INTRODUCTION TO SEROLOGY

Safety rules:

- Wear lab coat
- It is a must to wear gloves
- Never mouth pipette
- Cover any cuts or burns
- Do not eat or drink in lab
- In case of accident report to instructor
- Avoid hand to face operations
- Wash hands before you leave

What is serology?

- Is a branch of immunology dealing with study of Ag –Ab interactions in Vitro by different serological tests.
- Ag/Ab
- Importance of Lab diagnosis:

1- Save patient's life

2- Prevent spread of disease

3- Treatment therapy

4- Confirm clinical diagnosis

Lab diagnosis of infectious diseases:

1. Isolation and identification of causative agent by:

a. Morphological tests (microscopy)

b. Biochemical reactions

c. Cultural identification

d. Serological reactions

e. Biotechnology: PCR-DNA probe- DNA finger printing

2. Detection of specific Ab in sera of infected .patients using serological techniques

Serological Reactions:

- Primary 1st : It measures the direct interaction between Ag and Ab in Vitro(test tube).

Example: Elisa, IFA, RIA tests.

- Secondary 2nd : It measures the consequences of interaction between Ag and Ab in Vitro.

Example: Agglutination, CFT, Precipitation, Neutralization tests.

- Tertiary 3rd: It measures Ag and Ab interactions in Vivo (in body)

Terms:

- **Validity** : A serological test should provide an indication of which individuals actually have the disease and which do not.
- **Specificity**: ability of a test to identify correctly those who do not have the disease.(have least cross reactivity)
- **Sensitivity**: Ability of a test to identify correctly those who have the disease(can detect v. small amounts)

Example:

Sensitivity : True positive rate of the test(no false –ve)

Specificity: True negative rate of the test (no false +ve)

	Test result positive	Test result negative	Total No of people
Really have AIDS	**99**	**1**	**100**
Do not have AIDS	**199**	**9701**	**9900**
Totals	**298**	**9802**	**10,000**

Sensitivity =99/100 x100 = 99%

Specificity= 9701/9900x100= 98%

- **Quantitative test:**

It measures the amount of Ag or Ab.

- **Qualitative test :**

It detects the presence or absence of Ag or Ab.

- **Seroconversion:**

is development of detectable specific Ab to microorganisms in serum as a result of infection or immunization

- **Sero reversion :**

is the opposite of seroconversion .

This is when the test can no longer detect Ab or Ag in patient's serum

Criteria for Diagnosing:

Primary infection:

- Seroconversion
- Presence of IgM
- 4 fold rise or more in IgG titer

Re-infection:

- Absence to slight increase of IgM
- 4 fold rise increase in IgG

Serum Separation:

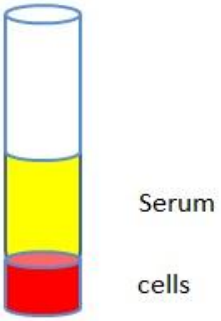
What is serum ?

Serum :Blood- cells and clotting factors

Plasma : blood – cells

Separation:

- Use plain tube (no anticoagulant)
- Leave blood for 1 hour at room temp.
- Separate the clot
- Centrifuge at 3000rpm for 10 min.



Serum preservation:

- Aliquoting : Must aliquot the serum into different tubes to

avoid freezing and thawing

- Keep serum in fridge at $4C^0$ for 1 day
- Keep in freezer at -20 C^0 for more the 1 day.
- Use frozen serum only once, discard after use.

Disposal of serum and contaminated lab ware:

- Dispose used serum tubes, microtiter plates ,universal tubes , bijou bottles in autoclave bags.
- Dispose used serological pipettes, microtiter tips, slides in disinfectant jars.
- Do not throw tissue , gloves, paper in disinfectant jars.

