Practical immunology Lab-1-

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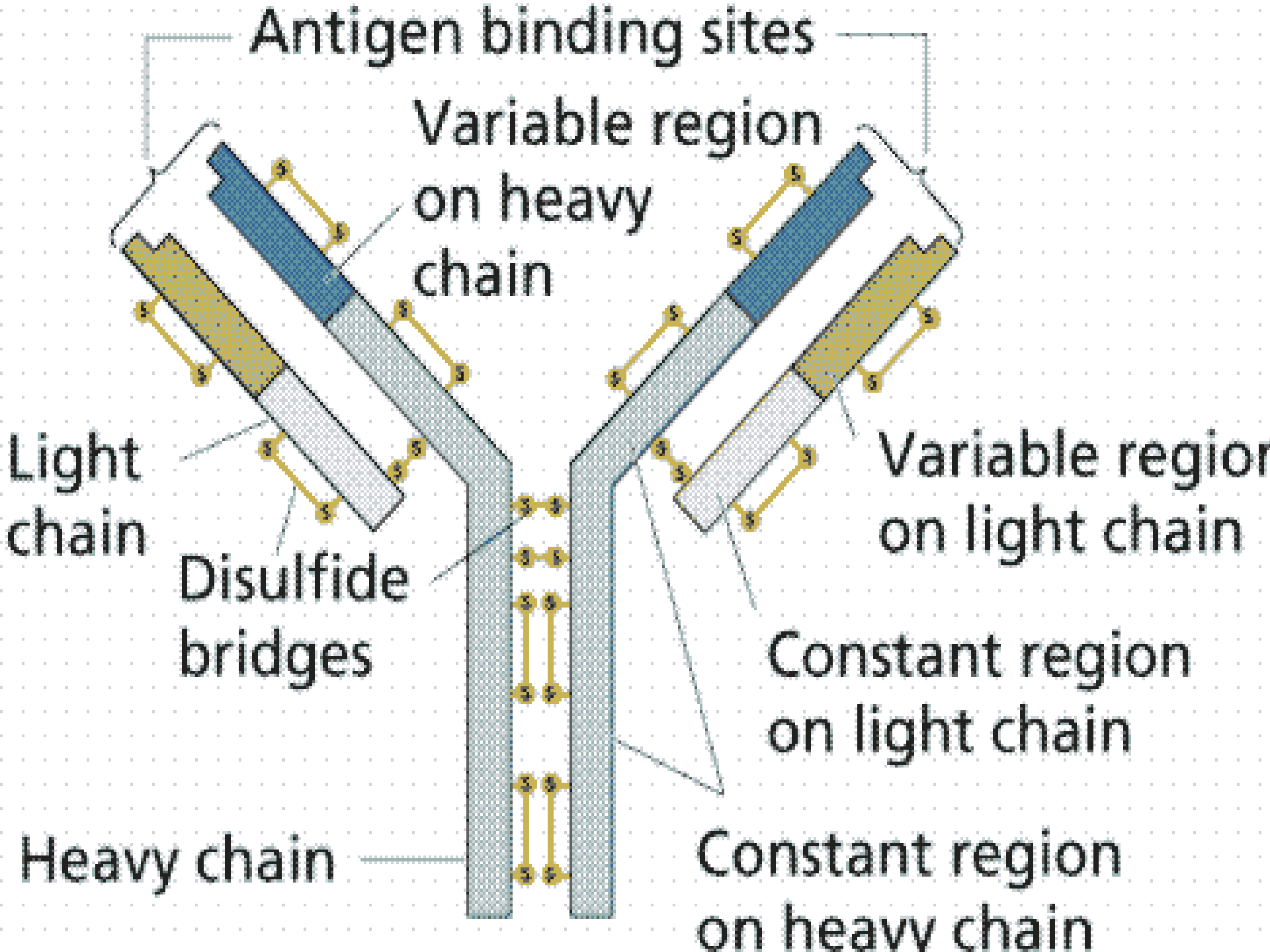
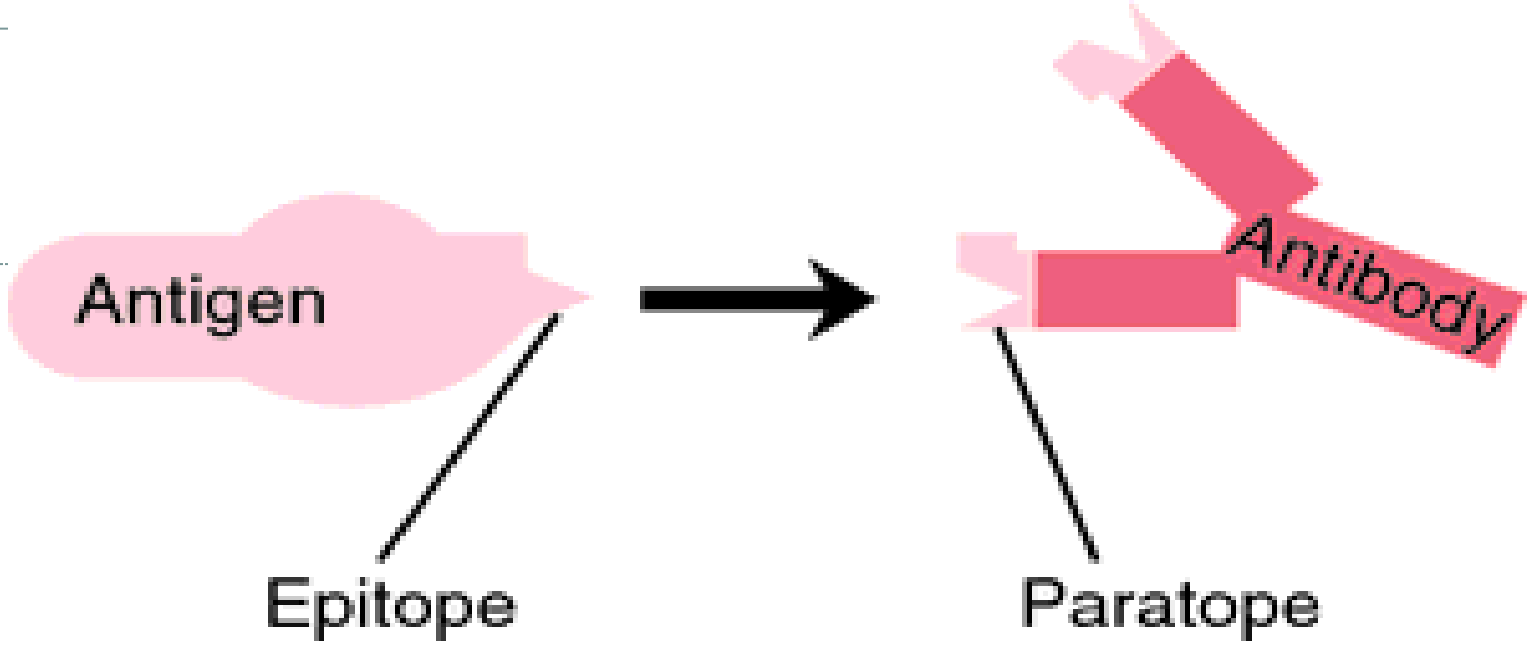
Lab(1)

