SEROLOGICAL REACTIONS

- Antigens and Antibodies combine with each other specifically and in an observable manner.
- Reaction between antigens and antibodies serve several purposes.
- In the Body

  1) Form the basis of antibody mediated immunity.

  2) tissue injury in some types of hypersensitivity and autoimmune diseases.

- In the laboratory

1) They help in the diagnosis of infections.

2) Identification of infectious and of non infectious agents such as enzymes, hormones.

3) Epidemiological surveys.

- In general these reactions can be used for the detection and quantitation of either antigens or antibodies.

- Antigen antibody reactions in vitro are known as *serological reaction*.

General features of antigen antibody reactions

- The reaction is specific and antigen combine with its homologous antibody and vice versa.

  The specificity however is not absolute.

  Cross reaction may occur due to antigenic similarity or relatedness.
- Entire molecules react and not fragments.

- No denaturation of the antigen or the antibody during the reaction.
- The combination occurs at the surface.
- The combination is firm but reversible.
- This firmness is influenced by the affinity and avidity of the reaction.
- Affinity refers to the intensity of attraction between the antigen and antibody molecule.
- Avidity refers to the strength of bond after antigen antibody complex formation.

- Both antigens and antibodies participates in the formation agglutinates or precipitates.

- Antigens and antibodies can combine in varying proportions.

Measurement of antigen and antibody

- Many methods are available.
- Measurement in terms of mass or units or in terms of titre.
- Antibody titre : denotes the highest dilution of the serum at which antibody activity is demonstrable.

Antigen antibody interaction

Occurs in stages:

- Primary interaction : Rapid and invisible, but reversible. Combination of antigen with Ab is effected by weaker intermolecular forces –Van der Waal's, Hydrogen, loinic
- Secondary stage : demonstrable events examples : precipitation, agglutination, complement fixation.
- Tertiary stage : in vivo

Antigen antibody reaction

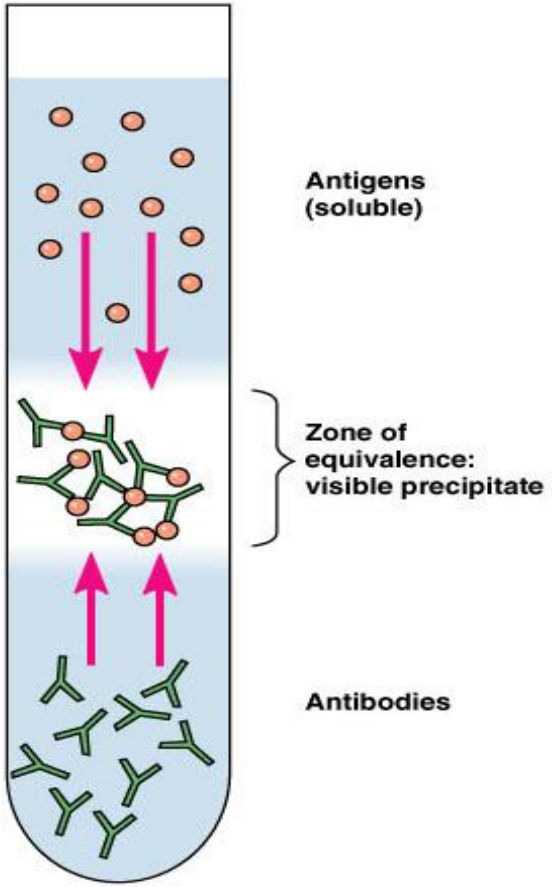
- Precipitation reaction
- Agglutination reaction
- Complement reaction
- Neutralization test
- Immunofluoroscence
- Enzymeimmuno assay
- Radioimmunoassay

Precipitation reaction

- Soluble antigen reacts with its specific antibody in presence of electrolytes at suitable temperature & pH, antigen antibody complex forms an insoluble precipitate
- When precipitation remains suspended instead of sedimenting,the reaction is called flocculation.
- Precipitation reaction may occur in liquid medium or semisolid medium like agar gels,agarose or polyacrylamide.

Precipitation Reactions

- Involve soluble antigens with antibodies



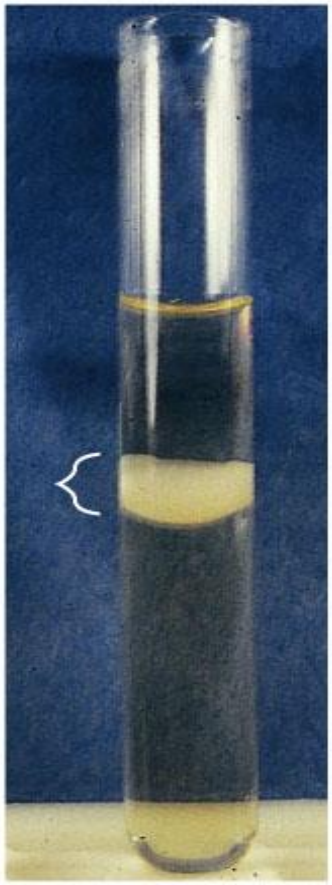
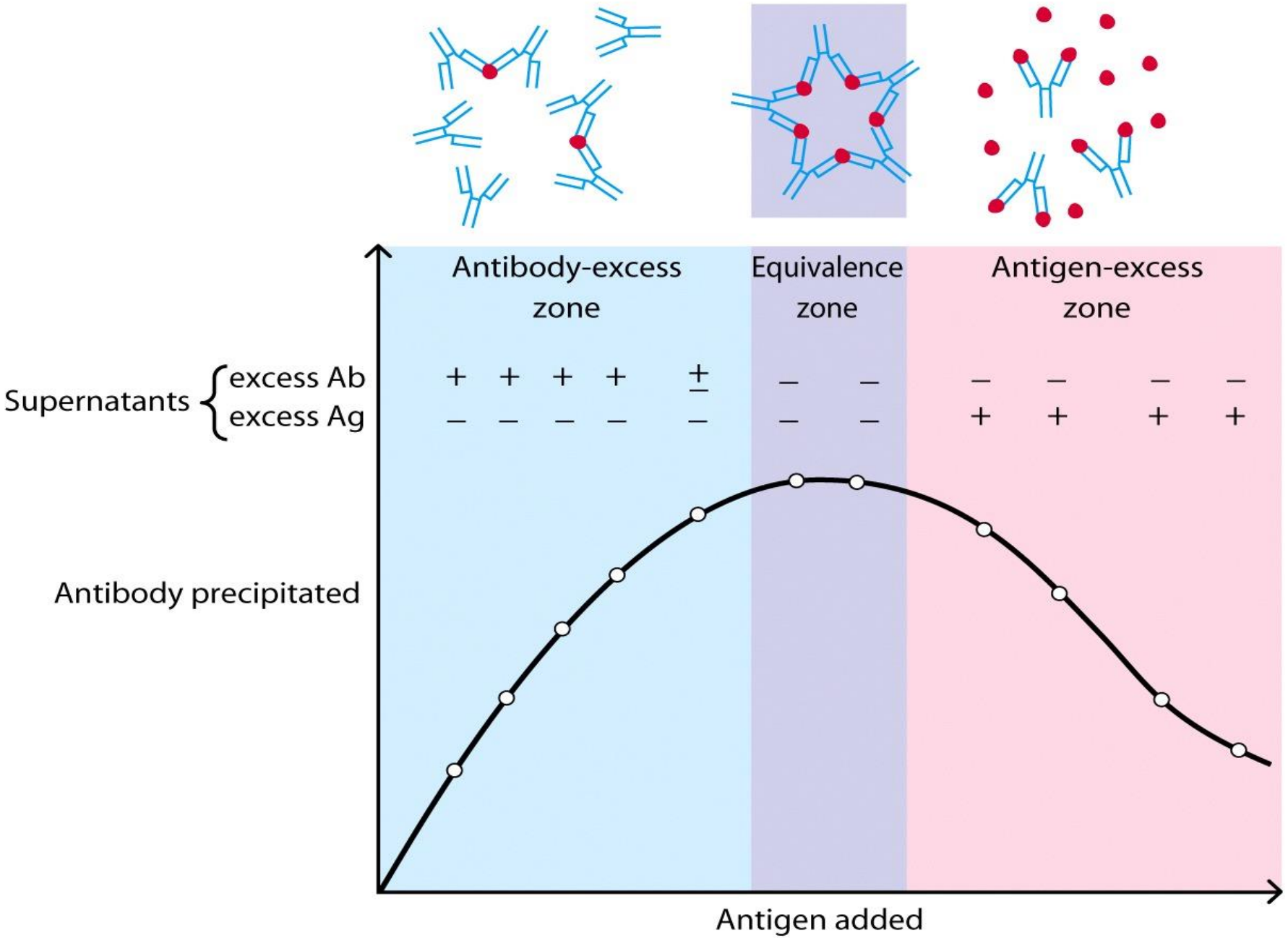


