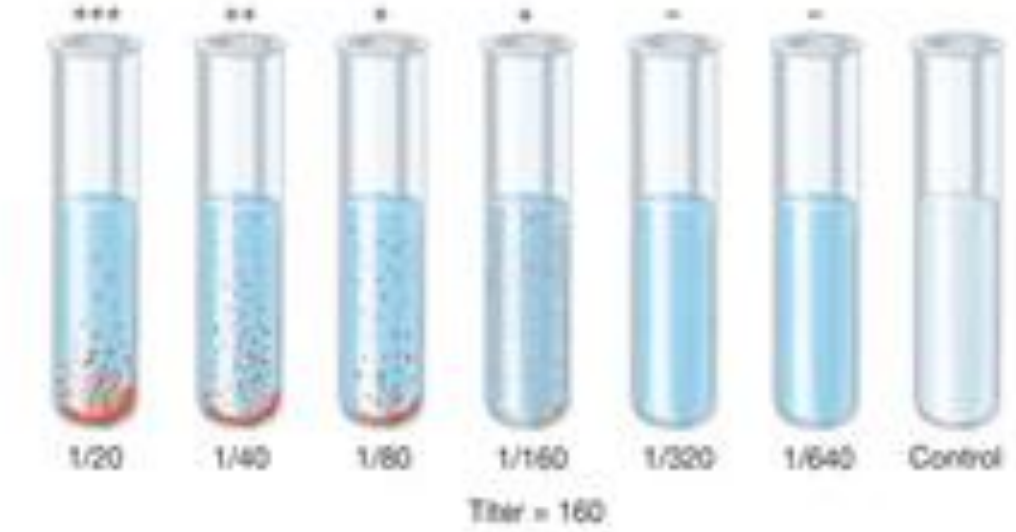
Unknown Antibody

1) Applications of Direct agglutination

3)Identification of an unknown antibody.

Tube agglutination (Widal test)