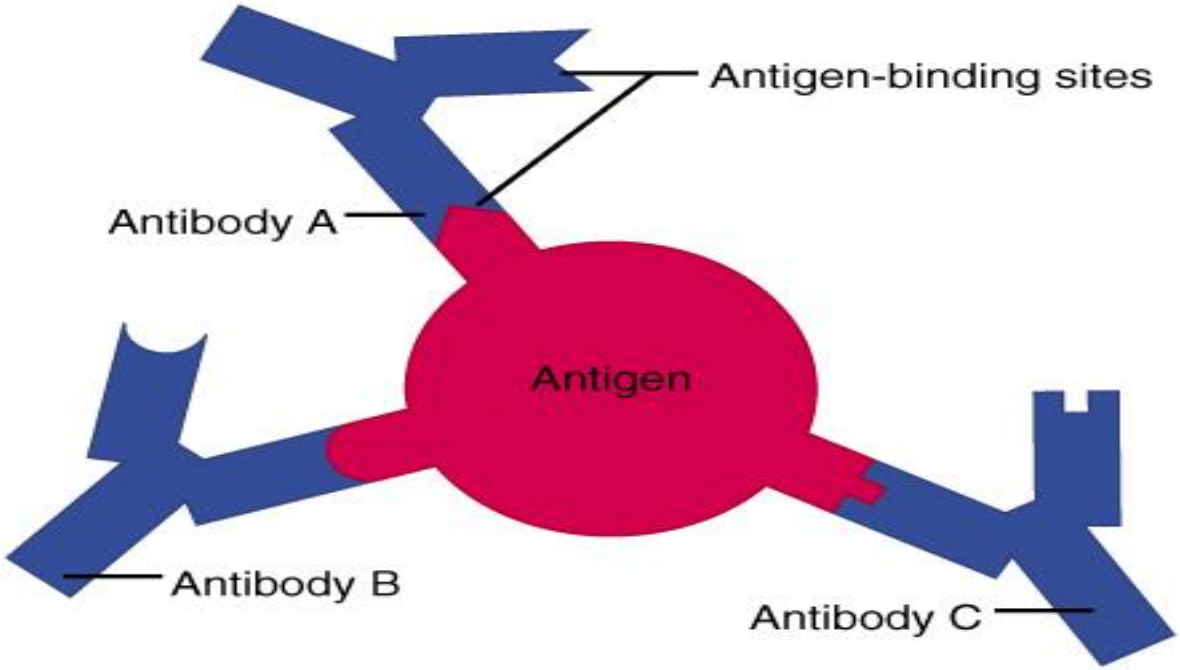
- **ANTIGENS :The foreign substances interact with antibodies .**
- **IMMUNOGENS: The foreign substances that induce an immune response and interact with antibodies**

- **ANTIBODY are Y-shaped proteins found in sera which are produced in response to a specific antigen, Antibodies are composed of two heavy peptide chains and two short peptide chains .**
- **EPITOPE:** is the unique part of the antigen recognized by an antibody.

PARATOPE: Is a unique parts of the Abs that recognized Ags (epitops)


Antigen binding sites
Variable region on heavy chain
Light chain
Disulfide bridges
Variable region on light chain
Constant region on light chain
Heavy chain
Constant region on heavy chain