Figure 18.3

Quantitative precipitation in liquid medium

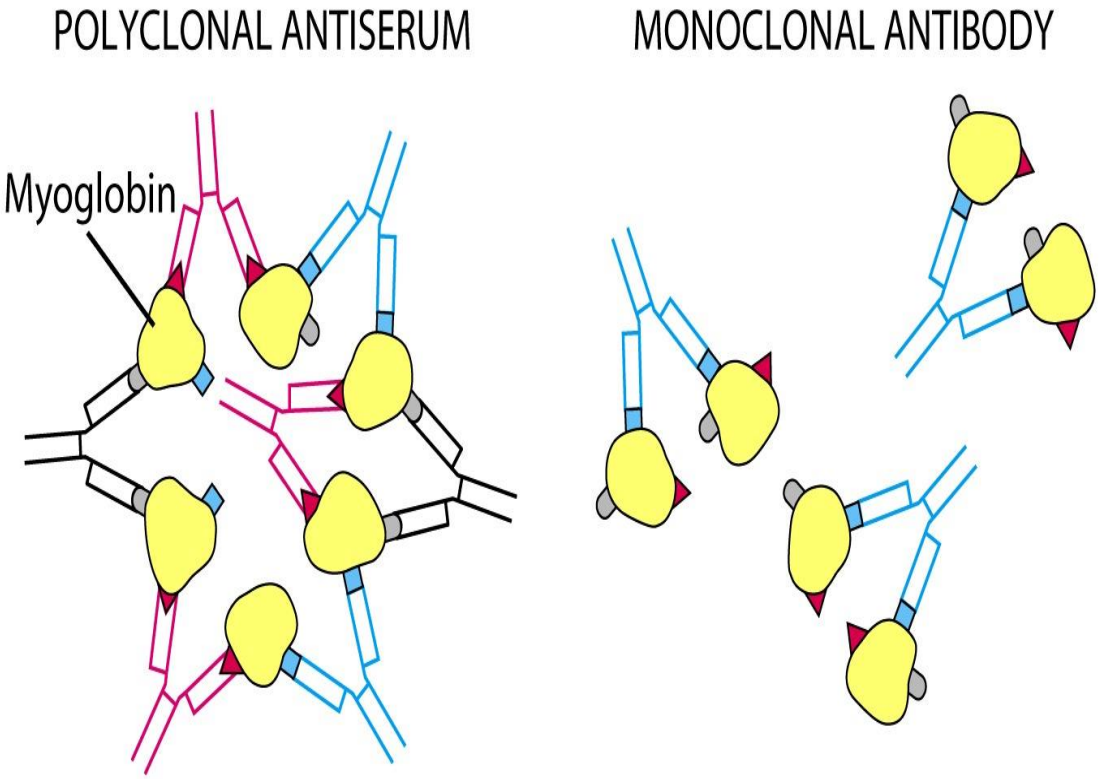
- When same amount of antiserum is mixed with increasing amount of corresponding pure soluble antigen in test tubes precipitation occurs most rapidly and abundantly in middle tubes. when the results are plotted in a curve three zones can be observed.
- A zone of antibody excess
- A zone of equivalence
- A zone of antigen excess


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

Mechanism of precipitation

- Marrack proposed the lattice hypothesis to explain the mechanism.
- Multivalent Ag combines with bivalent Ab in varying proportion.
- Precipitation results when a large lattice is form which is possible in the zone of equivalence.
- In the zone of Ag/Ab excess the lattice does not enlarge.
- This mechanism is also applied in agglutination.

3. Precipitation Reactions



(Lattices or large aggregates)

(no precipitate is formed if an Ag contains only a single copy of each epitope)

Precipitation reactions in fluids yield a precipitin curve.

Techniques of precipitation

- Simple precipitation test in liquid medium–slide and tube technique

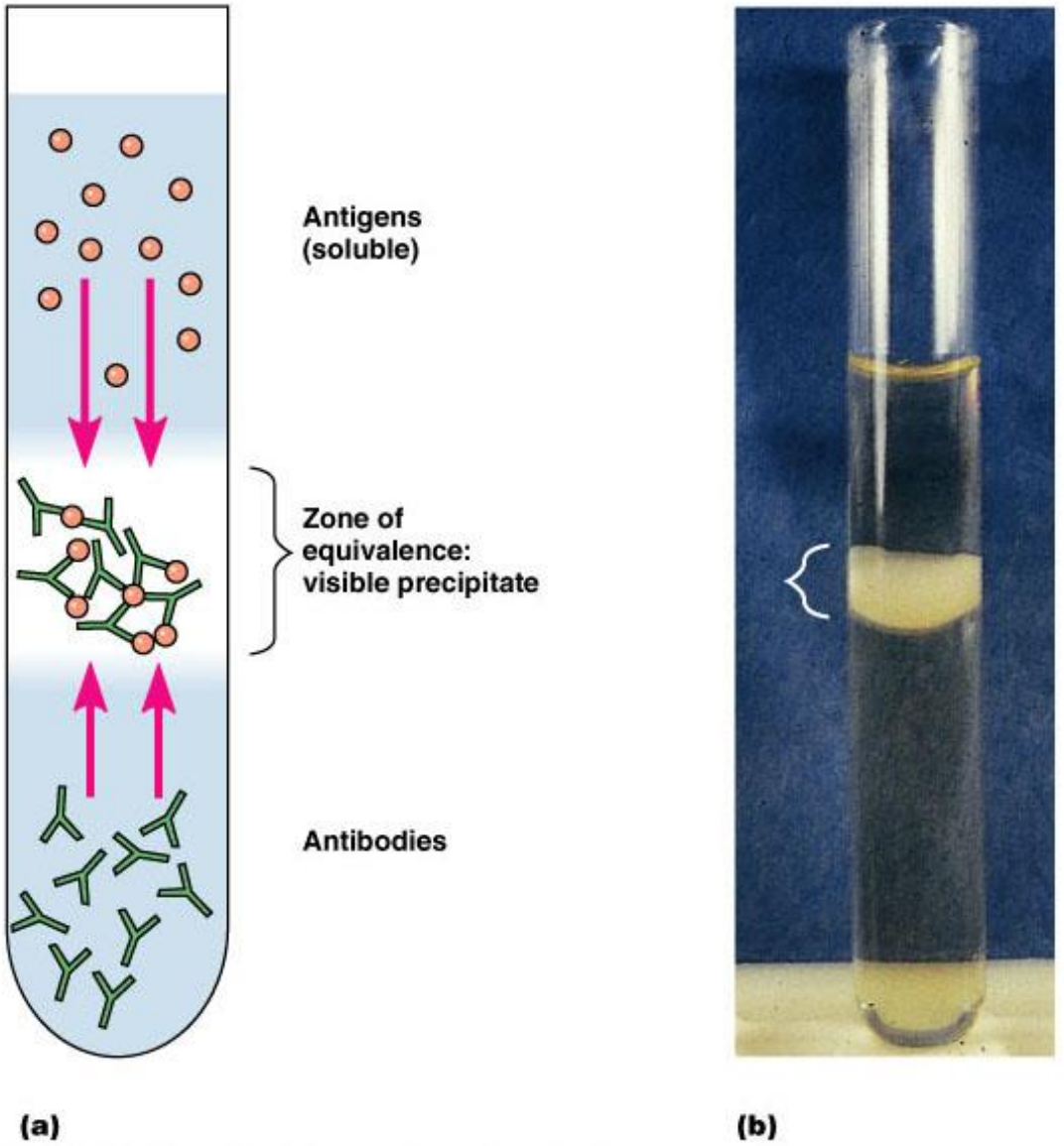
1) Tube test: Kahn test for syphilis, Ascoli's thermoprecipitation test, Lancefield technique for streptococcal grouping.
2) Slide test: VDRL test.(Slide floculation)

Modification of VDRL test:
(1) RPR Test–Black flocculation
(2) TRUST Test– Red flocculation

- Gel diffusion test
- Immunoelectrophoresis

Simple precipitation tests

- **Ring test** : A clear solution of an extract of the test antigen or organism is layered slowly on to a clear antiserum in a narrow test tube. Following a period of incubation, a white ring of precipitation appears at the junction of two clear fluids.
- Examples : Ascoli's thermoprecipitation test, lancefield method of grouping beta haemolyitc streptococci.