Serum dilution

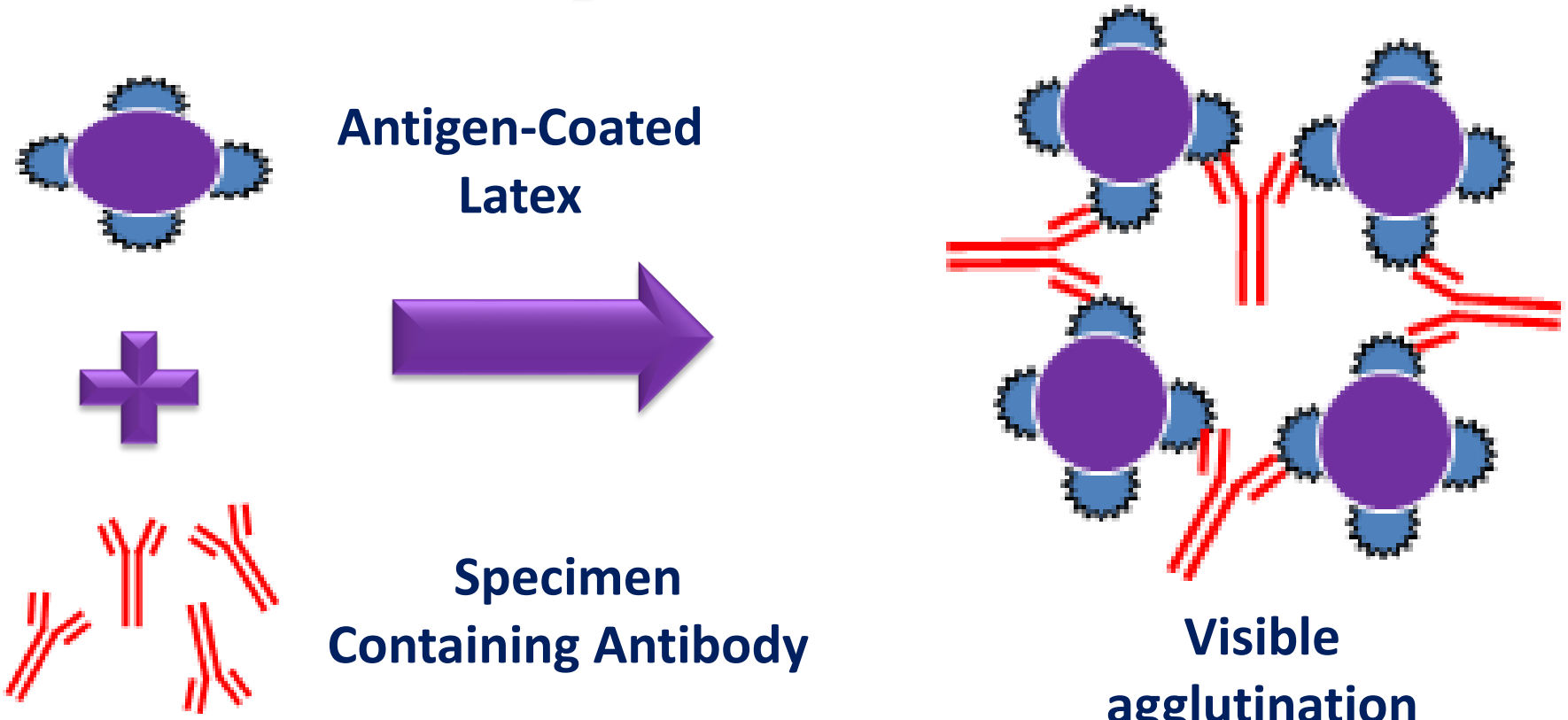


2) Indirect agglutination

So Ag or Ab must be converted into a particle to get visible agglutination

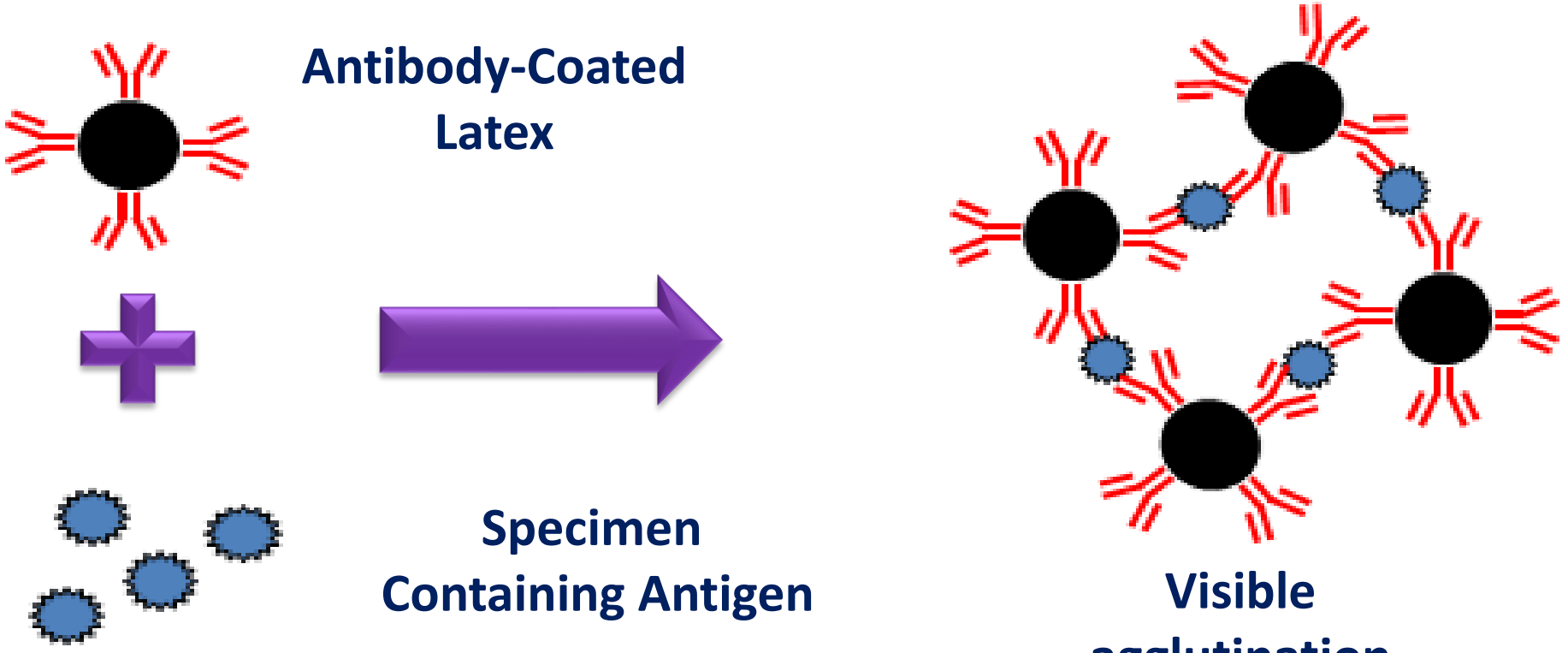
2) Indirect agglutination

Antibody test



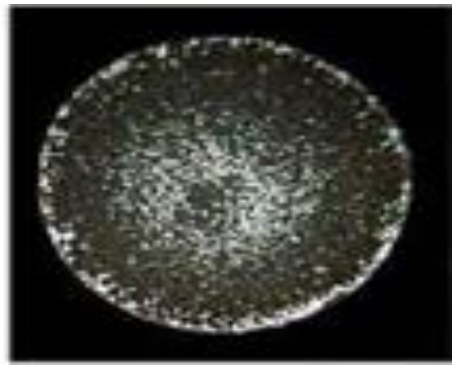
2) Indirect agglutination

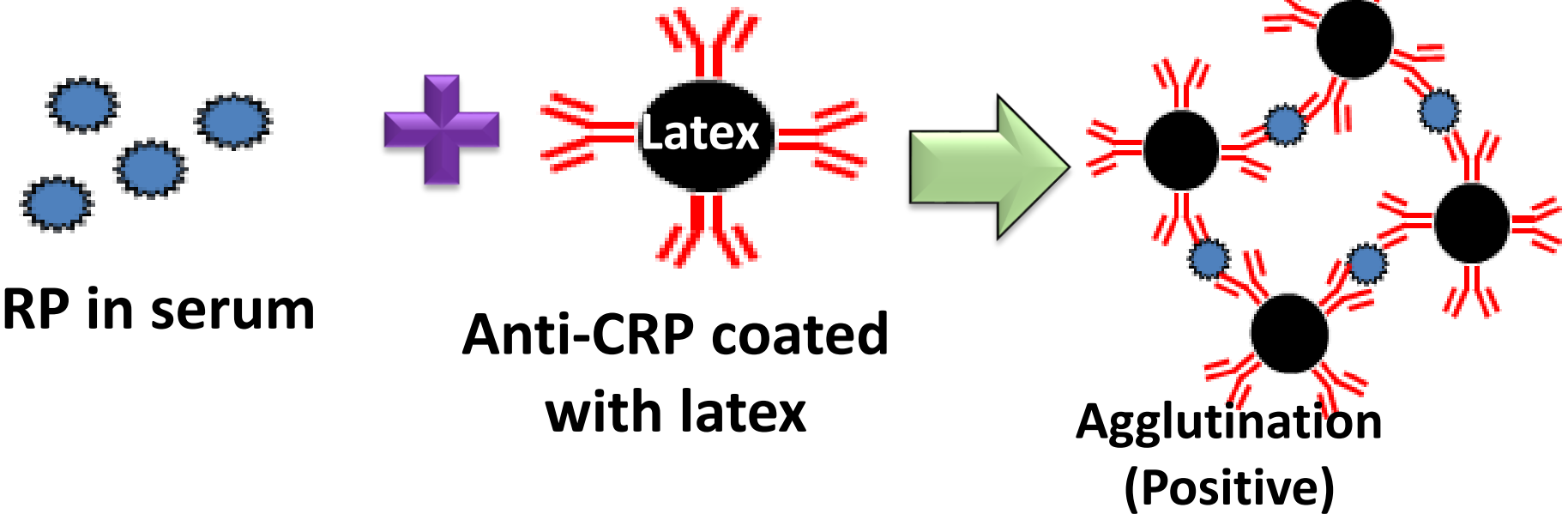
Antigen test



2) Indirect agglutination

1) Detection of CRP





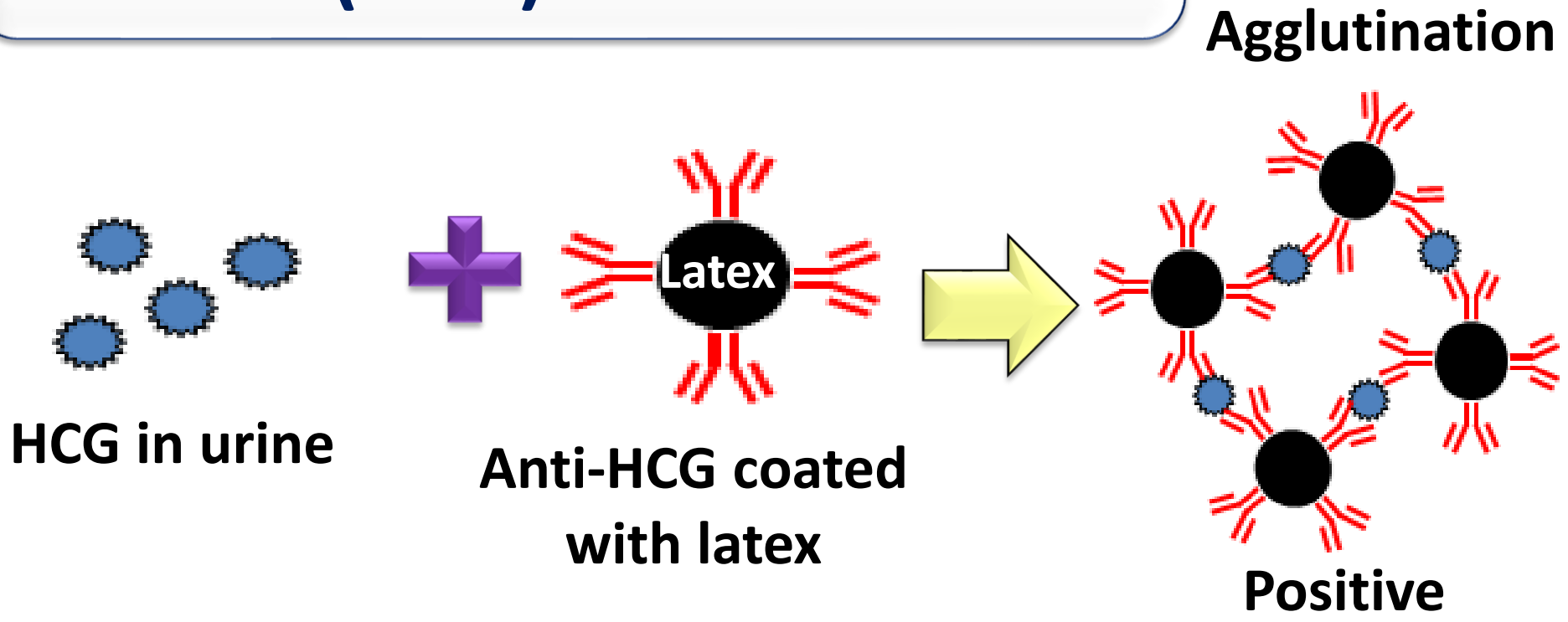
CRP in serum

Anti-CRP coated with latex

Agglutination (Positive)

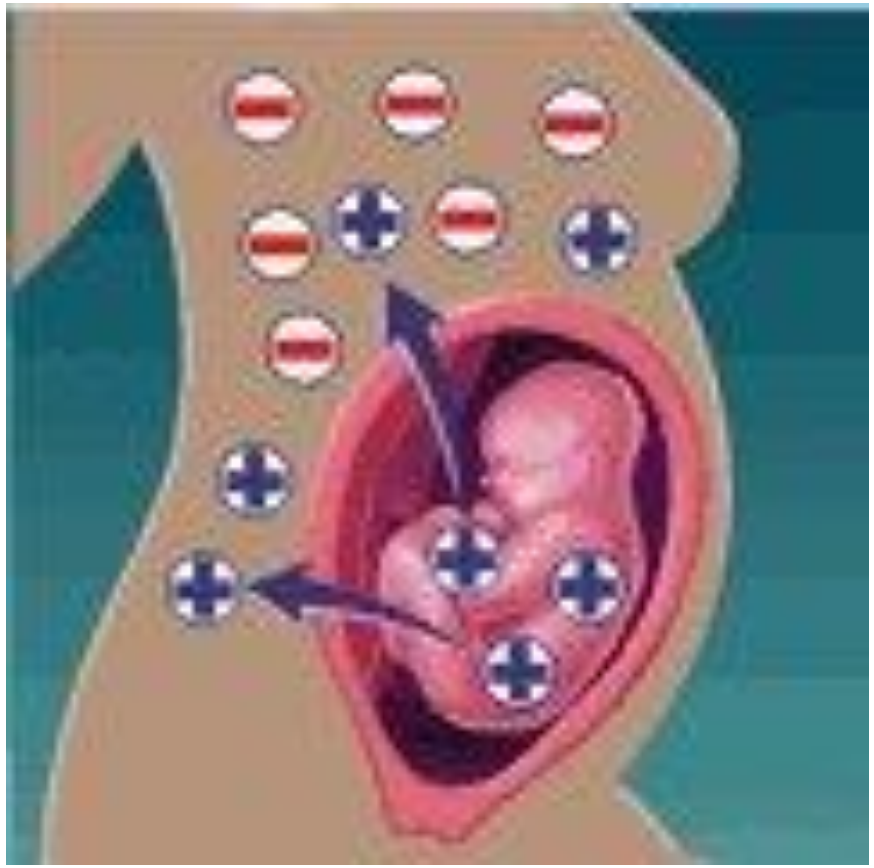
2) Indirect agglutination

2) Detection of Pregnancy test (HCG)in urine

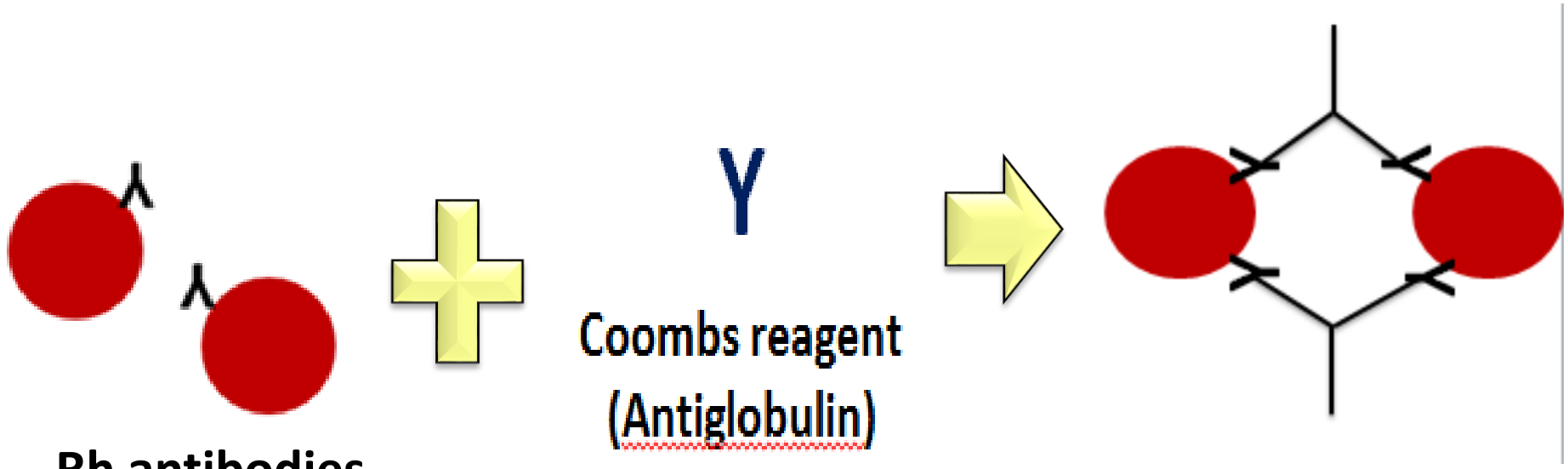


3) Direct Coomb’s test

This test to detect the presence of Rh incompatibility.