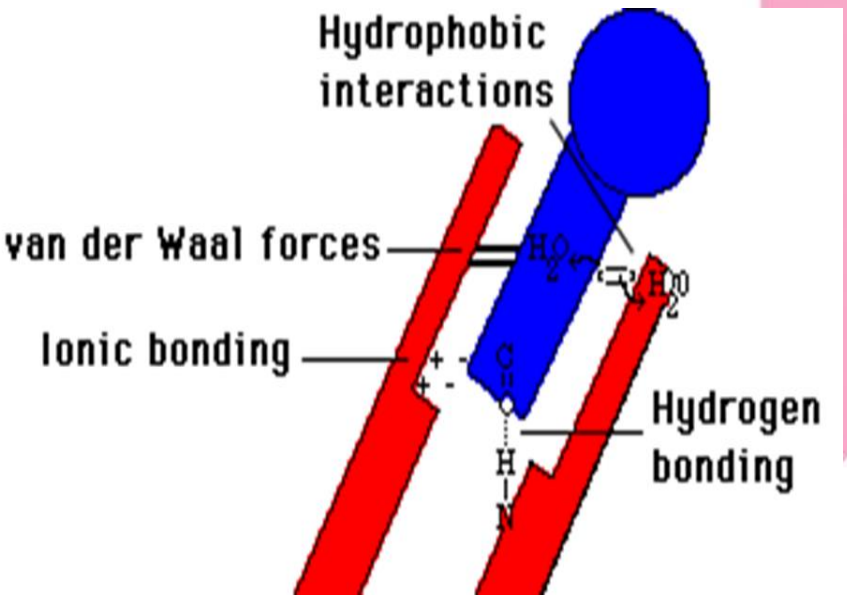


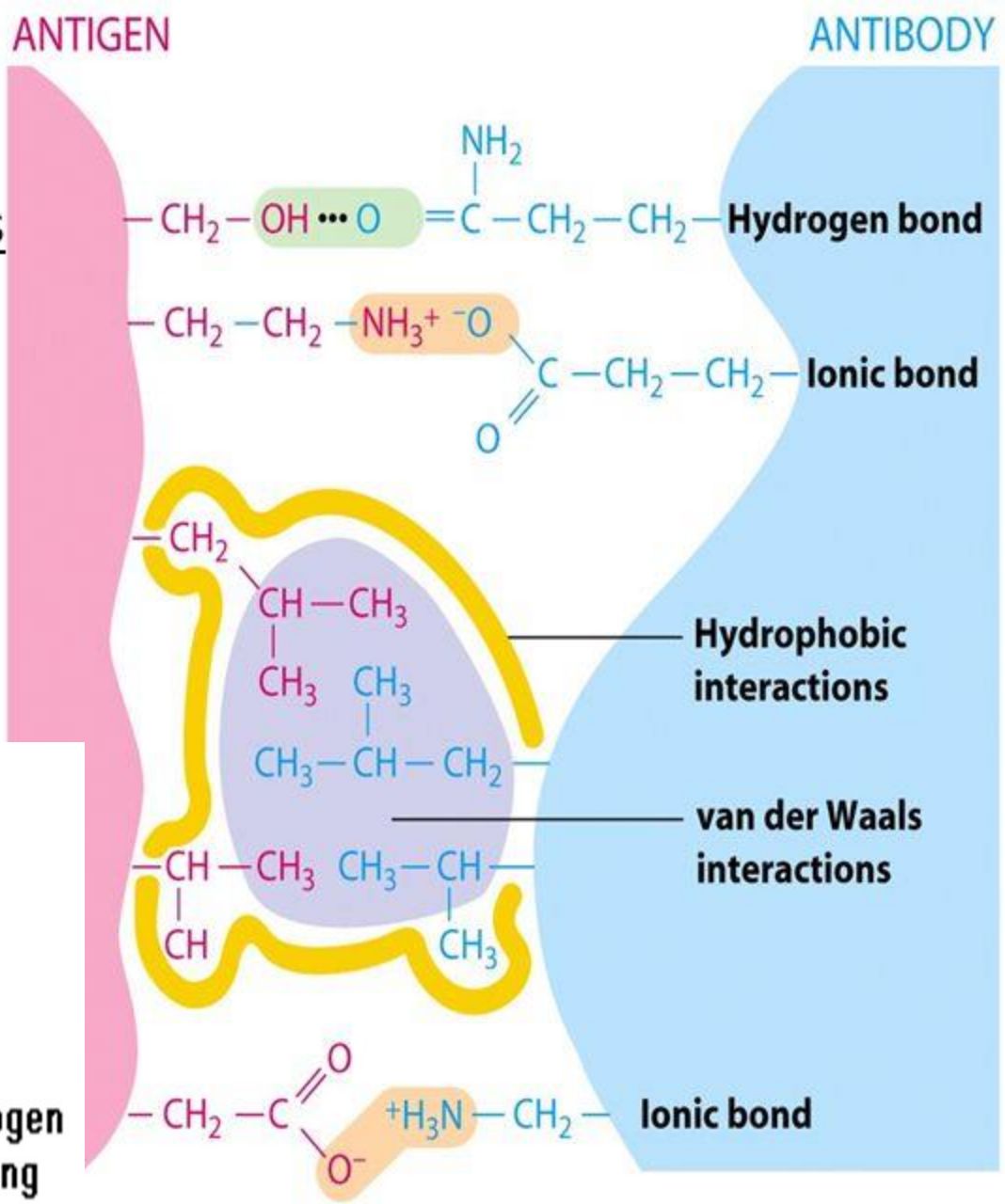


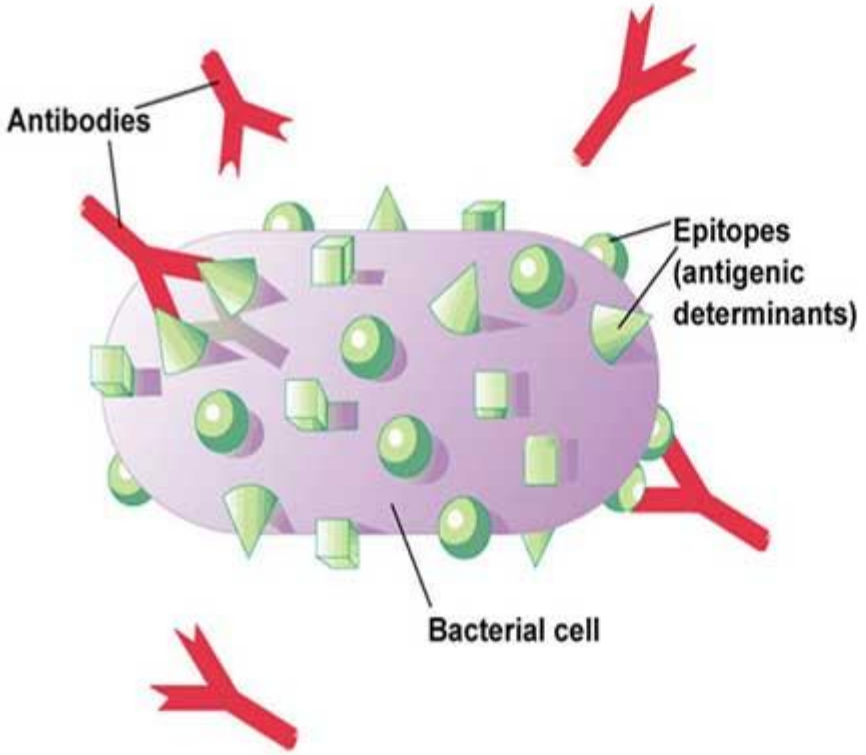
2- Non – covalent Bonds:

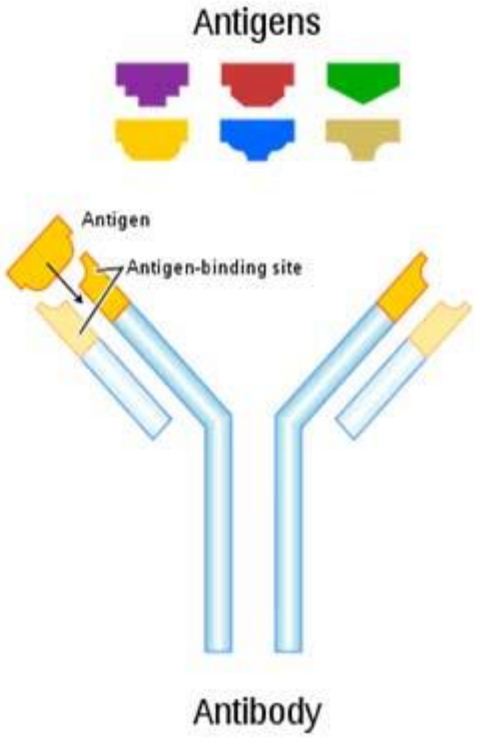
The Ag-Ab interaction is due to lots of non-covalent bonds include :

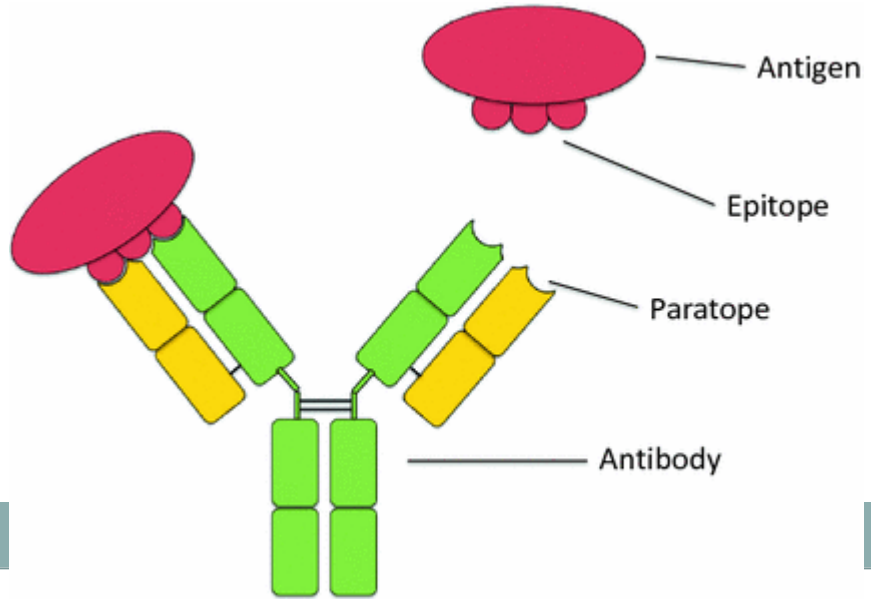
- Hydrophobic bonds.
- Hydrogen bonds.
- Electrostatic bonds.
- Van der Waal interactions.
- Ionic bonds.






Antibodies
Epitopes
(antigenic
determinants)
Bacterial cell


Antigens
Antigen
Antigen-binding site
Antibody

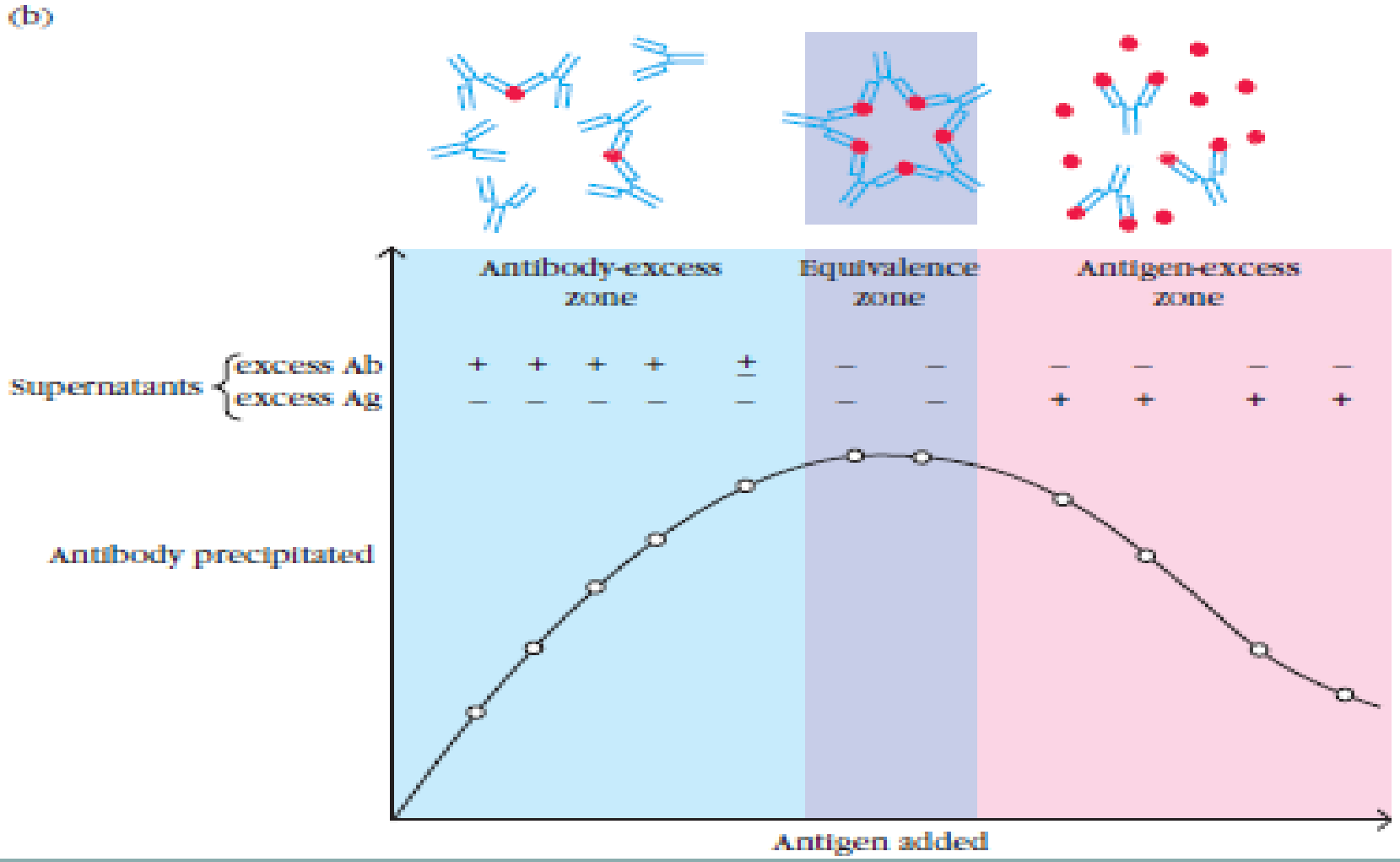

Antigen
Epitope
Paratope
Antibody

Antigen to antibody ratio

- The ratio between the antigen and antibody influences the detection of antigen-antibody complexes because the size of the complexes formed is related to the concentration of the antigen and antibody.