Antigens
(soluble)
Zone of
equivalence:
visible precipitate
Antibodies
(a)
(b)

- **Flocculation test**: done in slide and in tube

- In slide test a drop of VDRL antigen solution is added to a drop of decomplemented serum on a slide, mixed well and shaken for a few minutes. visible clumps appear in positive cases.

- Tube flocculation test (Kahn test) was done previously in diagnosis of syphilis.

- Advantage of RPR test over VDRL

1) Commercially available ready to use kit
2) Unheated serum is tested
3) Reaction can be read visually
4) Either plasma or serum can be used

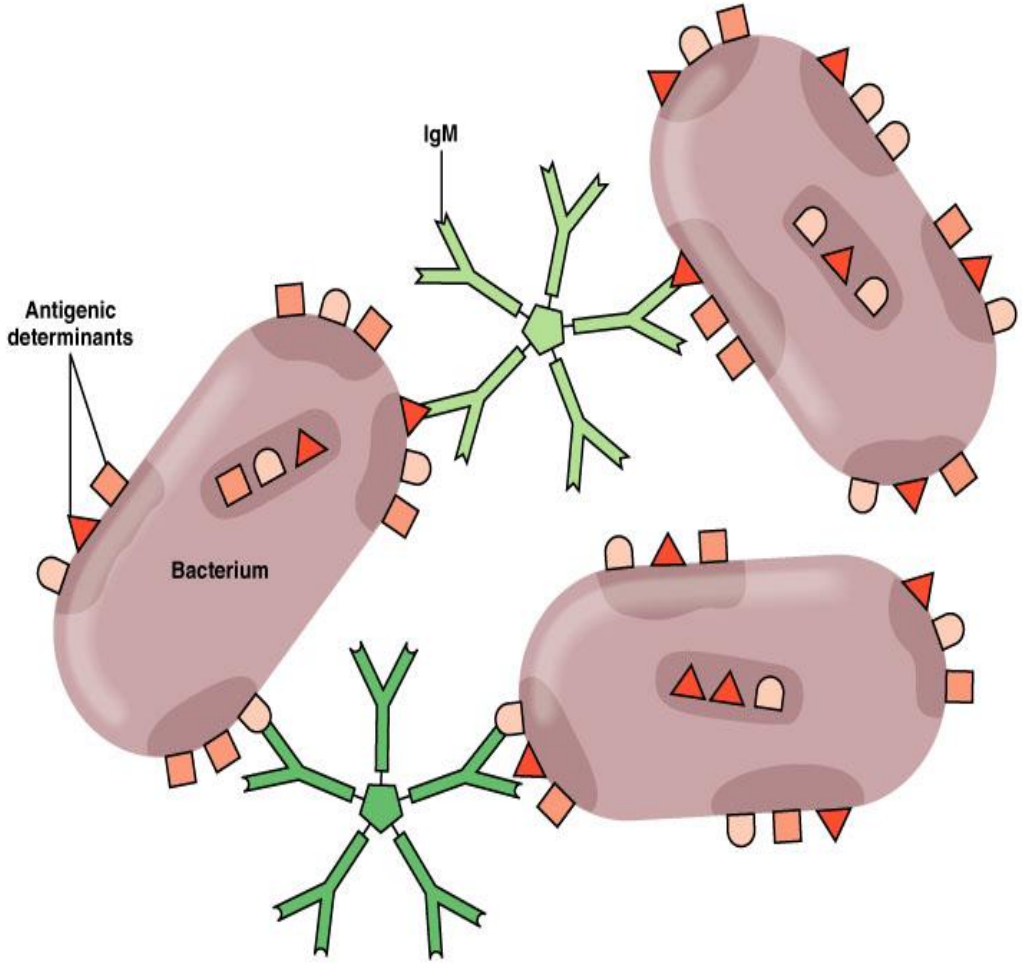
*Disadvantage : can not be used with CSF

Agglutination reaction

- When a particulate (insoluble) antigen mixed with its antibody ,the particles are agglutinated.
- More sensitive than precipitation for the detection of antibodies.
- Reaction occur optimally in zone of equilibrium.
- Incomplete antibodies do not cause agglutination though they combine with the antigen.

Agglutination Reactions

- Involve particulate antigens and antibodies
- Antigens may be:
- On a cell (**Active** agglutination)
- Attached to latex spheres (**Passive** agglutination)



Agglutination of two types :

Active agglutination :

direct agglutination of antigen with its corresponding antibody occur,

- Example : slide agglutination of salmonellae, vibrio cholerae using specific antibody.

Passive agglutination :

soluble antigen is attached to the carrier particle and thereby converting precipitation into agglutination reaction.

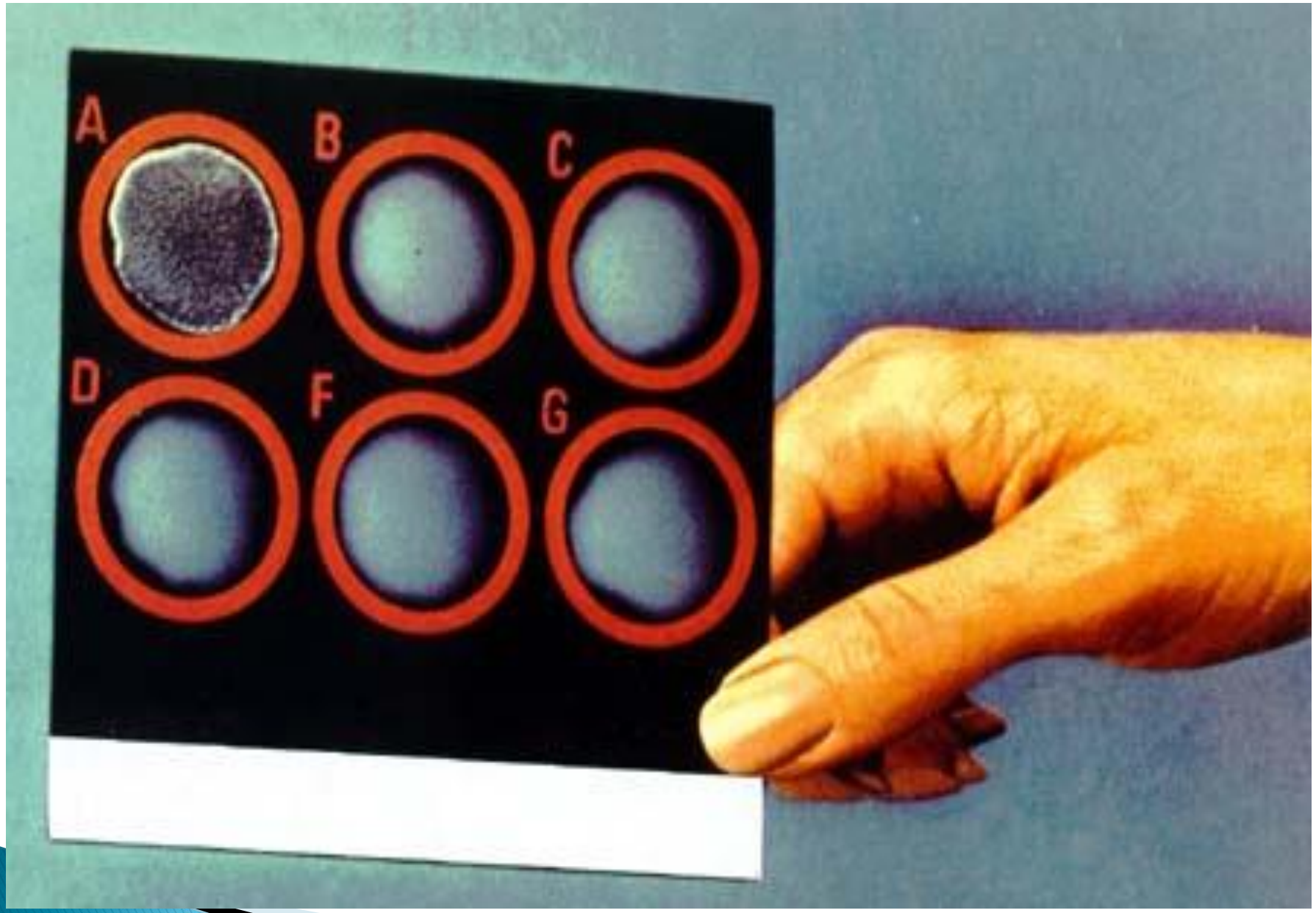
Carrrier particles used are Latex particles, Carbon particles,RBC

- Examples : ASO,CRP,RA

Application of Agglutination Reaction

Slide Agglutination :

- When a drop of antiserum is added to a smooth, uniform suspension of a particulate antigen in a drop of saline on a slide or tile agglutination takes place.
- Rapid test and results is obtained in minutes or even seconds.
- Routine procedure for the identification of bacterial isolates from clinical specimens.
- Used for blood group and cross matching.