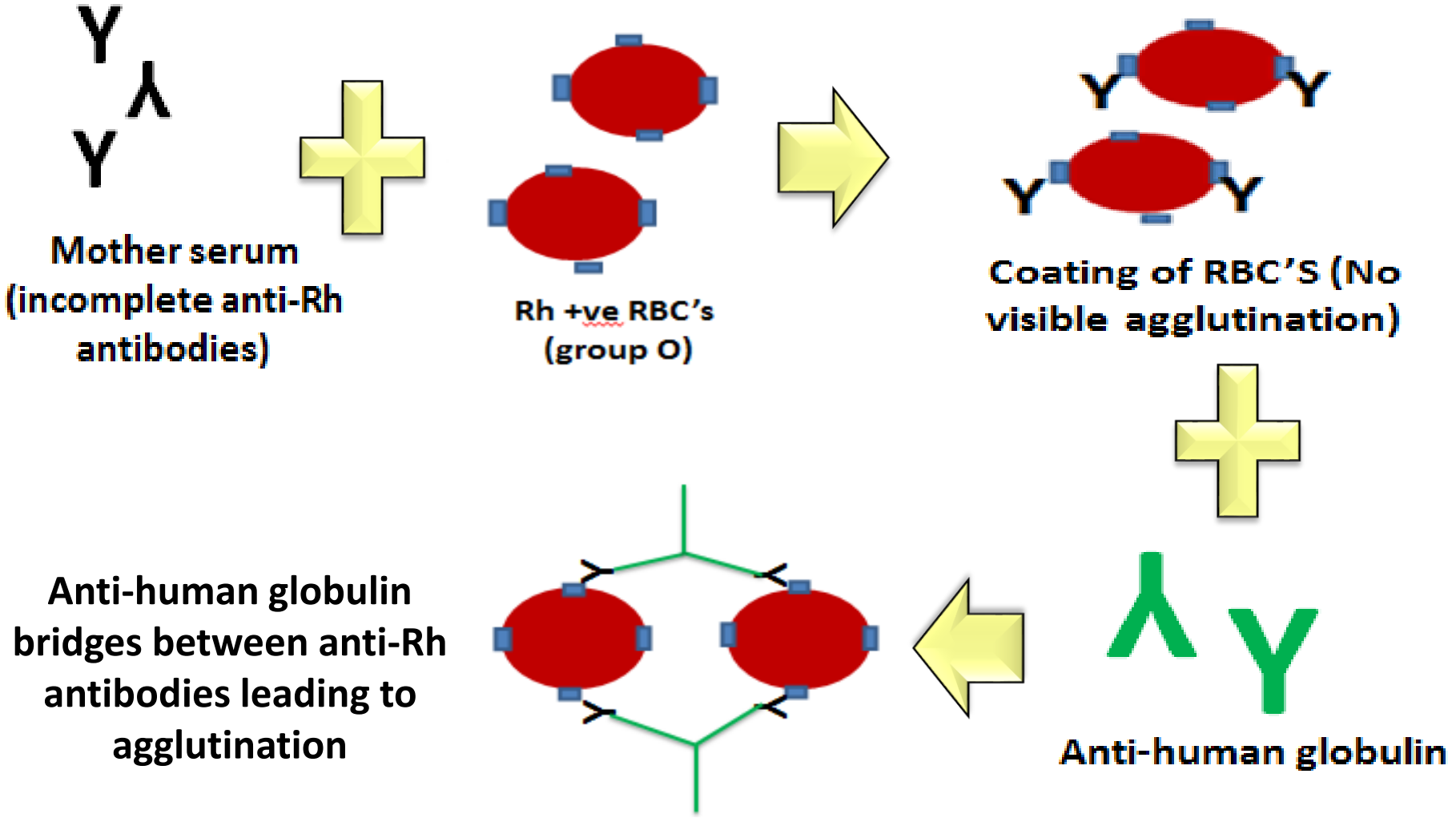


3) Direct Coomb's test



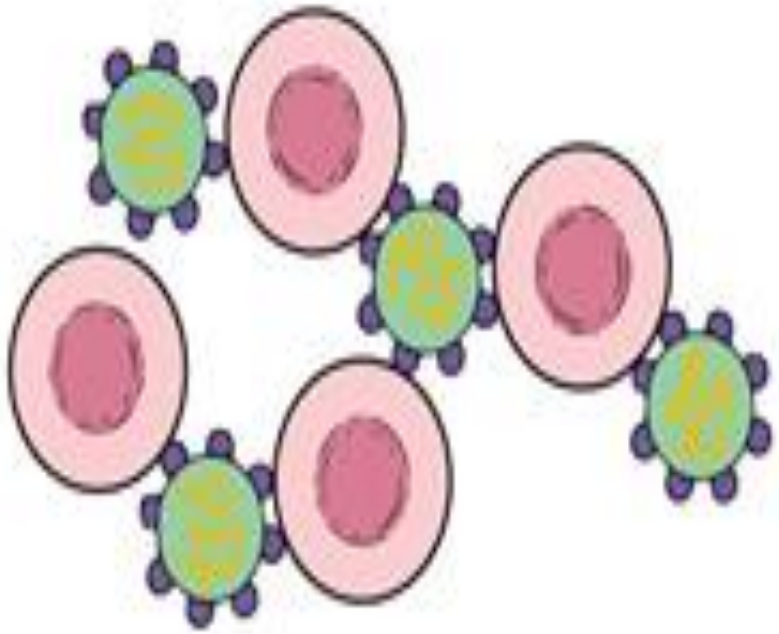
Rh antibodies coating the RBC'S of new born

3) Indirect Coomb's test

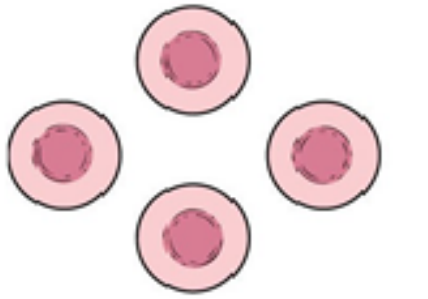


4) Hemagglutination test

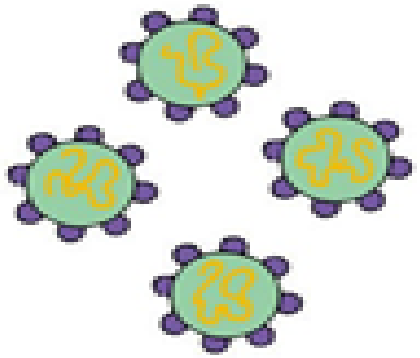
Detection the ability of some viruses to hemagglutinte RBCs.