Antigen to antibody ratio

- prozone: When there is an excess of antibody, reaction is not observed.
- equivalence zone: The size of the aggregation complex increases as the optimal ratio of antigen to antibody is achieved.
- Postzone: where too little antibody is present to produce an aggregation, each antibody molecule only binds to one antigen


(b)
Antibody-excess zone
Equivalence zone
Antigen-excess zone
Supernatants
excess Ab
excess Ag
+ + + + ±
– – – – –
– – – – – – – –
– – – – + + + +
Antibody precipitated
Antigen added

Phases of antigen-antibody reactions

- **The antigen-antibody reaction takes place in two phases .**
- **In the first phase, combination of the reactants occurs; this is followed by**
- **second phase, an aggregation (precipitation or agglutination).**

- Immunoassay: is a biochemical test that measures the concentration of a substance in a biological liquid, like serum, CSF or urine, using the reaction of an antibody or antibodies to its antigen.

- **The assay takes advantage of the specific binding of an antibody to its antigen.**

Immunological Methods

- Unlabelled **Immunoassay : Immunoassay that don't employs any types of labelssuch as Precipition, Agglutination** Ab, Immunodiffusion (Double and Single), Agglutinin Ab, Latex test, CFT.

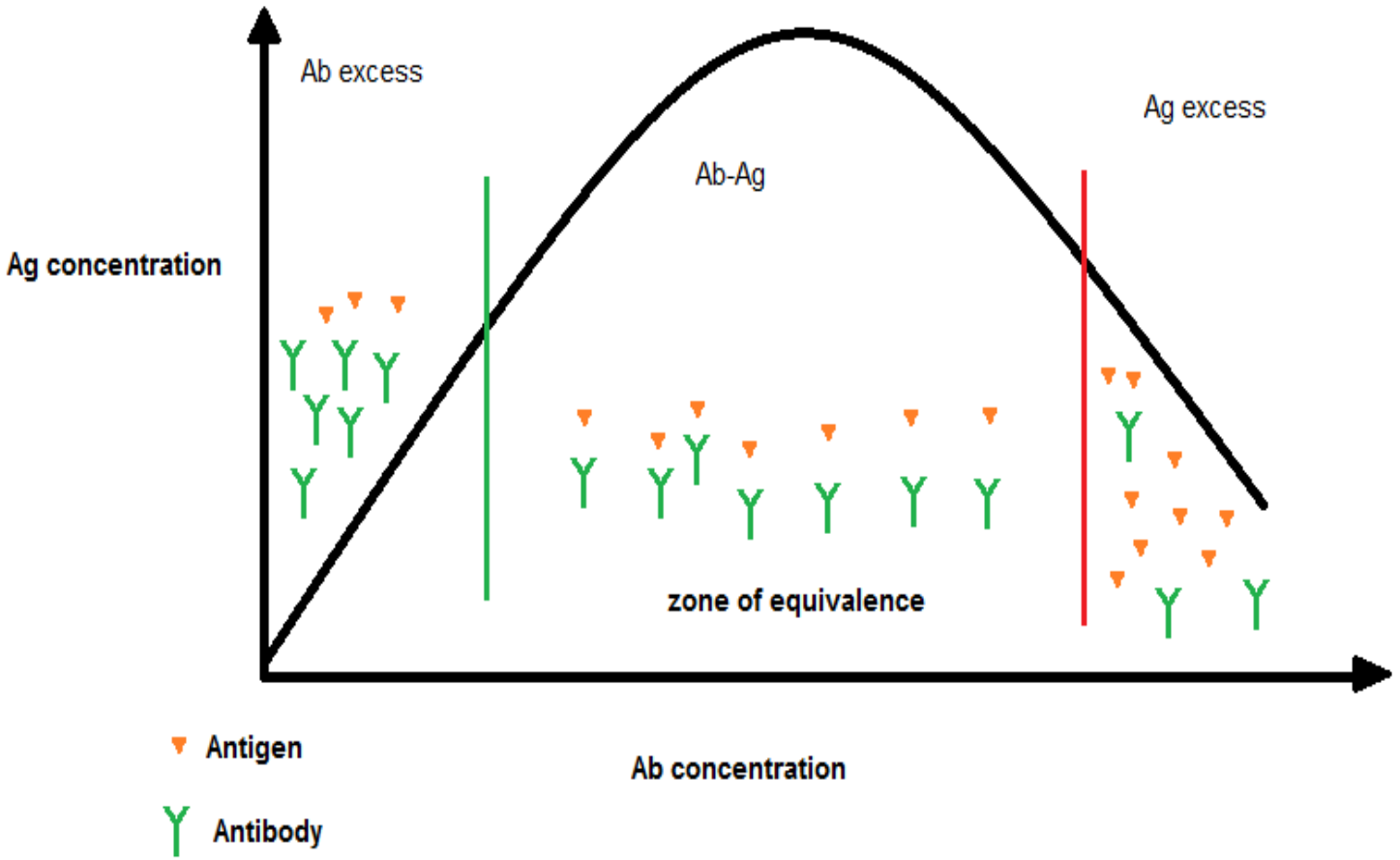
- Labeled **Immunoassay : Immunoassays employ a variety of different labels to allow for detection of antibodies and antigens. Labels are typically chemically linked or conjugated to the desired antibody or antigen**.Immunoflourescent assay (IFA), Radio-immunoassay (RIA), Enzyme linked immunosorbent assay (ELISA).

UNLABELLED AB

1-Precipitation test

This test is applied to soluble Ags, which when react with specific Abs, a precipitate will be formed in the zone of equivalence as shown in the precipitate curve.

The precipitation doesn't occur in the zone of Ag excess nor in the zone of Ab excess, where as the precipitation is maximal when optimal proportions of Ag & Ab combined


Ab excess
Ag excess
Ab-Ag
Ag concentration
zone of equivalence
Antigen
Ab concentration
Antibody

LAB(2) TYPES OF PRECIPITATIONS

- ***((1)). Precipitation in a solution (ring precipitation)***

This is done by layering a small volume of one over the other in a tube, a ring will appear at the interface; example : serotyping of ***group A β-Hemolytic Streptococcus.***