A
B
C
D
F
G

Tube agglutination

- More sensitive than slide test.
- Standard quantitative method for measurement of antibodies.
- Routinely employed for the serological diagnosis of typhoid, brucellosis and typhus reaction.

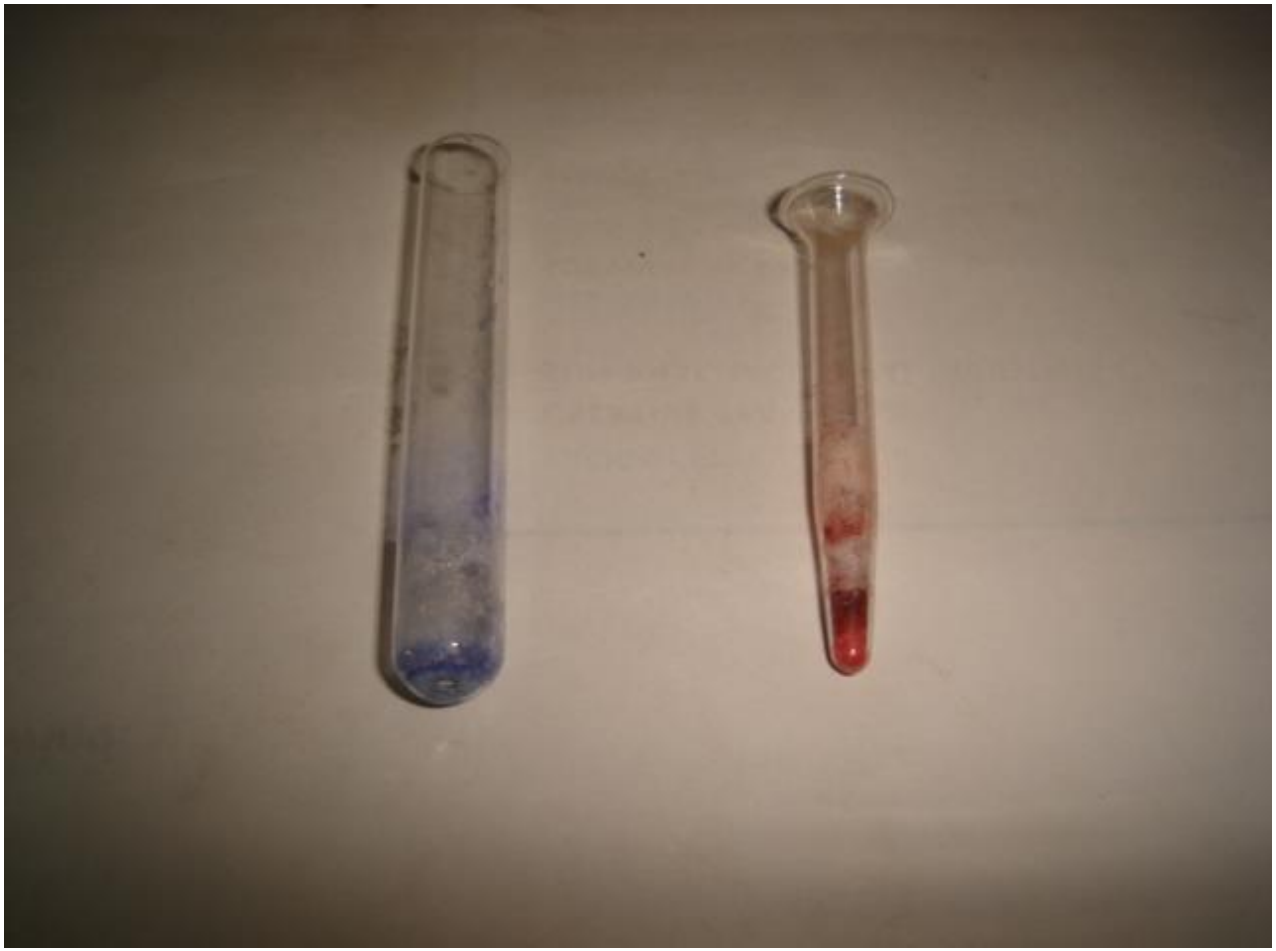
Examples

- Widal test – typhoid fever
  - Two antigen used – H & O
- Weil-felix reaction – typhus fever
  - Heterophile agglutination
  - OX2, OX19 & OX K antigen of proteus
- Paul Bunnel test – Infectious mononucleosis
- Cold agglutination test – mycoplasma
- Tube test for brucellosis is complicated by
  - Presence of blocking or monvalent antibodies
  - Prozone phenomena

Widal Test

- Widal Reaction : Slide & tube test
- Agglutination test
- Measurement of H & O agglutinin for typhoid & paratyphoid fever.
- Method : two types of tubes, narrow tube with conical bottom (Dreyer‘s for H agglutination) & Round bottom (Felix‘s) tube for O agglutination

Widal tubes



- Equal volume of serial dilution of serum(1:10 to 1: 640) mixed with equal volume of H & O antigen.
- Incubated in waterbath at 37°c overnight.
- Results :
- H agglutination forms loose,cottonwooly clumps
- O agglutination forms disc like pattern at the bottom.
- The antibody titre is measured.

- Preparation of widal Antigen :
- H Ag : Prepared by adding 0.1% formalin to a 24 hours broth culture or saline suspension of an agar culture
- O antigen : Culture org in cultured media contain phenol (1: 800)
- Strain : Used usually are the S typhi 901, O & H strain.

- Interpretation :
- Agglutination titre depends on the stage of disease.
- Rising agglutination titre is more meaningful.
- In a single test, a titre of > 1: 100 of O & a titre of >1: 200 of H agglutination signifies presence of active infection. but one should kept in mind about agglutinins level in 'normal sera' in different areas.

- Agglutinins may be present in case of Past infection, inapparent infection, immunisation with TAB vaccine
- Anamnestic reaction
- Bacterial suspensions used as antigen should be free from fimbriae
- Cases treated with antibiotics may show poor response.

ELISA

- ELISA is so named as the test technique involves the use of an enzyme system& an immunosorbent – an absorbing material specific for one of the components of reaction, antigen or antibody.

- The absorbing material may be pariculate,e.g. cellulose or agarose or a solid matrix,e.g. microwells,membranes.

- Widely applied in the detection of antigen or antibody such as hormones, toxins and viruses.

Principle :

Most ELISA developed for the detection of antigen or antibody consist of use of corresponding antibody or antigen in question which is firmly fixed on solid phase.

The enzyme system consist of :

1) an enzyme (Horse radish peroxidase, alkaline phoshatase) which is labelled or linked, to a specific antibody or antigen.

2) a specific substrate (O-phenyl-diamin-dihyrochloride for peroxidase, p-nitrophenyl phosphate for alkaline phosphate) which is added after the antigen antibody reaction. The enzyme catalyzes the substrate to give a colour end point.

- The intensity of the colour gives an indication of the amount of bound antibody or antigen.