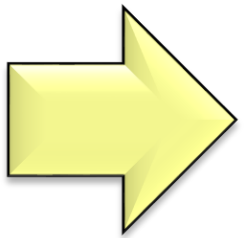
4) Hemagglutination test

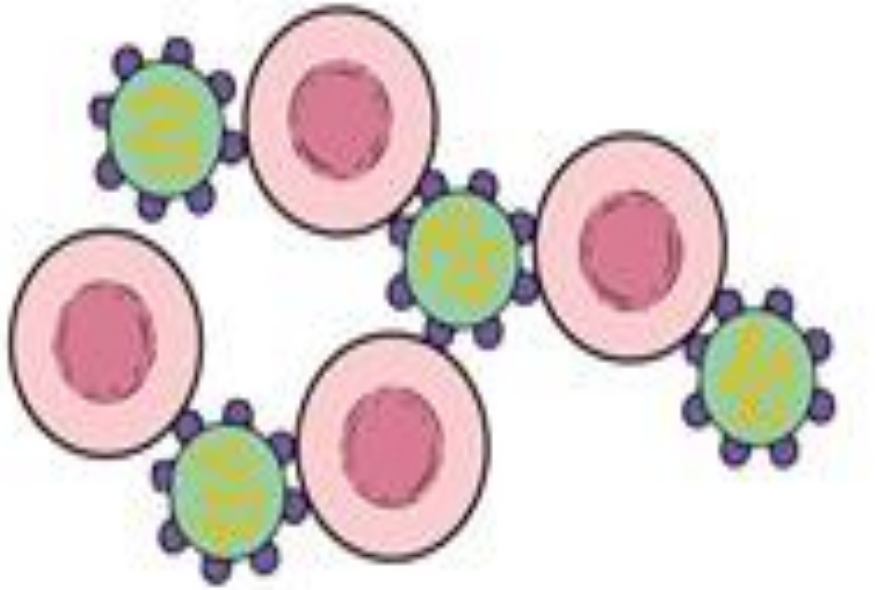


RBCs



Virus

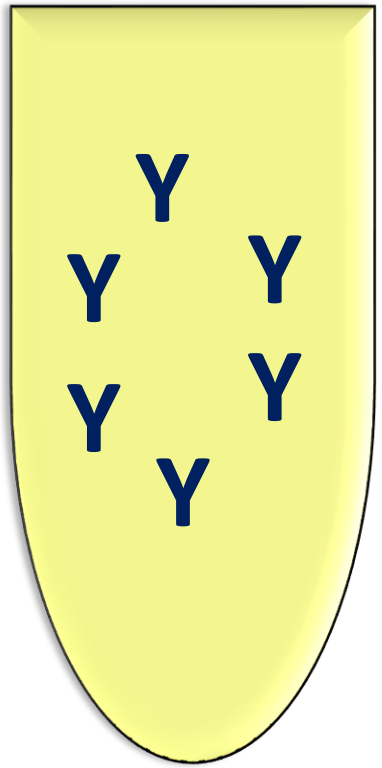




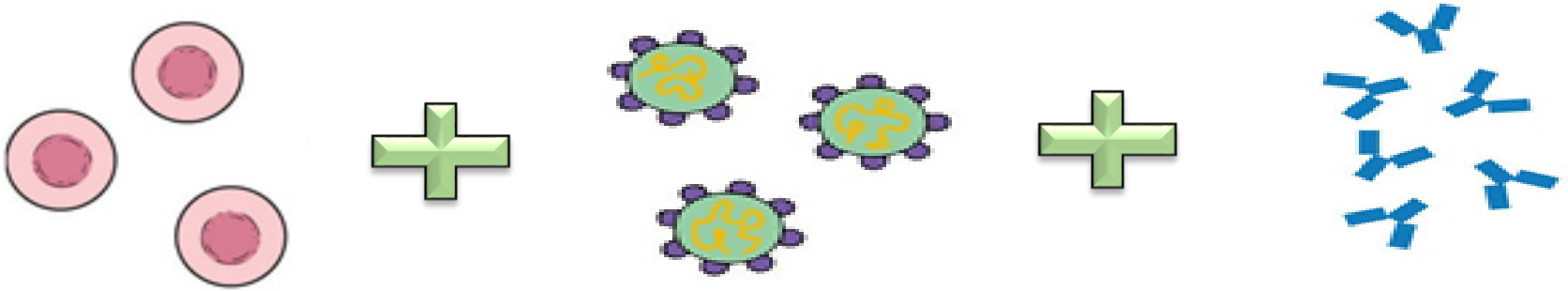
Haemagglutination

4) Hemagglutination Inhibition test

Detection of the presence of anti-viral antibody in the serum

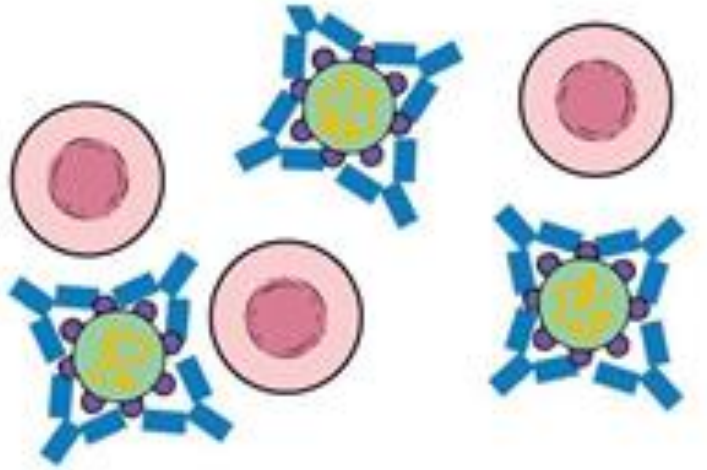


4) Hemagglutination Inhibition test



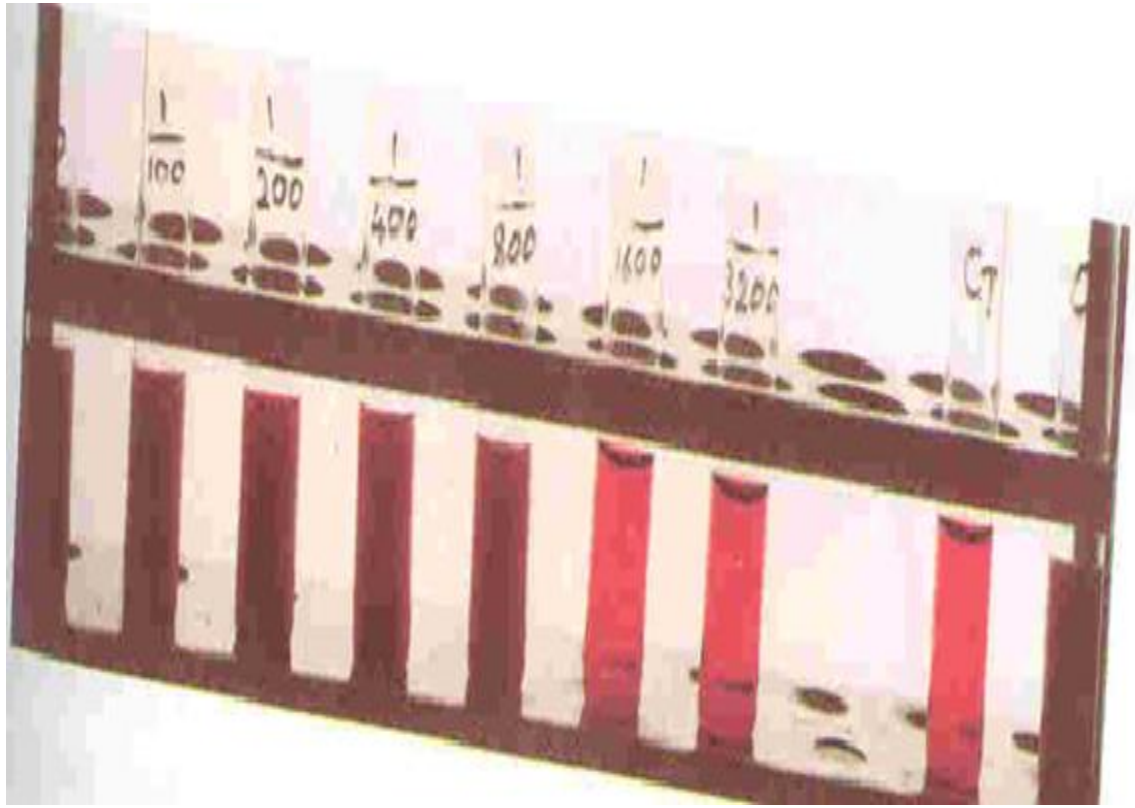
RBCs Virus Aby against virus

Haemagglutination Inhibition
(Ab neutralize the virus)



II) Toxin-antitoxin neutralization

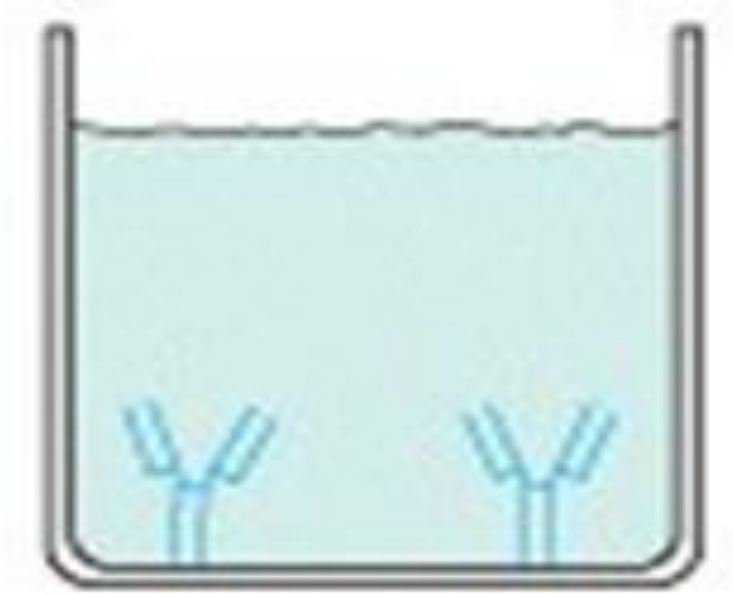
1- ASO (more than 200 units)