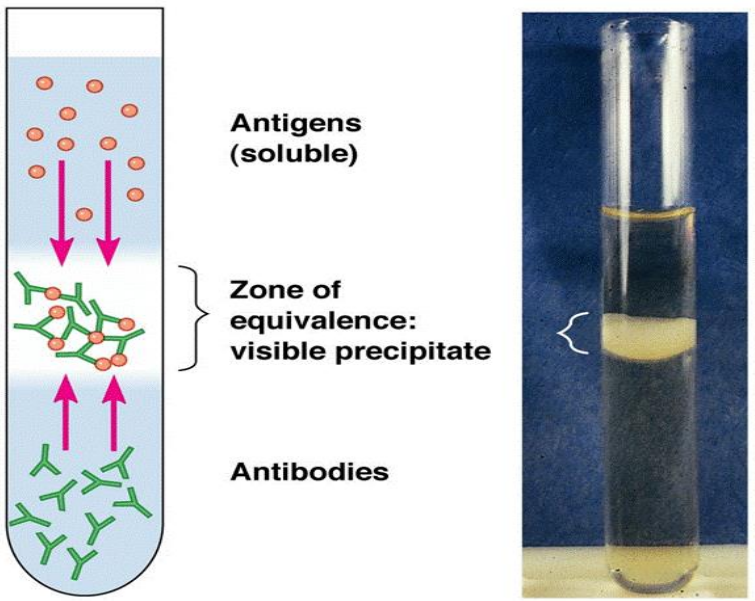
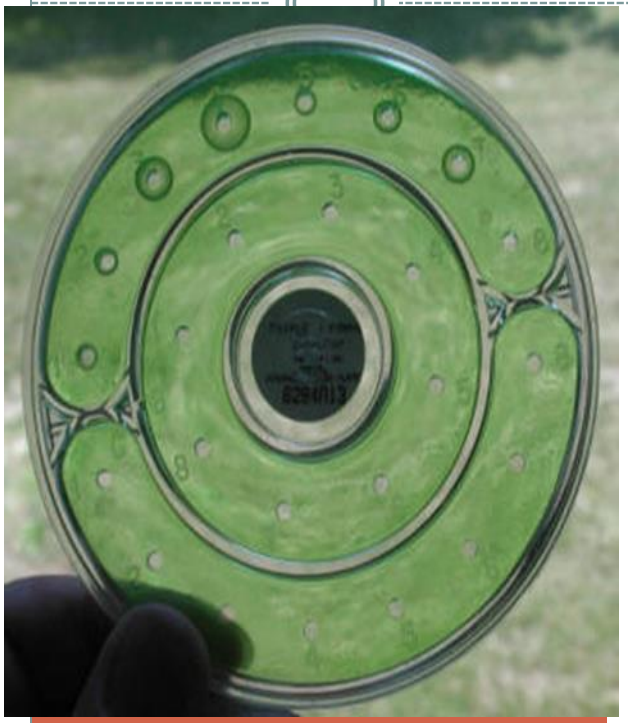


Figure 18.4 - Overview



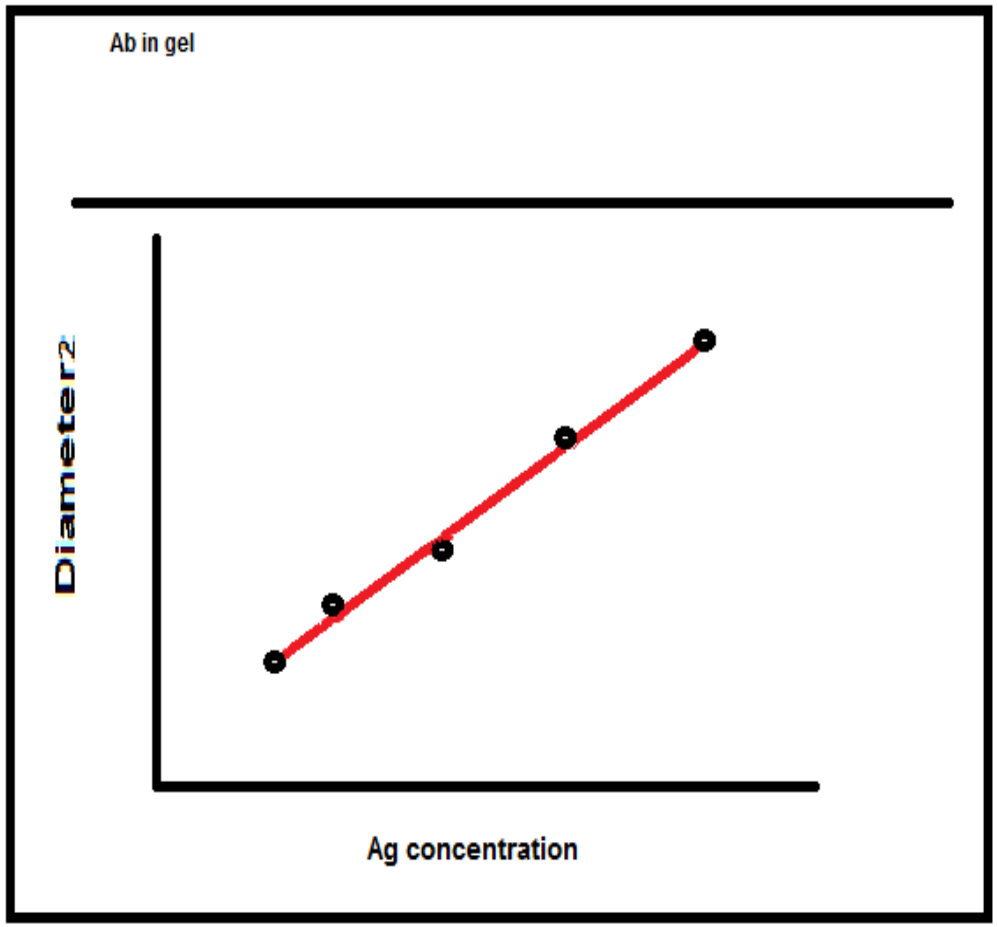
- ***((2). Precipitation in agar (agarose gel).***

The most important precipitation methods are those which occur on a gel. Involving the following types.

Single redial Immunodiffusion method (Mancini). It is a single diffusion technique whereby a solution containing the antigen is placed into wells in a gel or agar surface evenly impregnated with antibody.

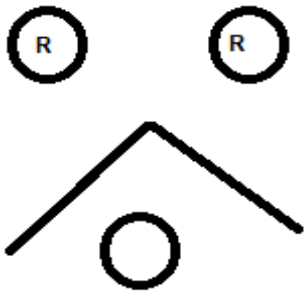
The diameter of the ring that precipitates around the well as a result of antigen antibody reaction corresponds to the amount of antigen in the solution..

Used for the quantitative measurements of Abs. the anti-sera is incorporated in to the agar. Ring of precipitation will form after 24-48 hrs of incubation at room temperature. The square diameter of this precipitate is proportional with the concentration of the Ab, correlated by using a standard calibrating curve


Ab in gel
Diameter2
Ag concentration

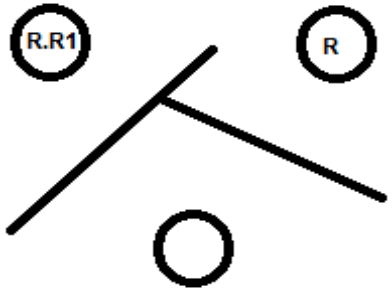
- ***Double Immunodiffusion method (Ouchterlony).***

A quantitative & qualitative method used for detection of Ag & Ab, or for identification of the **Ag- identity** (relation between different Ags). Ags plated in the central well, while different Ab are in the surrounding wells & all are allowed to migrate towards each other in gel & a line of precipitate is formed at the zone of equivalence.