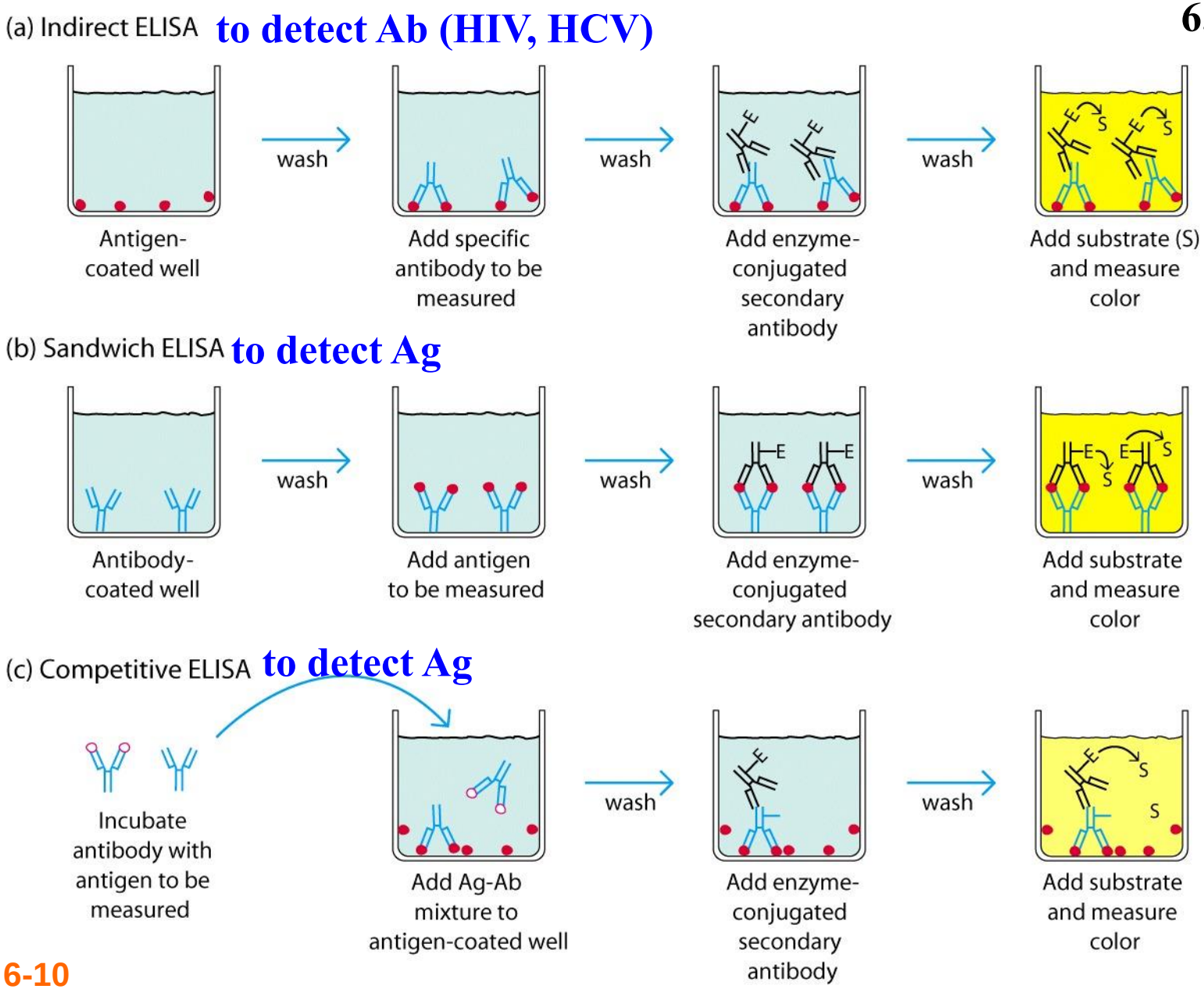


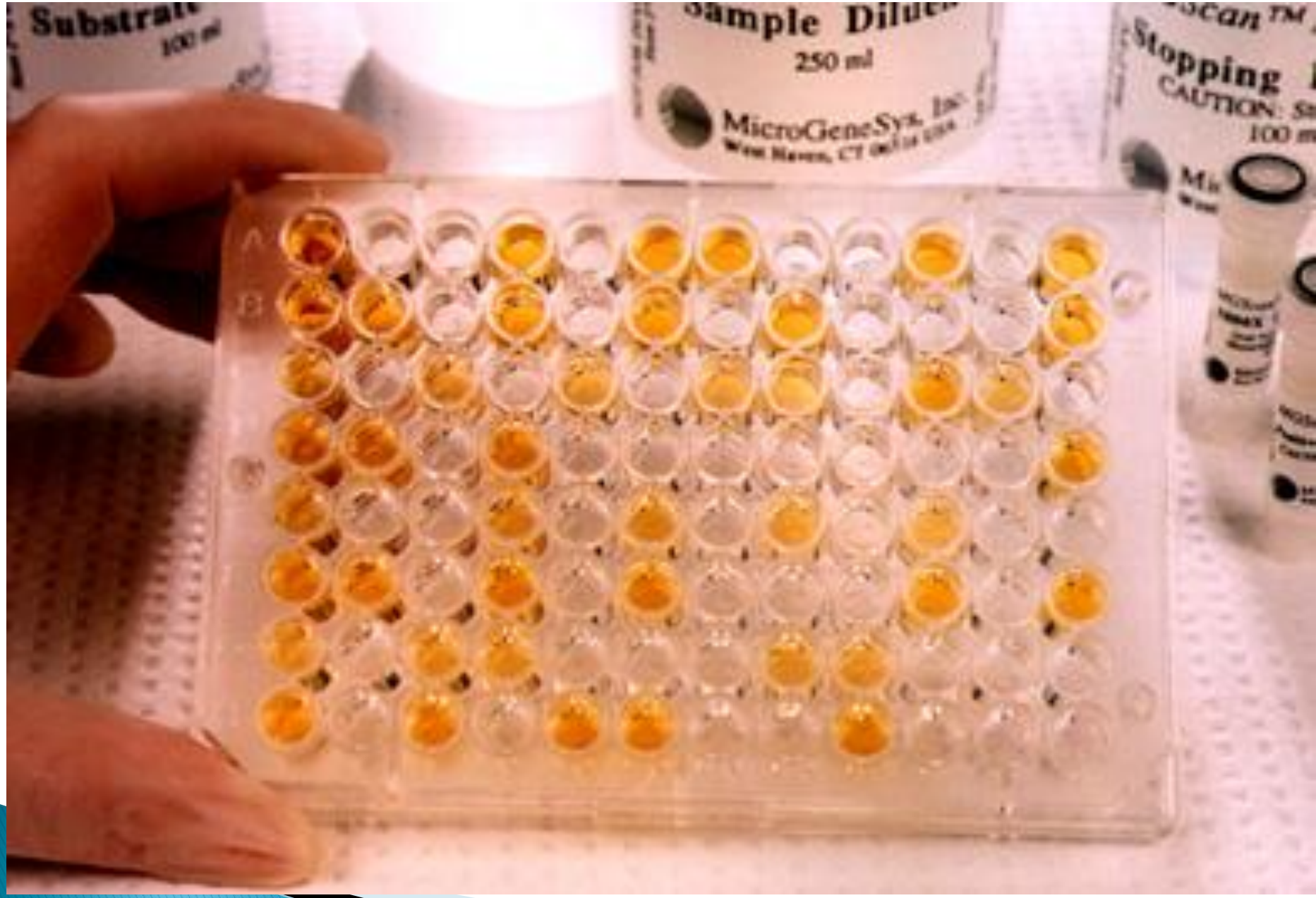
FIGURE 6-10

Variations in the enzyme-linked immunosorbent assay (ELISA) technique, similar to RIA except using an Enzyme (alkaline Ⓟ, horseradish peroxidase, & β-galactosidase) : safer & less costly.

- ELISA tests are commonly performed in Polystyrene tubes (Macro-ELISA) or in polyvinyl mocrotitre plates (Micro ELISA)
- ELISA is usually done in 96 well microtitre plates suitable for automation.
- An ELISA reader provides quantitative colour recordings.

Different HIV test kits available commercially

- **1st generation** - use antigen derived from disruption of viruses grown in human lymphocytes
- **2nd generation** - artificially derived recombinant antigens expressed from bacteria or fungi
- **3rd generation** - chemically synthesized oligopeptide of 15-40 amino acids-synthetic peptide
- **4th generation** - Ag & Abs detected simultaneously. Use a combination of recombinant & synthetic peptides as Ags.