III) ELISA

Step 1

Antibodies for specific Ag are fixed in the well

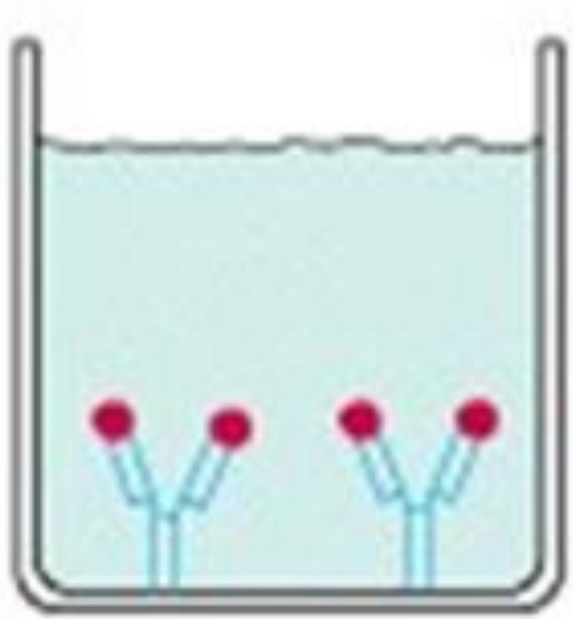


Antibody-coated well

III) ELISA

Step 2

Add specimen



Add specimen containing Ag

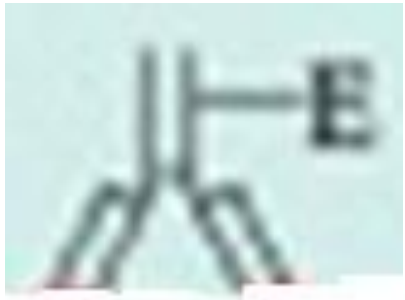
III) ELISA

Step 3

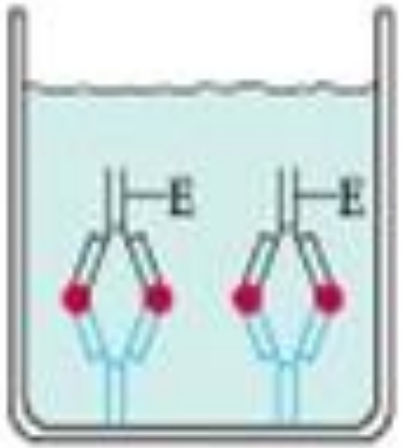
Wash

III) ELISA

Step 4



Add antibody linked with enzyme



Add enzyme-conjugated secondary antibody

III) ELISA

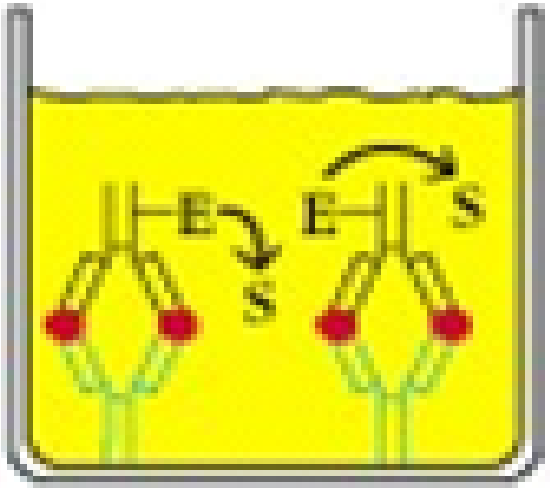
Step 5

Wash

III) ELISA

Step 6

Add substrate



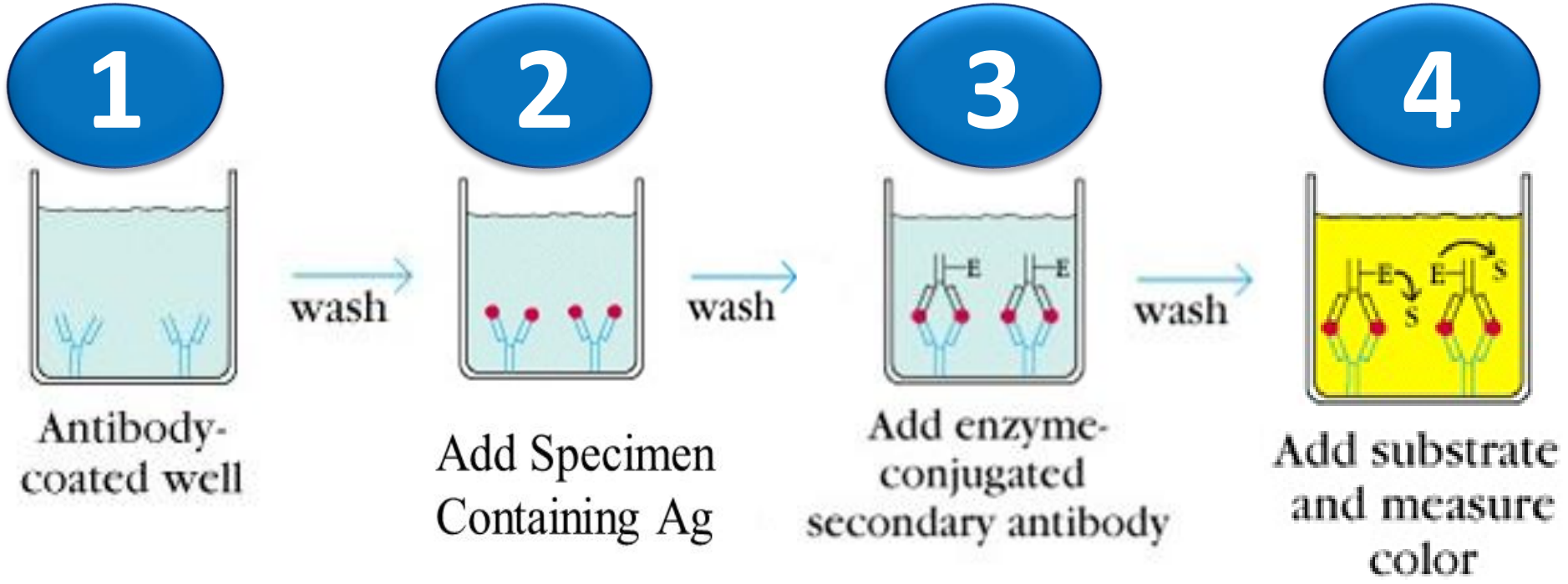
Add substrate and measure color

III) ELISA

Step 7

Read the change of color by spectrophotometer

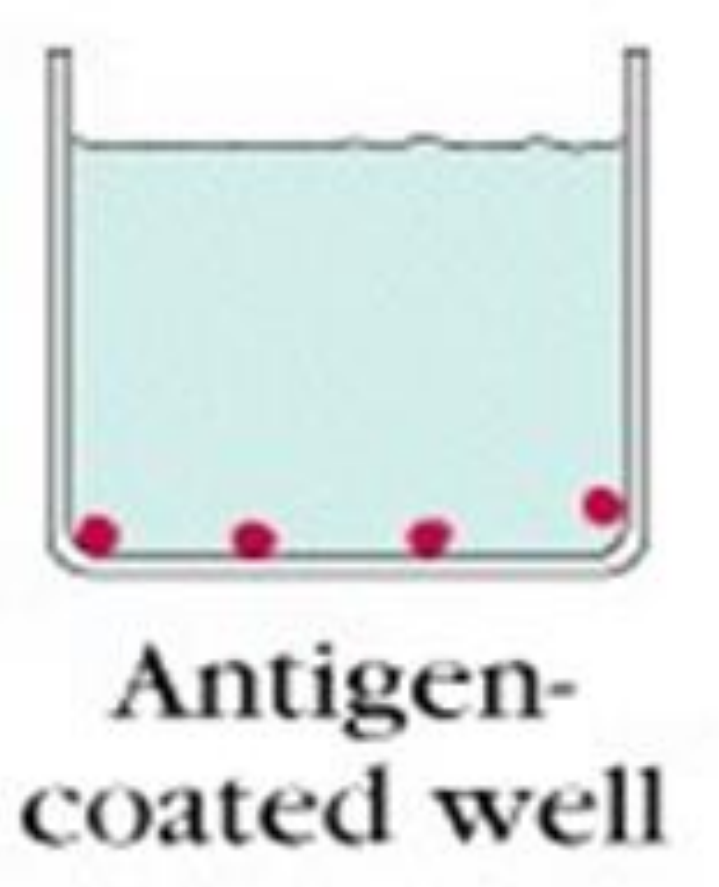
III) ELISA


1
2
3
4
wash
wash
wash
E
E
E
E
S
S
Antibody-coated well
Add Specimen Containing Ag
Add enzyme-conjugated secondary antibody
Add substrate and measure color

III) ELISA

Step 1

Known antigens are adsorbed to test well.

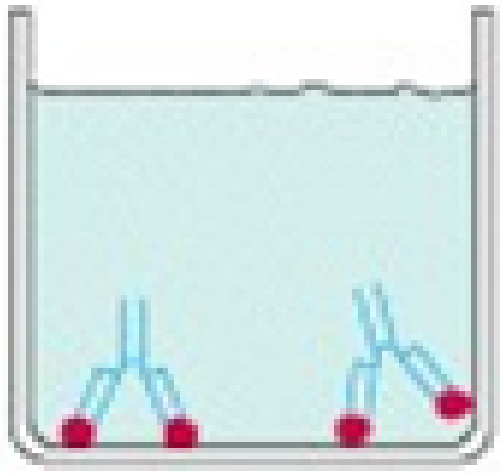


Antigen-coated well

III) ELISA

Step 2

The patient's serum is added (containing antibody)



Add specific antibody to be measured (Specimen)