Items:

- Micropipettes

Fixed volume /adjustable volume

Ejectable / non ejectable

Multichannel micropipette

- Microtiter plates

U -bottom

V- bottom

Flat bottom

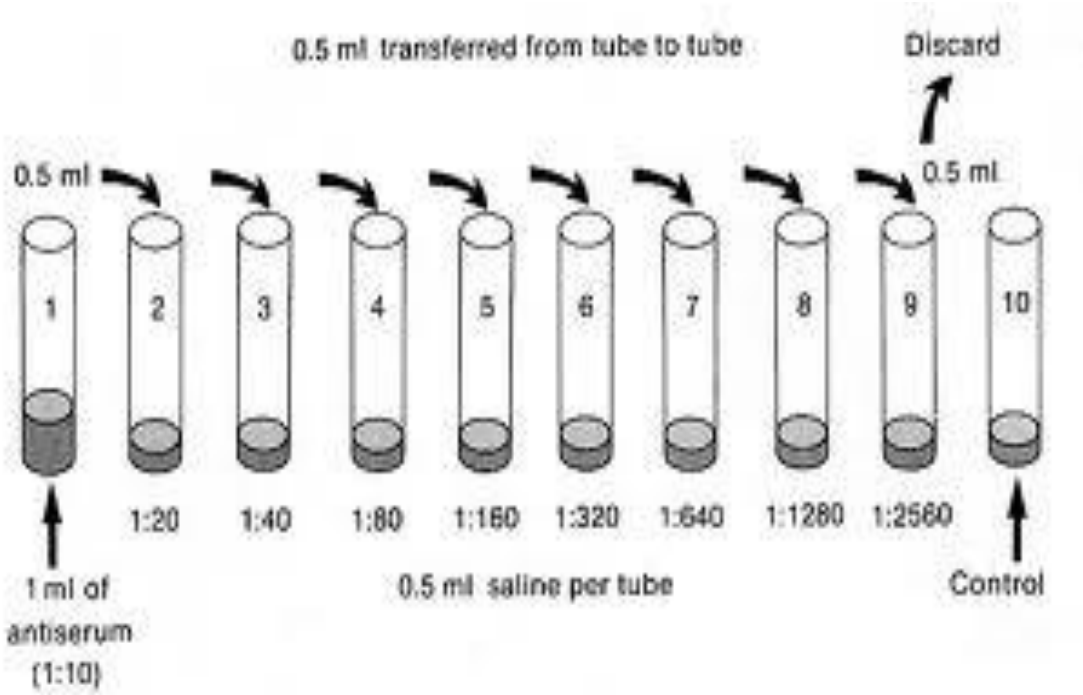
Dilution:

- It is important to dilute patient samples for serological tests
- Dilution= $\frac{\text{serum volume}}{\text{Total volume}}$
- Total dilution = serum volume + diluent volume

  Serum volume=Total volume-diluent volume

- Dilution $= dilution\ of\ preceding\ tube\ x\ \frac{\text{serum volume}}{\text{Total volume}}$

Doubling serial dilution:



Ammonium precipitation method:

Ammonium sulfate precipitation is a method used to purify proteins by altering their solubility. It is a specific case of a more general technique known as salting out .

Since proteins differ markedly in their solubilities at high Ionic strength , salting – out is a very useful procedure to assist in the purification of a given protein. The commonly used salt is ammonium sulfate , as it is very water soluble, and has no adverse effects upon enzyme activity.
It is generally used as a saturated aqueous solution which is diluted to the required concentration, expressed as a percentage concentration of the saturated solution (a 100% solution).

One of the principle decisions to make is the percentage saturation of ammonium sulphate to use . Although many IgG. (gamma – globulins) precipitate at a lower concentration of ammonium sulphate than most other proteins. It is difficult to be more precise as many factors such as the properties of other proteins present in the sample can influence the ammonium sulphate concentration required to precipitate your target protein.

Procedure:

1- Dissolve in 315 g ammonium sulphate per liter of serum or sample , while stirring. This gives 50% saturation.

2-After all the ammonium sulphate has dissolved, stir for another 2 hours to allow complete equilibration between the dissolved and aggregated proteins.

3-Centrifuge the sample at 10,000 rpm for minutes to pellet the precipitated pr otein containing your target antibody.

4-Decant the supernatant and dissolve the precipitate in a small volume of suitable buffer . A volume of buffer twice that of the volume of the precipitate sufficient .

5-The dissolved precipitate contains a lot of ammonium sulphate. This must be removed, either dialyse against a suitable buffer or use gel filtration- de-salting columns.