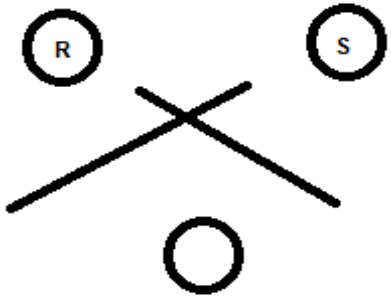
Anti- R

Called line of identity i.e. had similare Ag determinant



Anti-R,R1

Called line of semidentity i.e. share some Ag determinant



Anti-R,S

Called line of non identity i.e. doesnt share any Ag

2. Agglutination

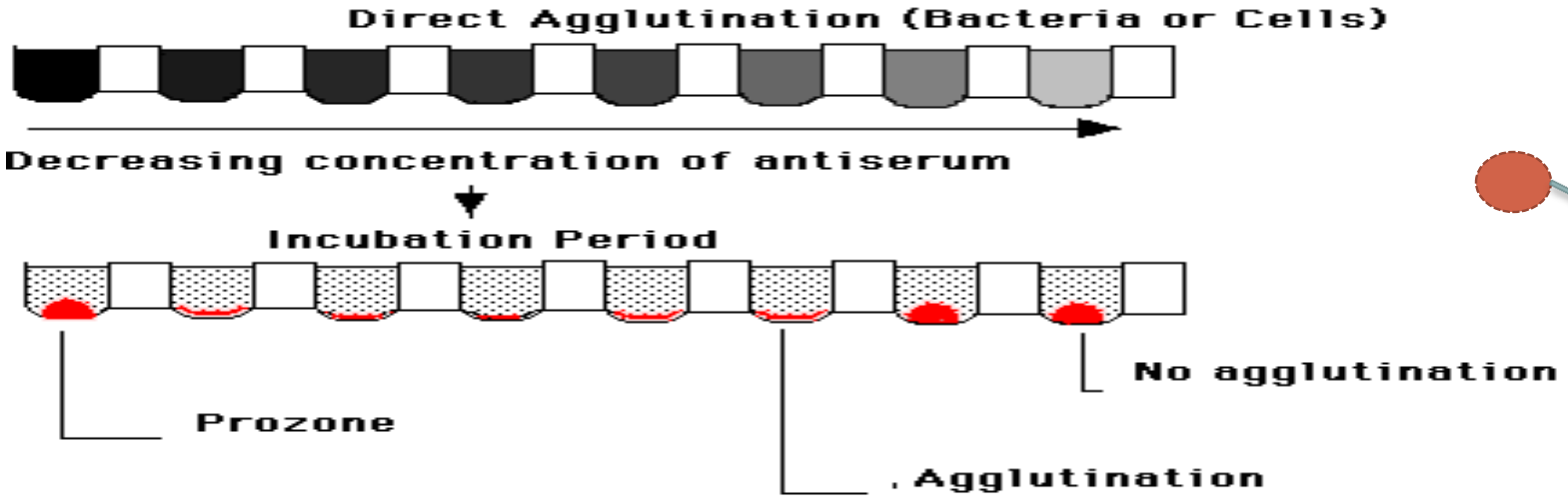
- *In this test the Ag is a particulate. These Ag will be clumped or agglutinated by specific Ab. Involve the following tests. Ags are (bacteria , RBC) or is an inert particle (latex- beads) coated with an Ag*
- *Why does agglutination take place?*
- *Cross linking of the Ag bearing particles by Ab disrupts the homogeneity of the suspension ((clumping))*

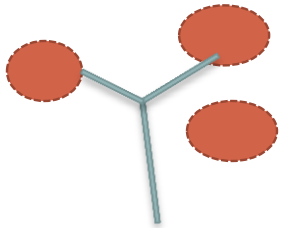
(A) Haemagglutination test ((Particulate Ag is an erythrocyte)) which also is of two types:

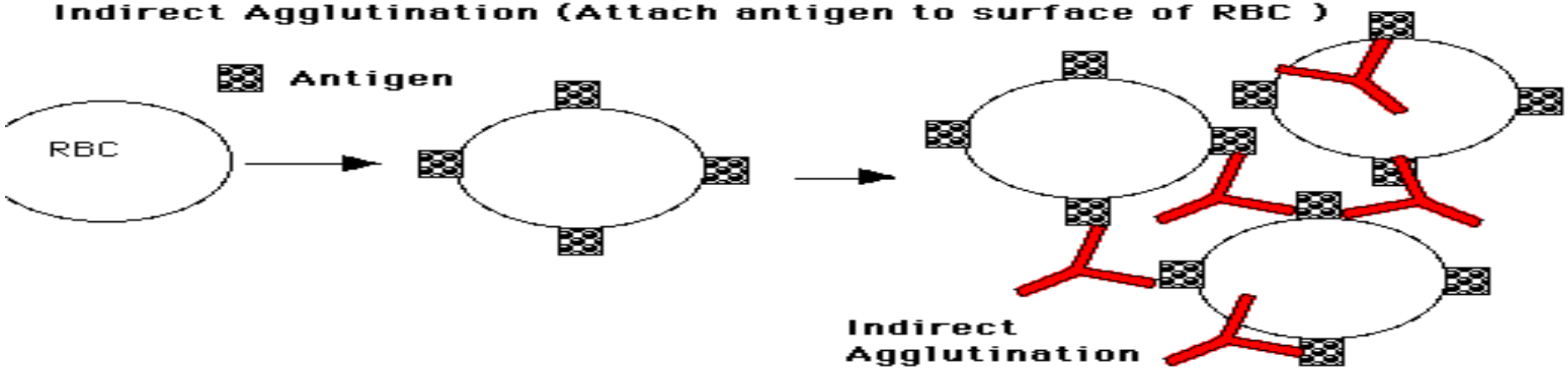
1- Direct Haemagglutination test:

2- Indirect Haemagglutination test.