III) ELISA

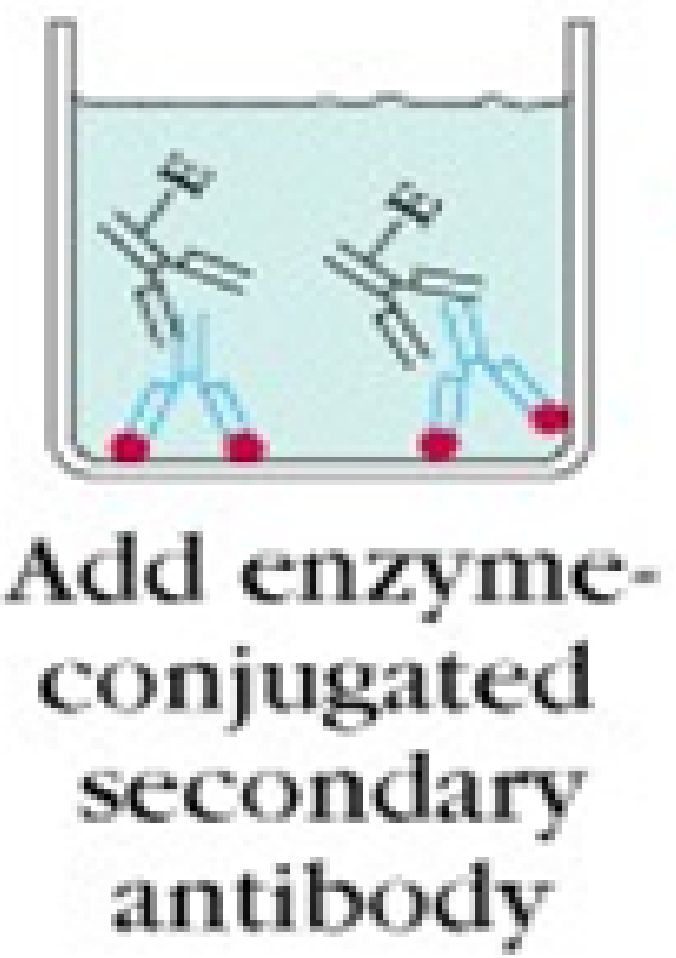
Step 3

Wash

III) ELISA

Step 4

Anti-HGG linked with enzyme is added to the well



III) ELISA

Step 5

Wash

III) ELISA

Step 6

Add substrate

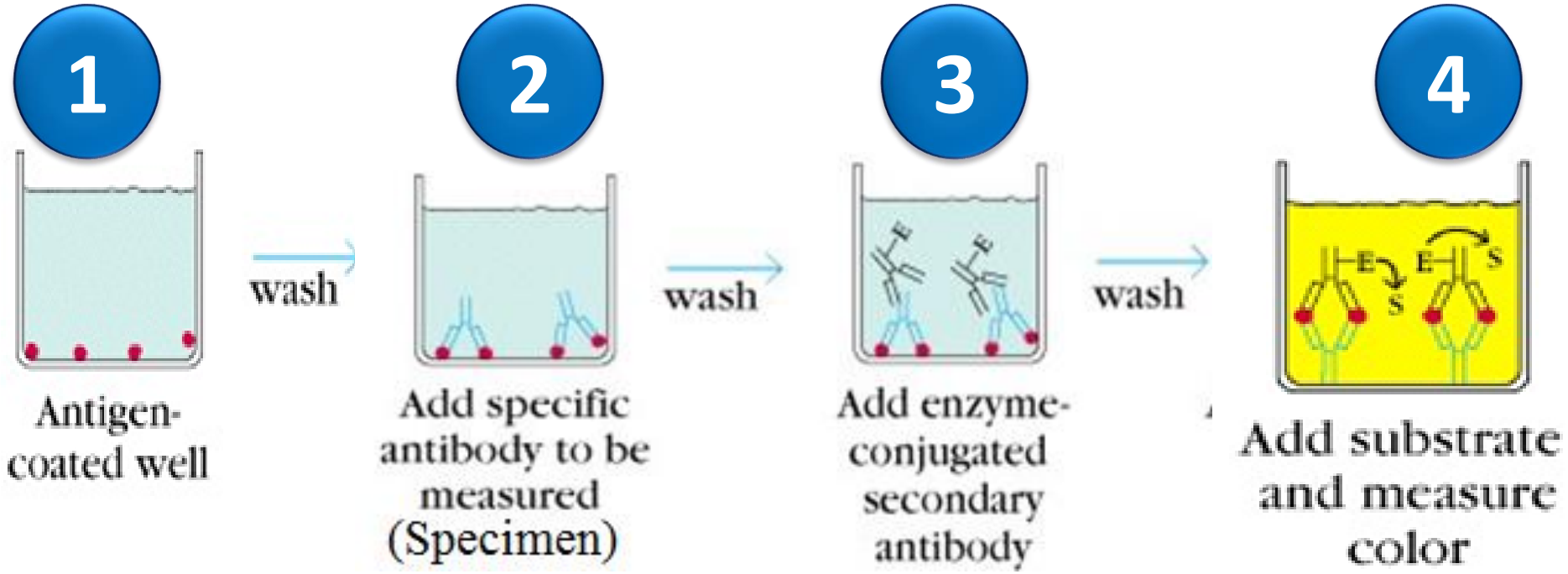
Add substrate (S) and measure color

III) ELISA

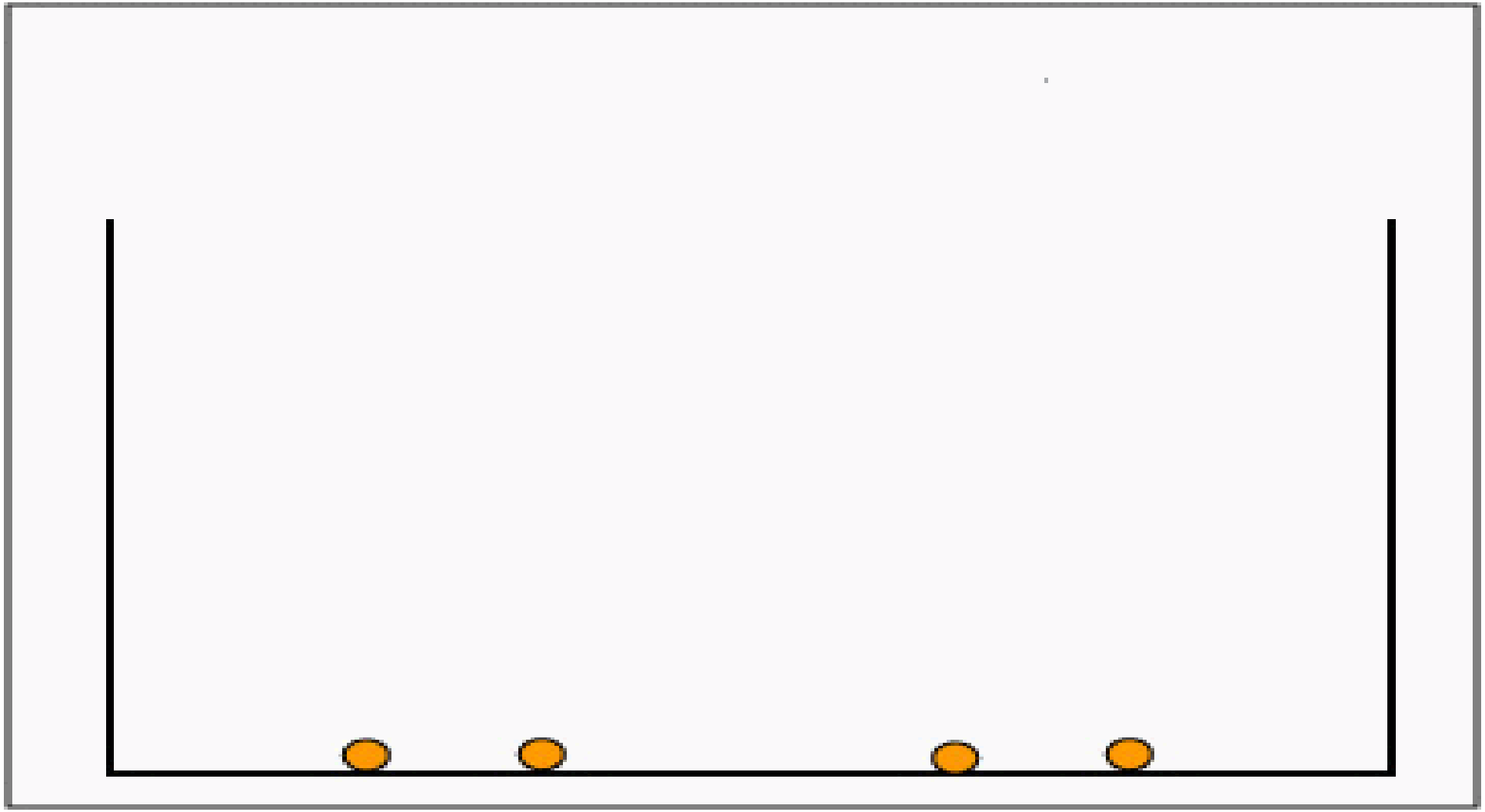
Step 7

Read the change of color by spectrophotometer

III) ELISA


1
2
3
4
wash
wash
wash
E
S
Antigen-coated well
Add specific antibody to be measured (Specimen)
Add enzyme-conjugated secondary antibody
Add substrate and measure color

III) ELISA



V) Direct Immunofluorescent

The test is used to detect Antigen (bacteria or Ag in tissues)

V) Direct Immunofluorescent

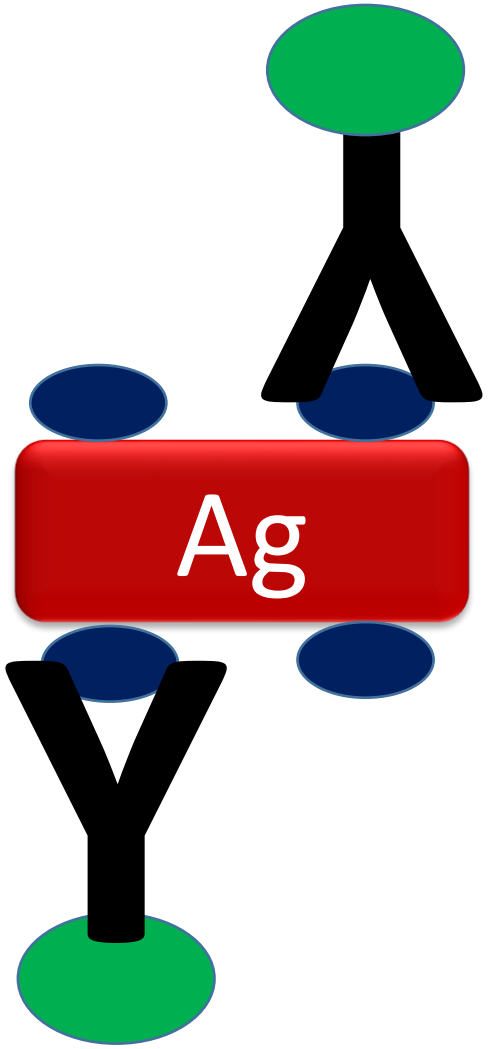
Ag fixed on a slid

+

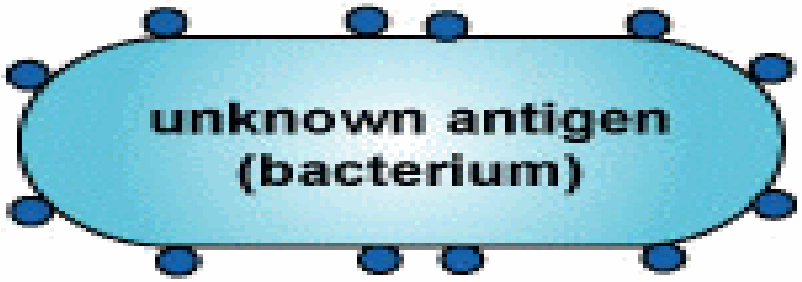
Fluorescin labelled antibodies are added