Substrate
100 ml
Sample Dilu
250 ml
MicroGeneSys, Inc.
Stopping
CAUTION:
100 m

ELISA DESIGN

- Can be designed in many ways
- Types
  - **Non-competitive**
    - Direct ELISA
    - Indirect ELISA
    - Sandwich ELISA
  - **Competitive / Inhibition**

Principle of ELISA

- **Indirect-**
- **Competitive-**
- **Sandwitch-**
- **Capture-**
  - All ELISA consist of either antigen or antibody attached on solid phase (matrix or support) and incorporated conjugate or /and substrate detection system
  - Viral antigen may be either whole virus lysate, recombinant antigen or synthetic peptide
  - Matrix can be wells or strips of micro plate plastic beeds or nitro cellulose paper
  - Conjugate are antibodies couple to enzyme –(alkaline phosphates or horse reddish peroxides), fluorochrome or other reagent can be used which can be visualized

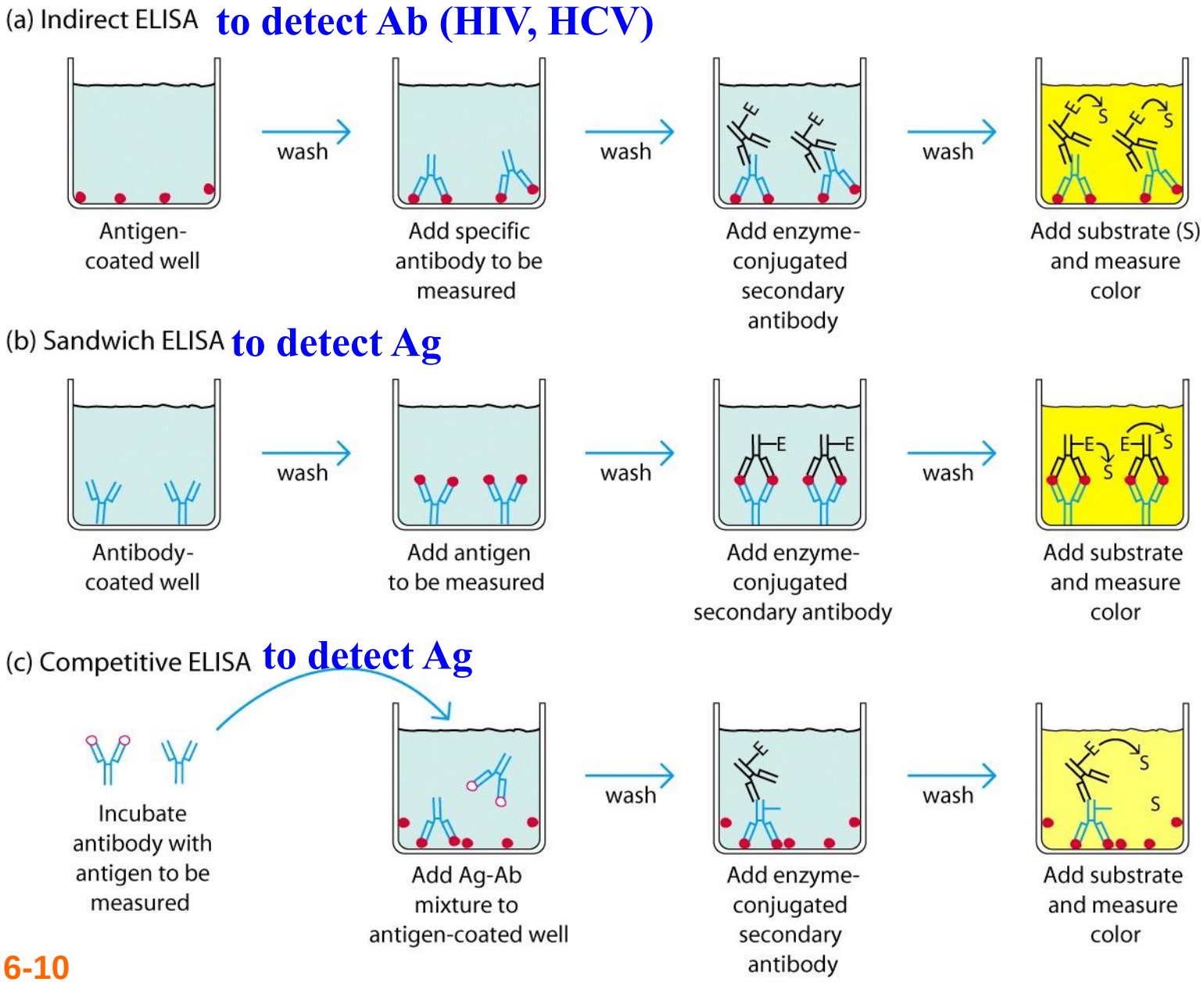
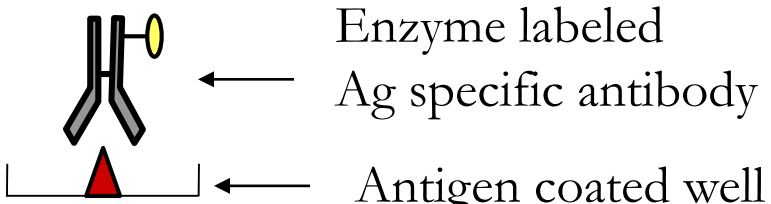


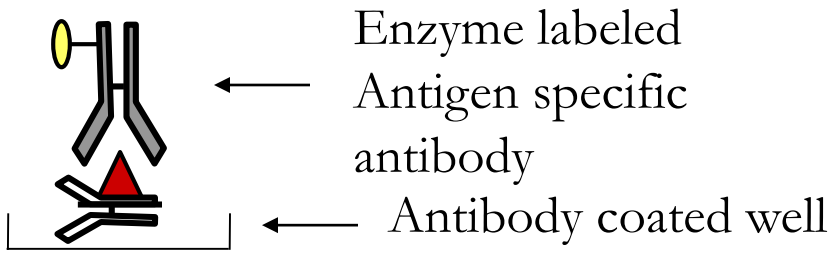
FIGURE 6-10

Variations in the enzyme-linked immunosorbent assay (ELISA) technique, similar to RIA except using an Enzyme (alkaline Ⓟ, horseradish peroxidase, & β-galactosidase) : safer & less costly.

Direct ELISA

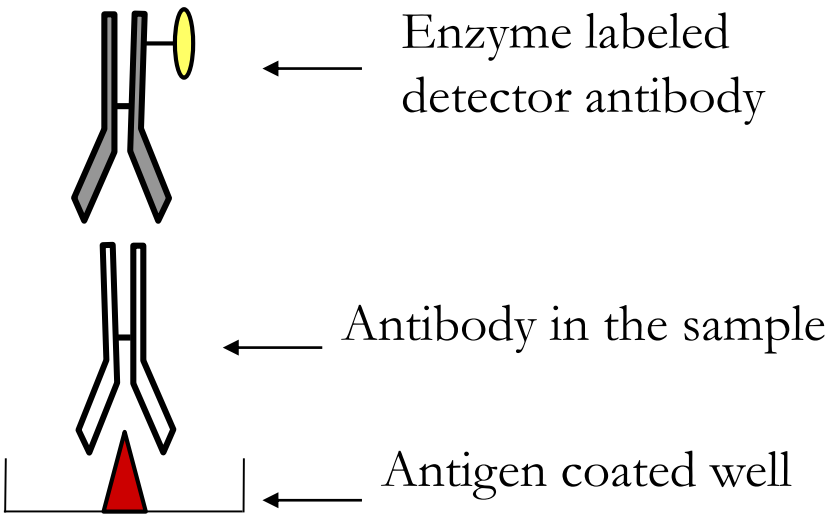


- Useful for antigen detection in culture fluids/ cell extract with virus etc.



- Useful for checking the specificity & optimal dilution of the enzyme - conjugate.