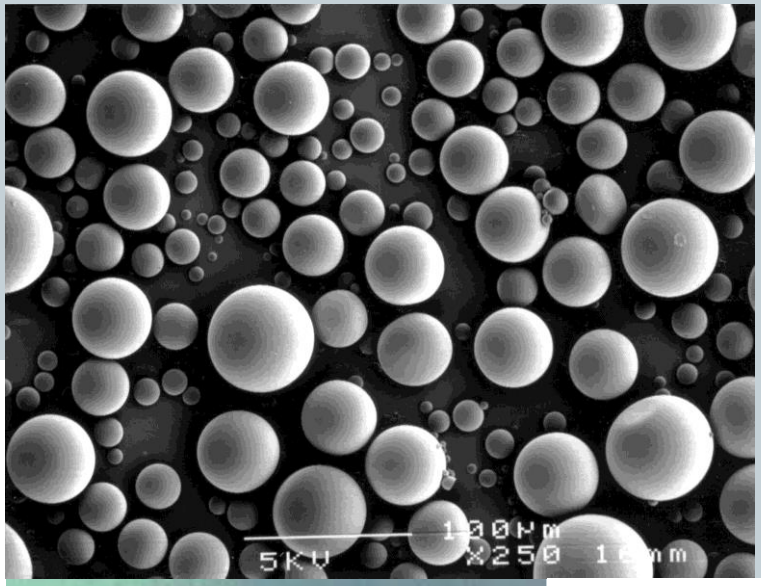
2-Indirect agglutination

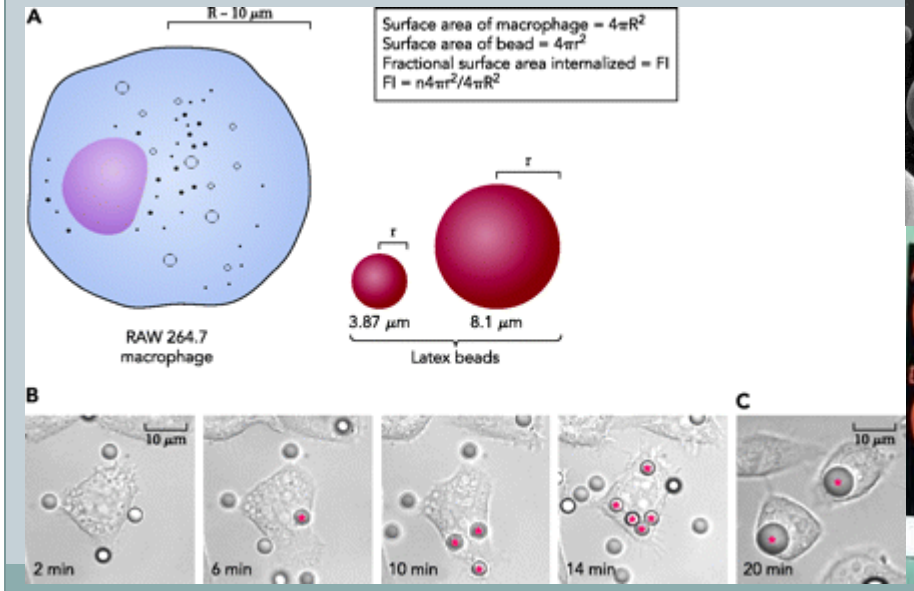


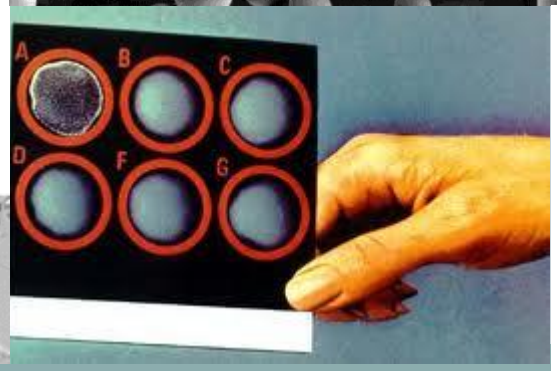




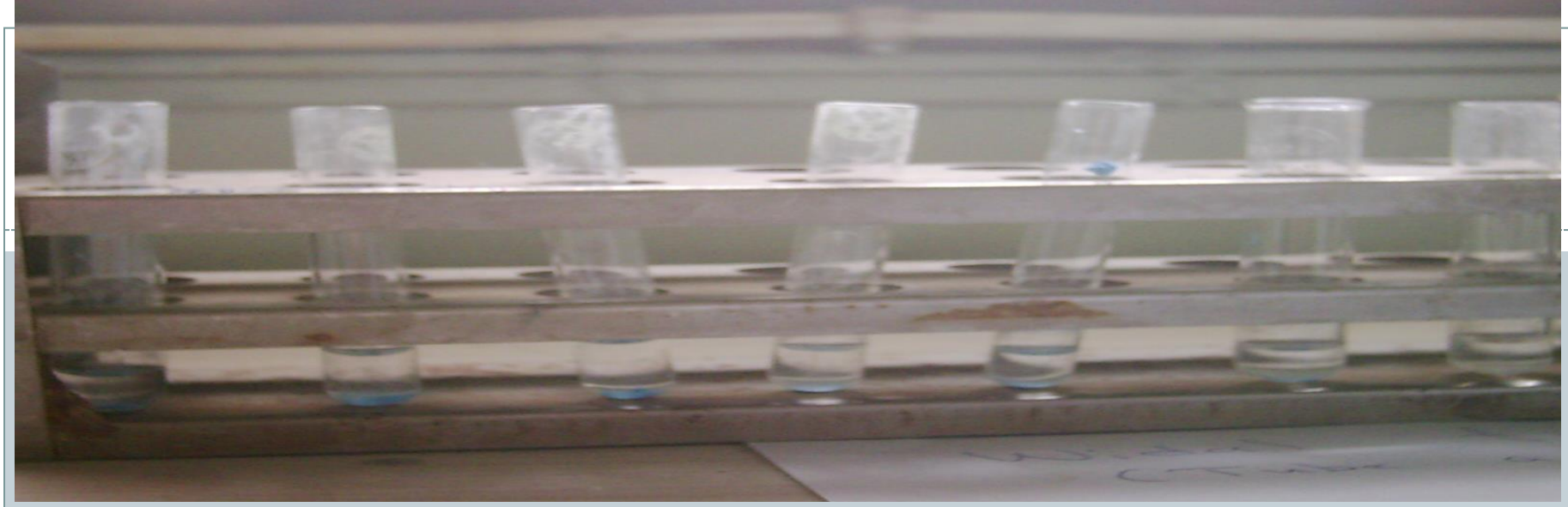
- *(B) Latex agglutination test:* *inert latex beads* ***provide a suitable carrier for Ag*** *e.g. particles coated with HCG + HCG in urine ; Rheumatoid factor (particles coated with IgG + IgM in the patients serum).*







Latex beads are coated with antigens



- ***(C) Agglutination test in suspension (Tube Agglutination Test):***

The most common complex in this category is Widal test .

This is a serological test to detect Ab against Salmonella spp. In the serum of a patient suspected to have typhoid fever . It is done as following

. Dilute the patients serum with normal saline (1/10; 1/20; 1/40; 1/80; 1/160; 1/320; 1/640; 1/1280).

. Add a fixed amount of the Ag (either O or H) for S typhi & S paratyphoid A& B.

. Mix and incubate for 24 hrs at 50C°.

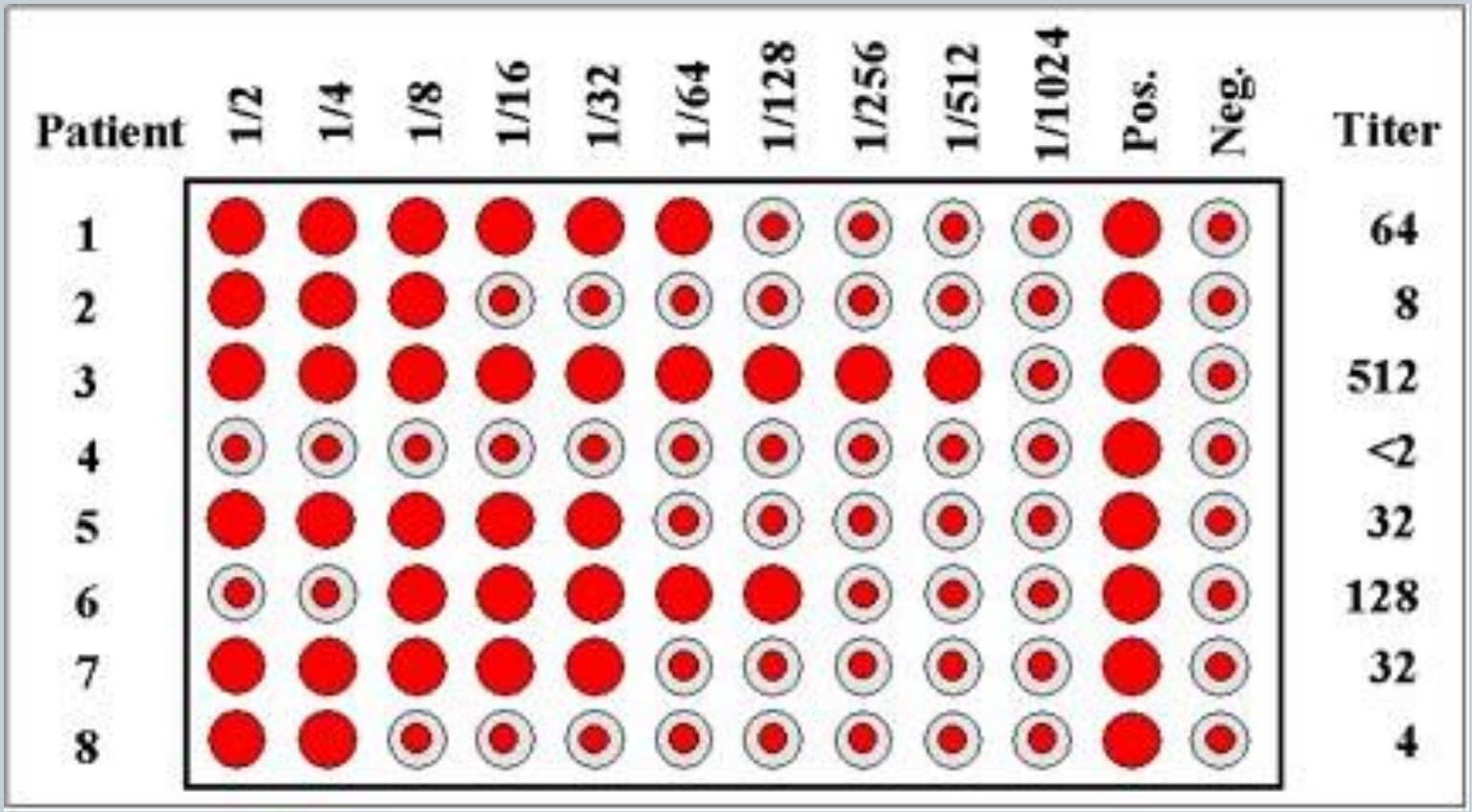
. Examine for agglutination at the bottom of the tube .