=

Apple green fluorescence

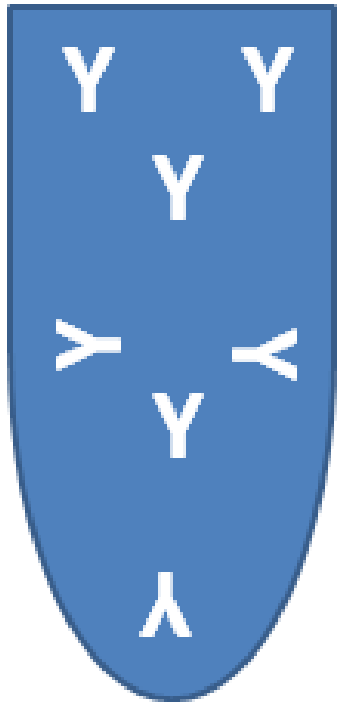


V) Direct Immunofluorescent



V) Indirect Immunofluorescent

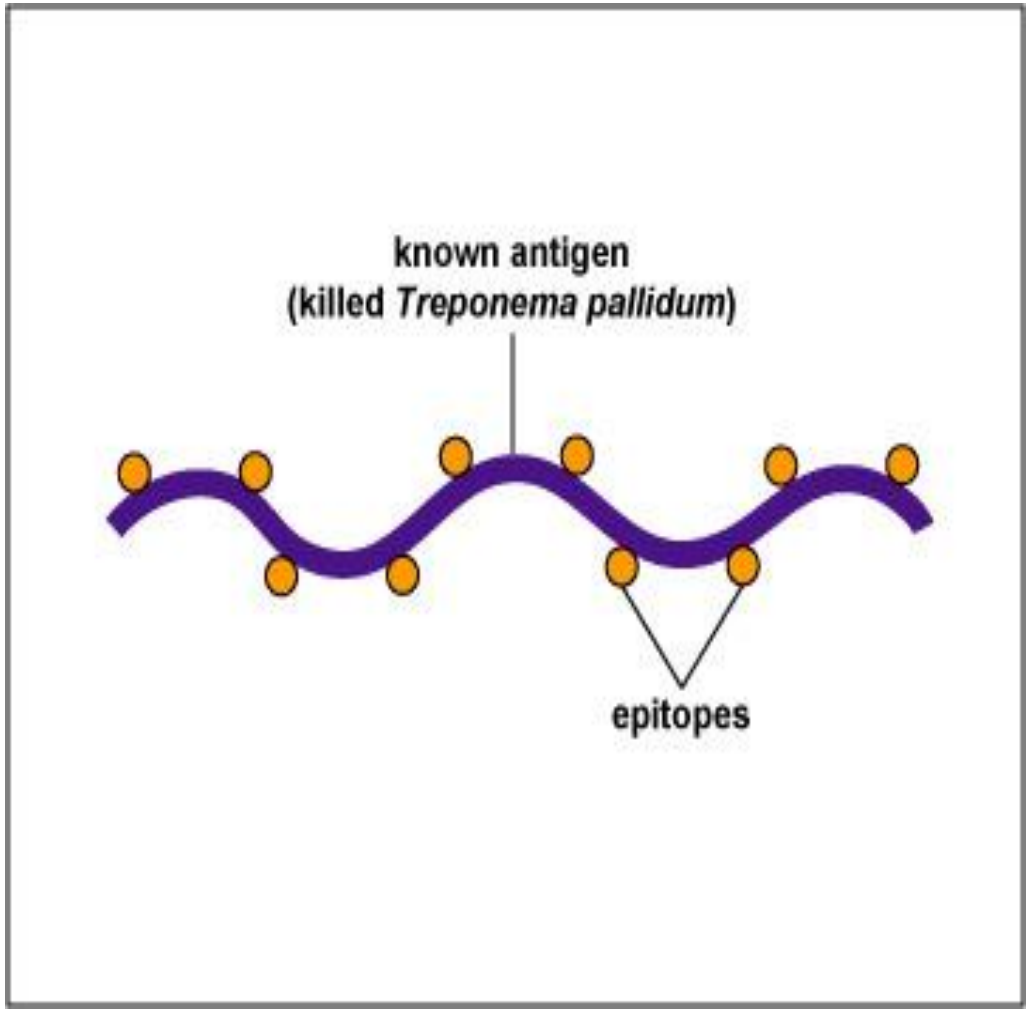
The test is used to detect antibodies in serum patients



V) Indirect Immunofluorescent

Step 1

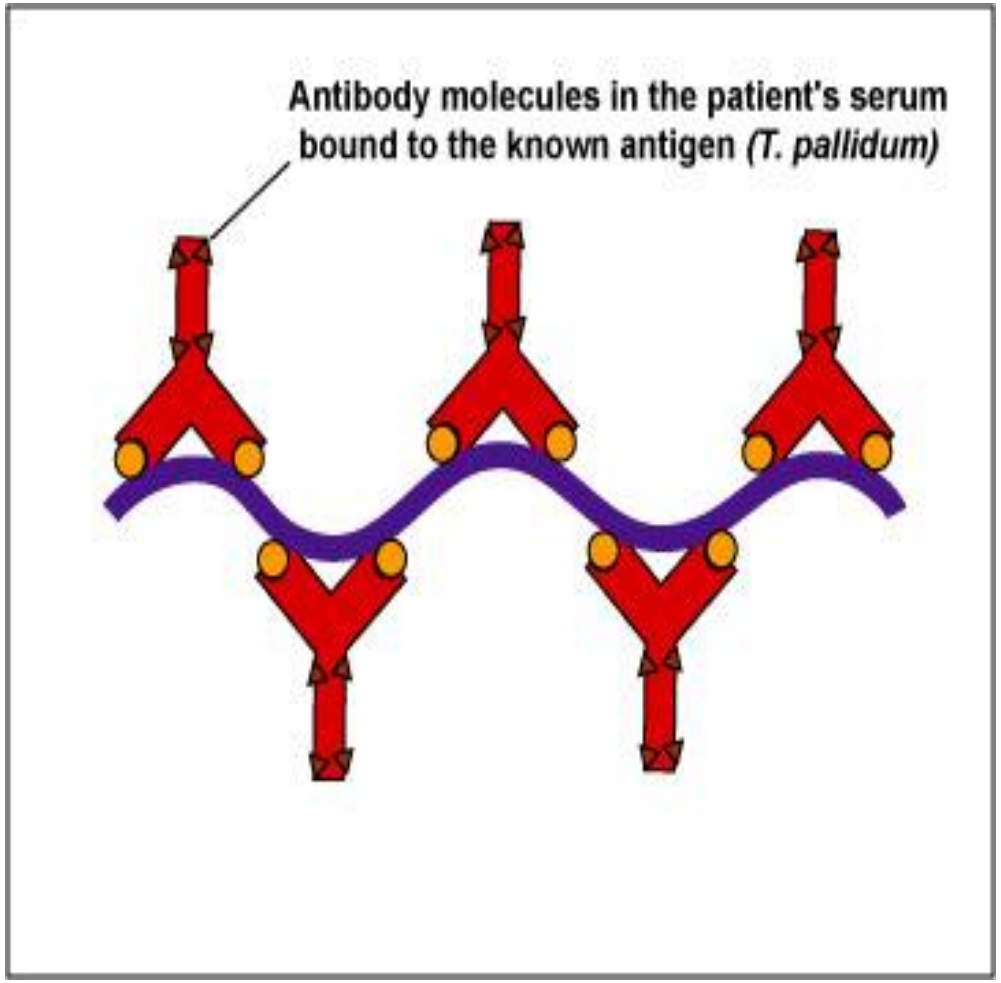
Ag is known



V) Indirect Immunofluorescent

Step 2

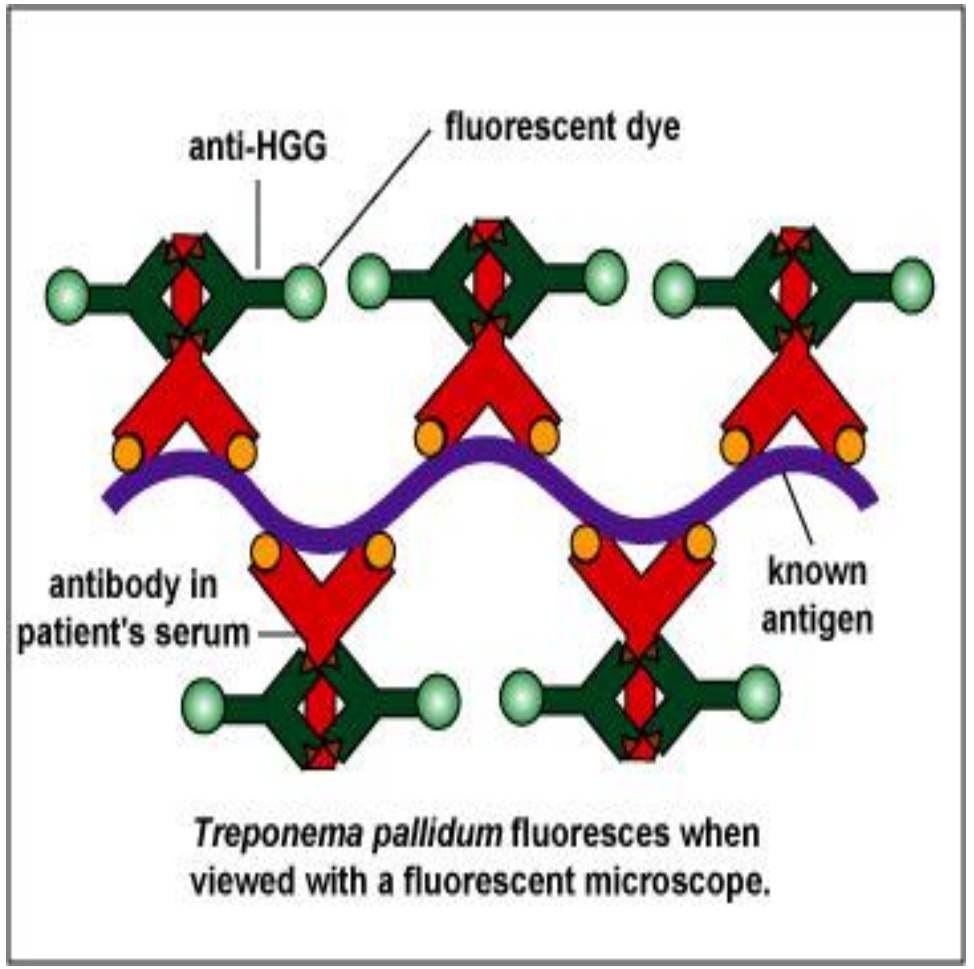
Patient Serum is added (containing Ab)



V) Indirect Immunofluorescent

Step 3

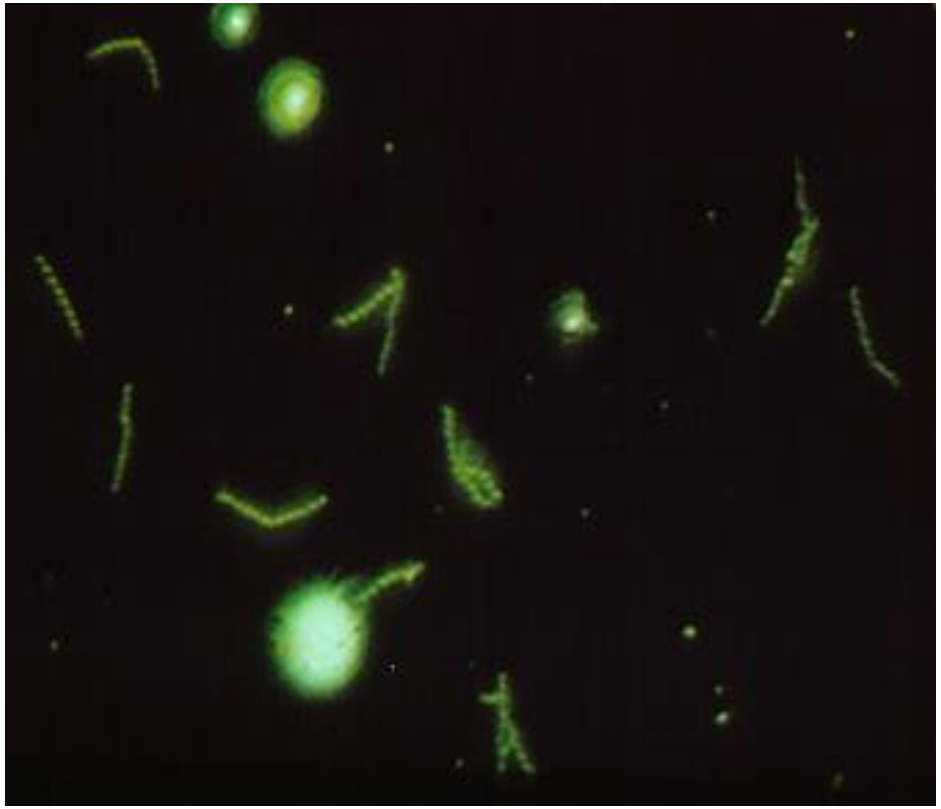
Anti-HGG linked with fluorescent is added to the well