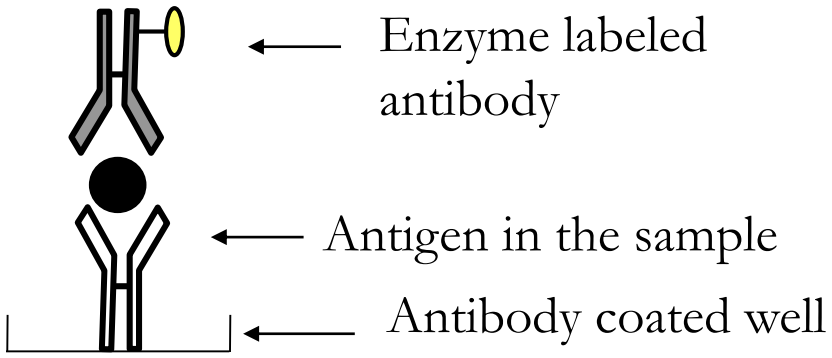
Indirect ELISA



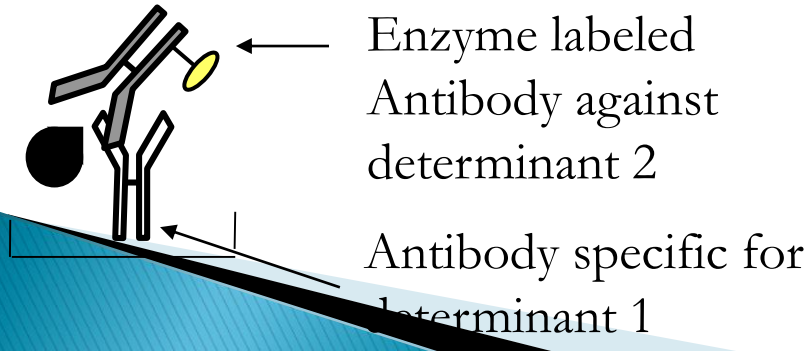
- Routine Screening test for antibodies in test sample

Sandwich ELISA

a) Symmetrical



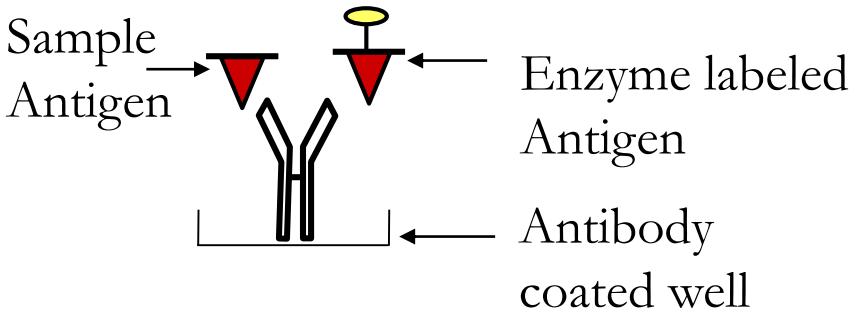
b) Asymmetrical



- Used as a test for antigen
- Symmetrical assays usually use polyclonal antibodies
- In asymmetric assays : Monoclonal capture antibody (high specificity) Polyclonal Detector antibody (high Sensitivity) are used

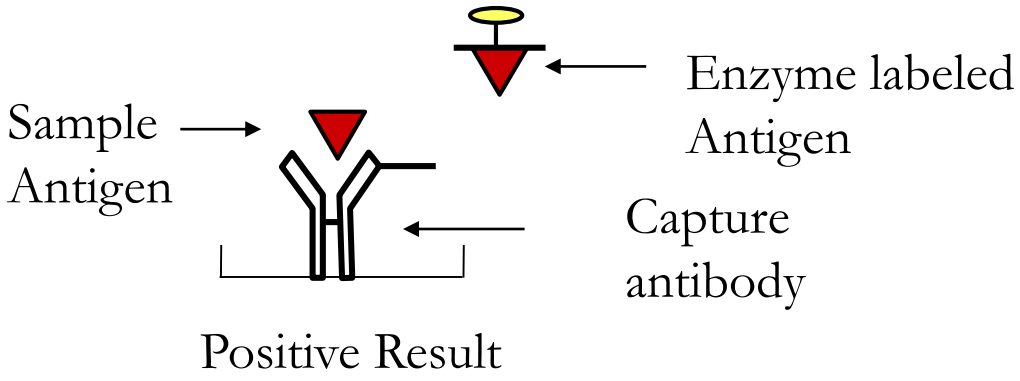
Competitive ELISA

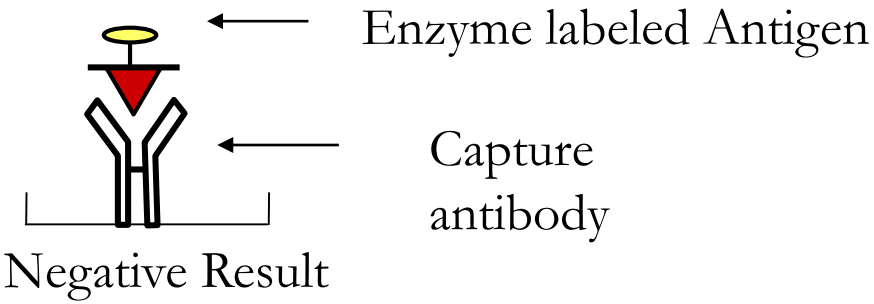
For detection of Antigen



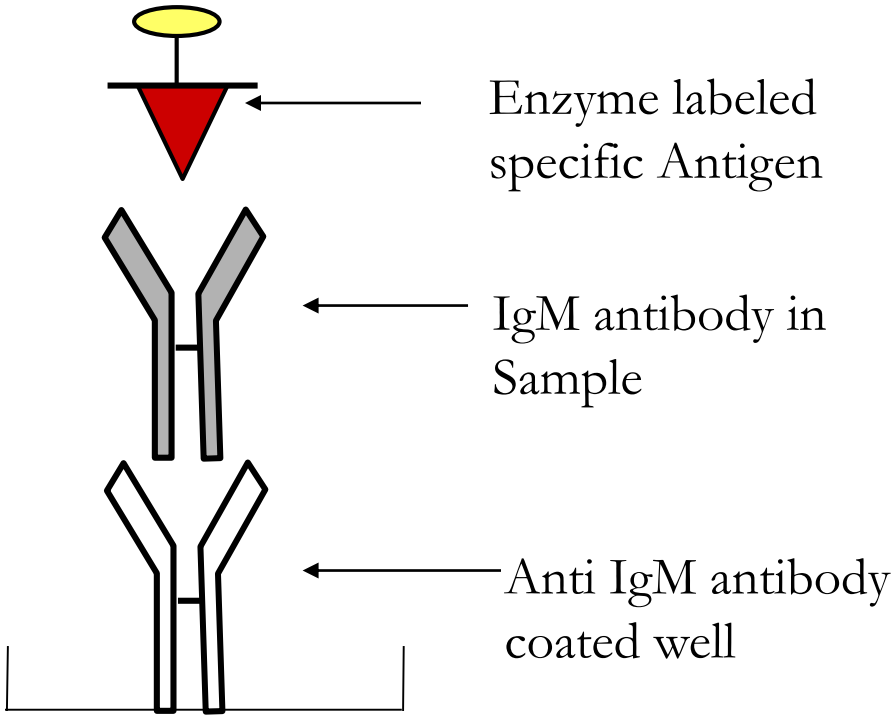
- Based on simple competition with standard labelled antigen for coated antibody. Useful to know the presence of common or distinct antigenic determinants.

Inhibition ELISA
For the detection of antigen





Capture Antibody ELISA
(For specific IgM/IgE antibodies)



- A simple modification of ELISA which has been found for testing one or a few samples of sera at a time is the cylinder or cassette ELISA.
- Each specimen is tested on a separate disposable cassette.
- Test is rapid, taking only 10 minutes
- Result is read visually.
- Inbuilt positive and negative controls are usually provided for validation of the test procedure.

HIV TRI – DOT TEST (continue)

- **INTERPRETATION:**

  – If only one control dot : Non reactive for either to HIV-1 or HIV-2.

  – If two dot, one for control and other for HIV-1 : reactive for HIV-1.

  – If two dot, one for control and other for HIV-2 : reactive for HIV-2.