Types of agglutinations

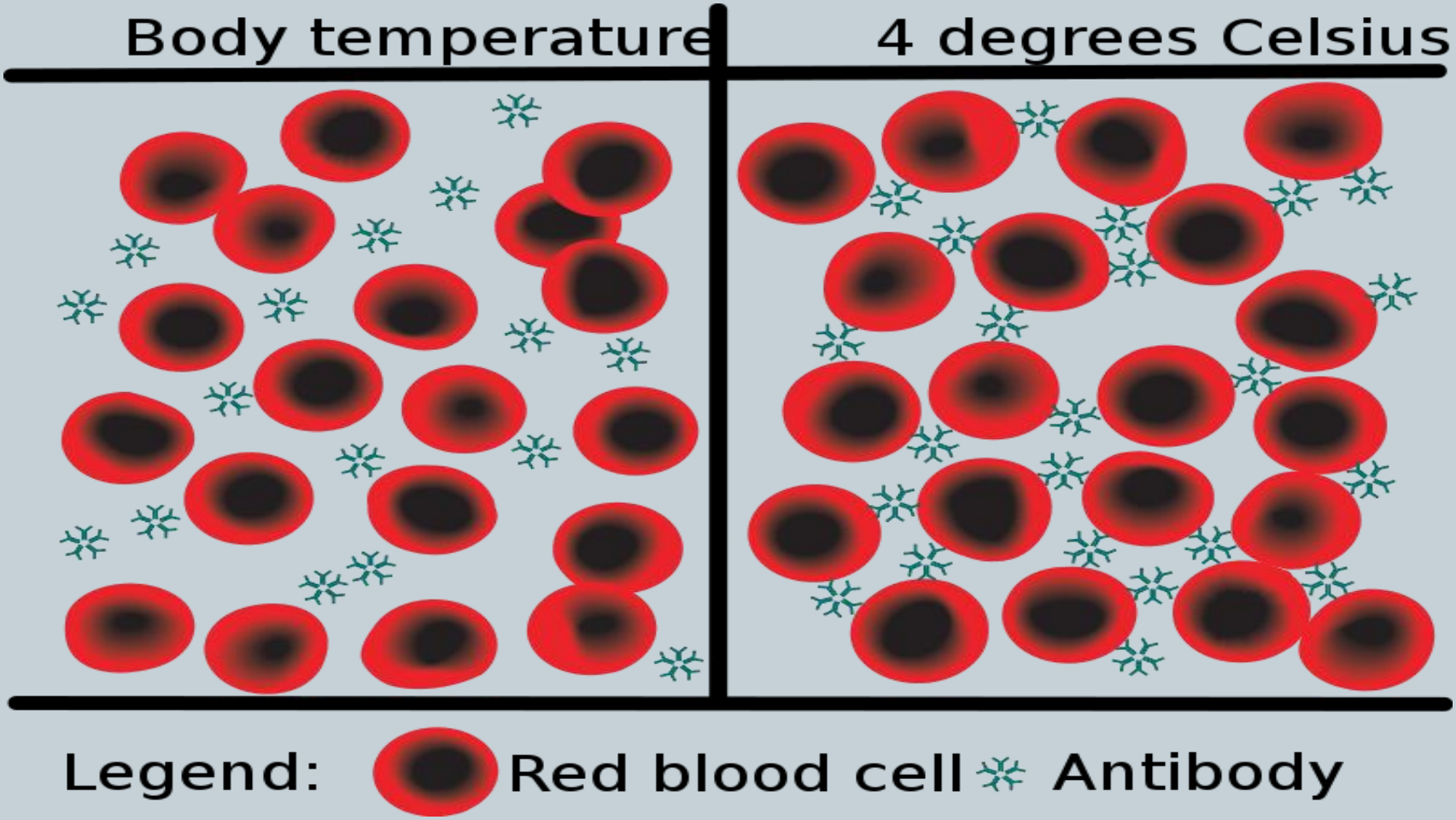
- **Qualitative agglutination test**
  Agglutination tests can be used in a qualitative manner to assay for the presence of an antigen or an antibody.

Quantitative agglutination test

- Agglutination tests can also be used to measure the level of antibodies to particulate antigens.
- In this test, serial dilutions are made of a sample to be tested for antibody and then a fixed number of red blood cells or bacteria or other such particulate antigen is added. Then the maximum dilution that gives agglutination is determined.
- Titer: the reciprocal value of the maximal dilution that gives visible agglutination


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

- **Cold agglutinins**: are autoantibodies produced by a person's immune system that mistakenly target red blood cells (RBCs). They cause RBCs to clump together when a person is exposed to cold temperatures and increase the likelihood that the affected RBCs will be destroyed by the body. This test detects and measures the amount of cold agglutinins in the blood.


Body temperature
4 degrees Celsius
Legend:
Red blood cell
Antibody

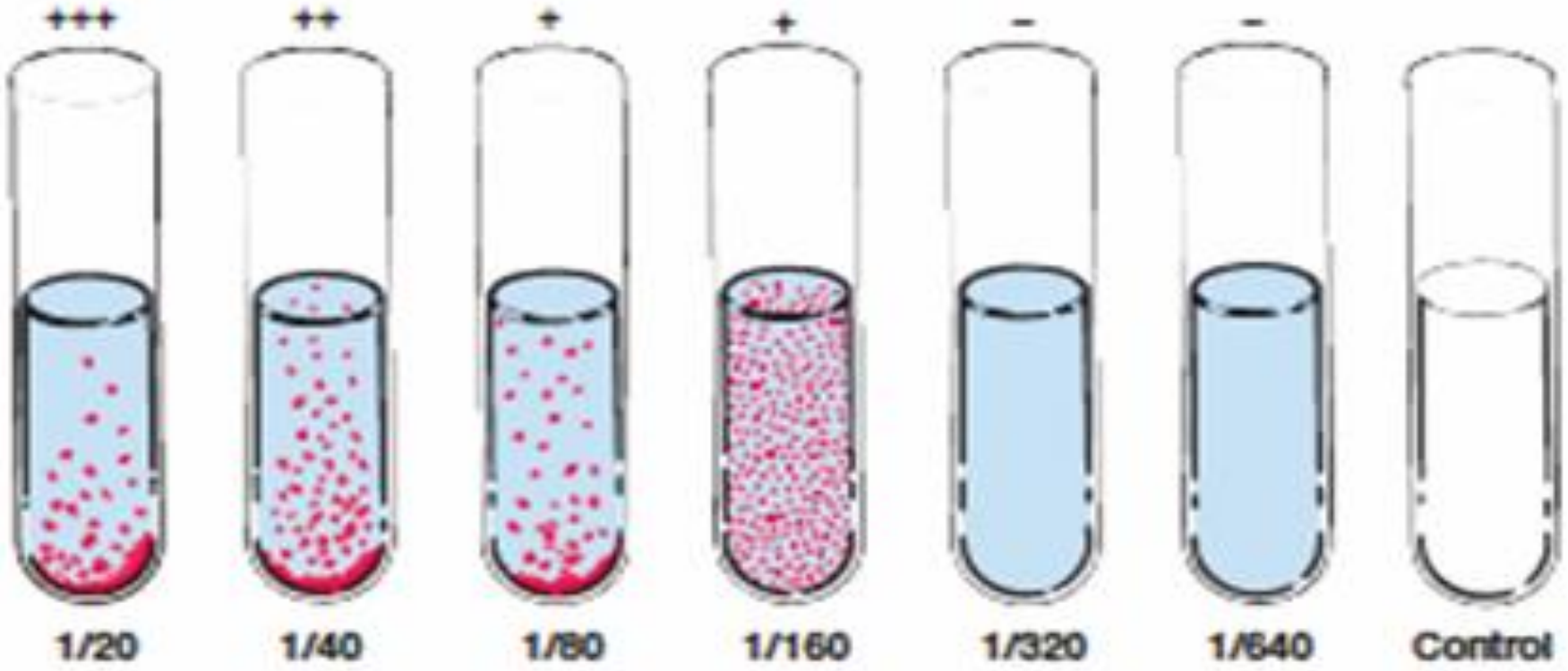
1- Cold agglutinins test

A serial dilutions of sera were mixed with 1% group O adult red cells and refrigerated at (4 °C)

a positive reaction of agglutination will occur in those tubes containing sufficient specific anti(I) antibody

The end point is determined as the last tube demonstrating the agglutination.

The reciprocal of the dilution was reported as titer



(a)

Titer = 160