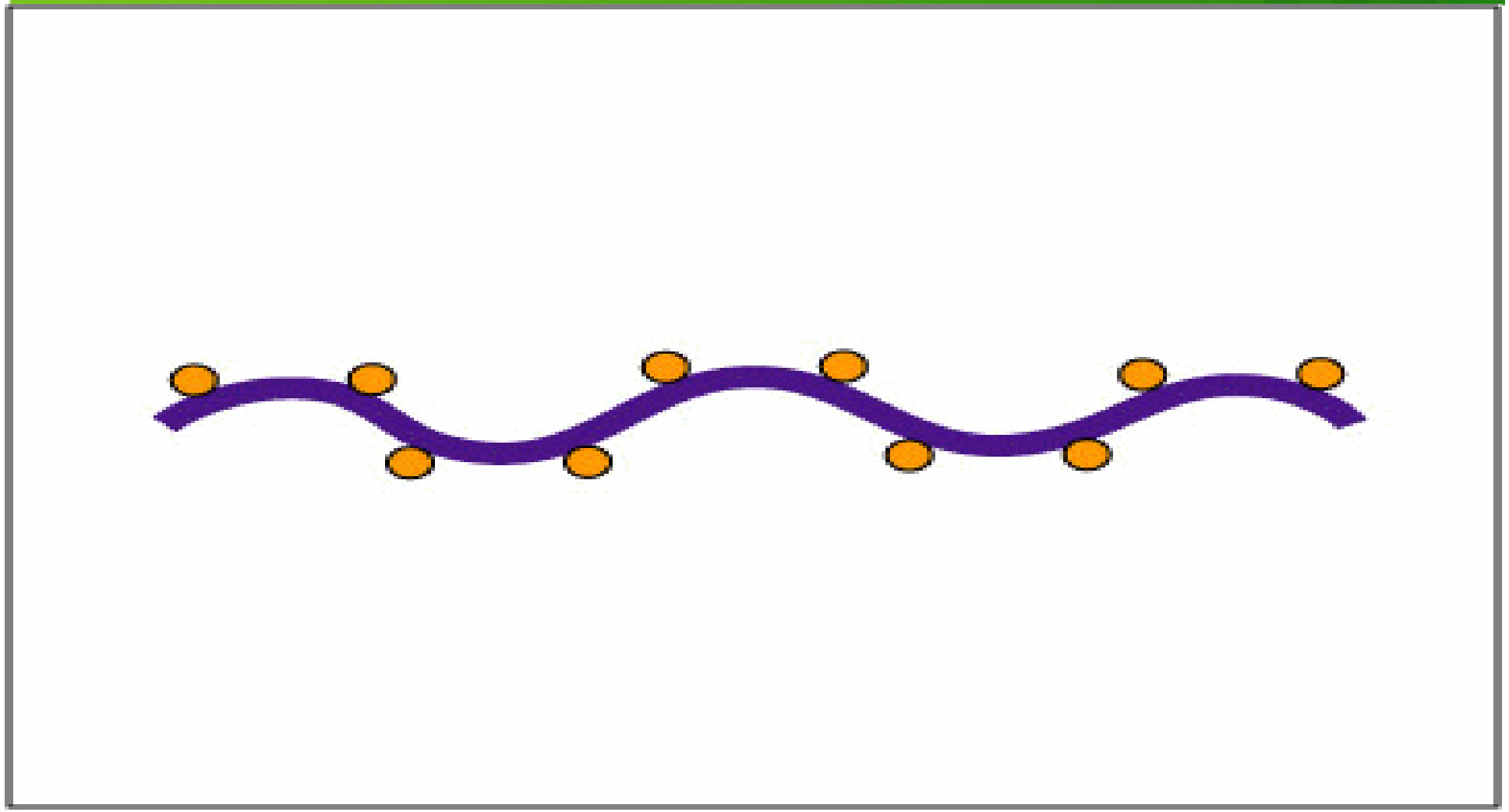
V) Indirect Immunofluorescent

Step 4

After incubation
See the fluorescent under fluorescent microscopy (green apple)



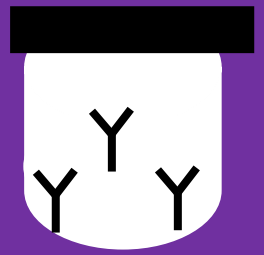
V) Indirect Immunofluorescent



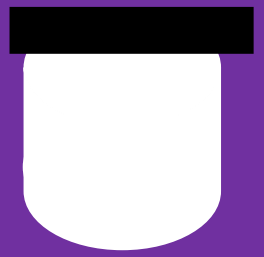
VI) Complement fixation test

It used for detection of antibody

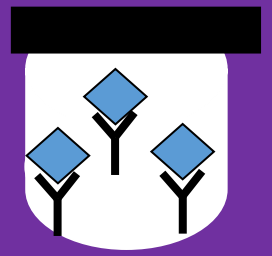
VI) Complement fixation test



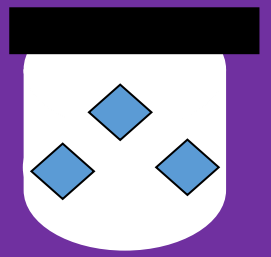
Serum with antibodies



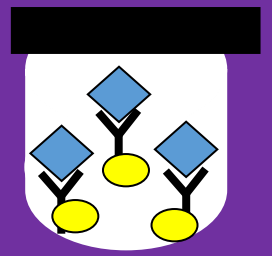
Serum without antibodies



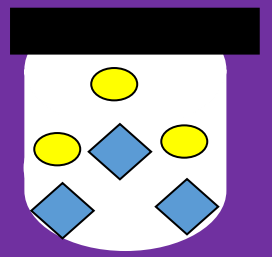
Antigen binds to antibodies



Unbound antigen

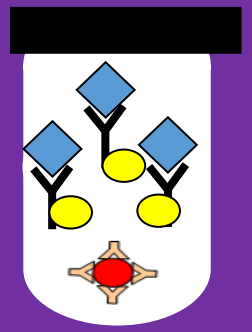


Complement binds to Ag/Ab complex

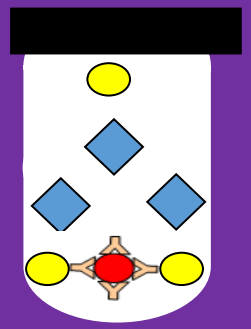


Unbound complement

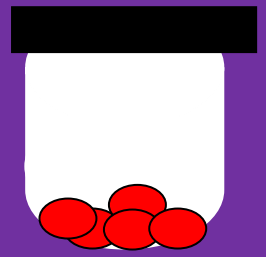
VI) Complement fixation test



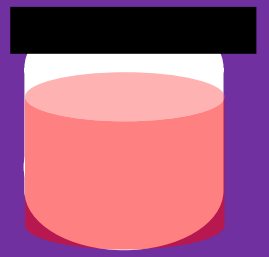
RBCs coated with Aby serve as an indicator is added



RBCs coated with Aby serve as an indicator is added



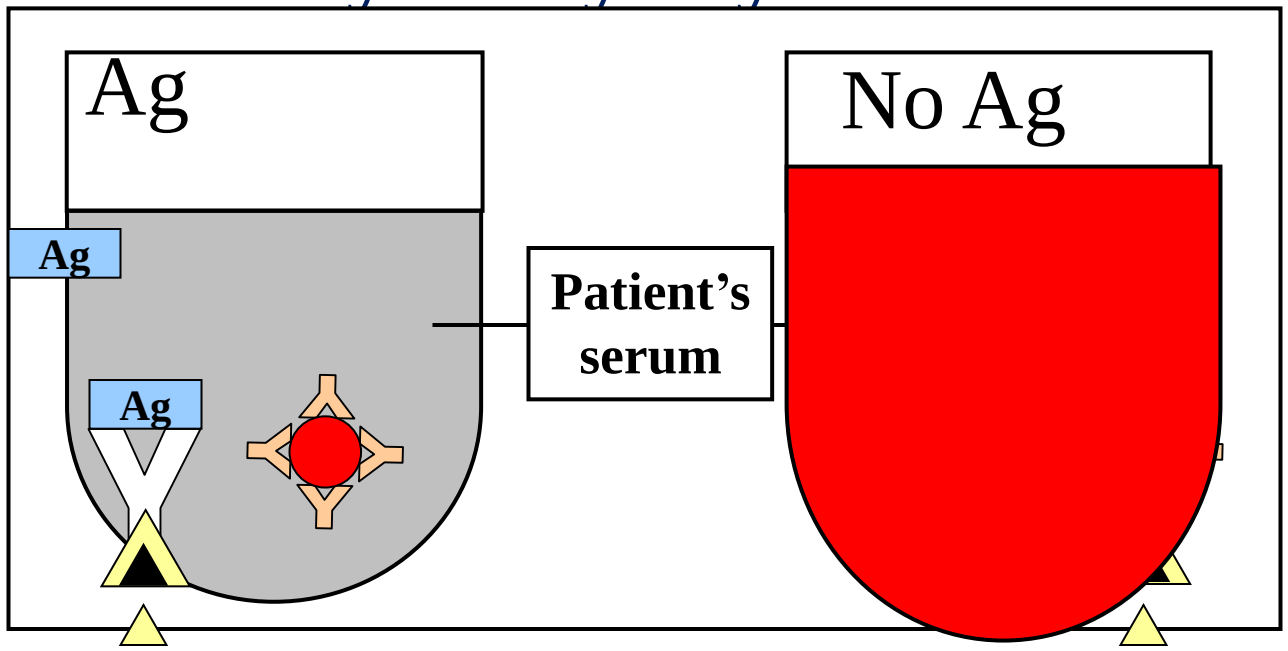
No lysis
Positive



Lysis
Negative

VI) Complement fixation test

- Ag mixed with test serum to be assayed for Ab
- Standard amount of complement is added
- Erythrocytes coated with Abs is added
- Amount of erythrocyte lysis is determined



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