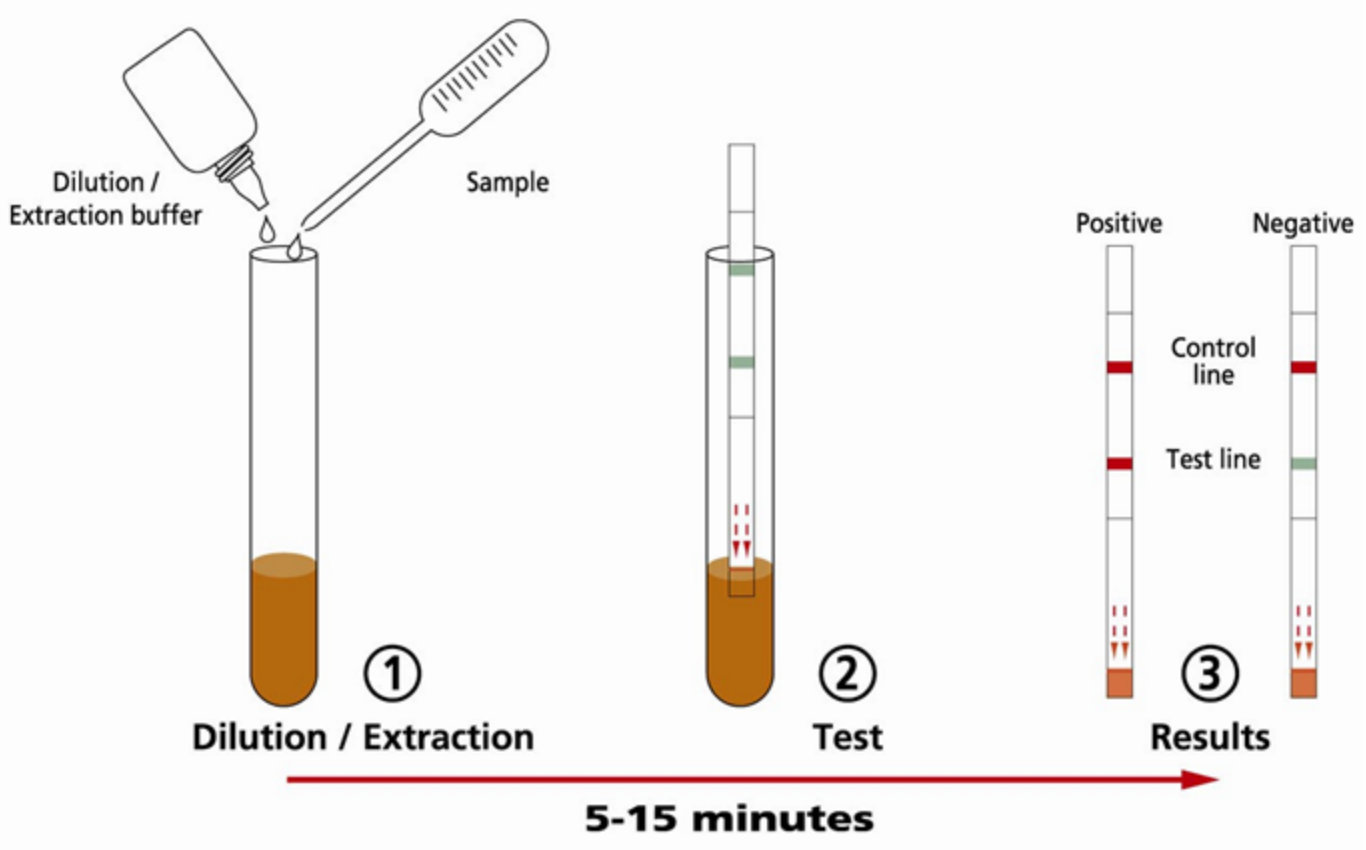
  – If three dots, one for control and others for HIV-1 & HIV-2 : reactive for HIV-1 & HIV-2.

  – If no dot appear : invalid.

Immunochromatographic test

- A one step qualitative immunochromatographic technique
- Simple, economical, reliable.
- The test is claimed to be nearly as sensitive and specific as EIA tests.
- Results within 10 – 15 minutes.

Methodology



Complement Fixation test

Principle :

- Antigen-antibody complexes fixes the complement. The coupling of complement with Ag-Ab complex does not have any visible effect like agglutination or precipitation.

- Therefore an indicator system is necessary consist of sheep red cell coated with anti sheep red cell antibody(ambocepter).

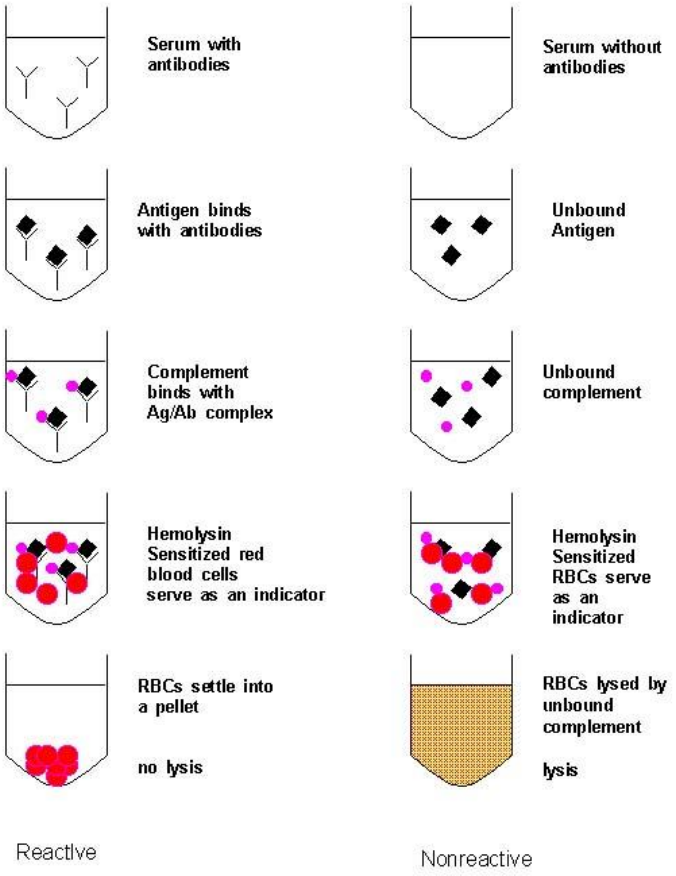
- CFT is a complex procedure consist of two steps and five reagents- antigen, antibody, complement, sheep erythrocytes and amboceptor.
- The use of CFT is considerably reduced with recent serological advances.
- The classical example of Wasserman reaction formerly routine method for the diagnosis of syphilis.

Procedure : two steps

- 1)Test system : Antigen (cardiolipin) and antibody (inactivated patient's serum) are mixed and measured amount of complement is added & incubated at 37° c for one hour. If antigen and antibody match, the complement will be fixed, otherwise it will remain free.

- 2) An indicator system consisting of sensitised red blood cells (Sheep erythrocytes coated with ambocepter) is added to the test mixture and incubated for 30 minutes in water bath at 37° c.

COMPLEMENT FIXATION TEST



Observation :

- Absence of hemolysis indicates that complement was used up during initial reaction.(Positive reaction)
- Presence of hemolysis indicates persistence of complement ,negative result.
- In the presence of appropriate antibodies, complement lyses erythrocytes, kills and lyses bacteria,immobilises motile organisms, immune adherence.

Neutralisation

- When an antitoxin combines with a toxin, the biological effect of the toxin are neutralized .
- Since toxin is an antigen in solution it is also precipitated.

Virus neutralization test

- Neutralization of viruses by their antibodies
- Test serum is mixed with a suspension of infectious virus particles of the same type as that with which the individual is suspected to have been infected.
- Control is put up by mixing normal serum with a suspension of viruses.
- The virus suspension are then inoculated into embryonated egg or tissue culture.

Result :

- Control suspension of virus show evidence of infection.

- Test suspension of virus does not show any evidence of infection as the antibody has neutralized the infectivity of the virus.

Neutralization of bacterial toxin

- Bacterial toxins are good antigens & can induce the production of neutralizing antibody following injection into living animal.

- Toxin-antitoxin neutralization can be measured in vivo and in vitro.

Neutralization test in vivo

Guinea pig inoculation test :

- Homologous antibodies prevent the biological effect of toxin.Toxigenicity test of C diptheriae consist of Intradermal inoculation of bacterial toxin in guinea pig previously protected by ADS. No biological effect of toxin is seen in the controlled animal but unprotected animal dies.
- Schick test is based on the ability of circulating antitoxin to neutralize the diphtheria toxin given intradermally,& indicates immunity or susceptibility to the disease.

- Toxin neutralization in vitro depends on the inhibition of some demonstrable toxic effect.

- Antistreptolysin O test, in which antitoxin present in patient sera neutralizes the hemolytic activity of streptococcal o hemolysin.

THANK YOU