Laboratory Immunological Tests

The Immune System:-

Immunology is the study of molecules, cells, and organs that make up the immune system. The function of the immune system is to recognize self antigens from non-self antigens and defend the body against non-self (foreign) agents. Through specific and non-specific defense mechanisms, the body's immune system is able to react to microbial pathogens and protect against disease. The first line of defense against infection is intact skin, mucosal membrane surfaces, and secretions that prevent pathogens from penetrating into the body.

When a foreign agent penetrates the first line of resistance, an immune reaction is elicited and immune cells are recruited into the site of infection to clear microorganisms and damaged cells by phagocytosis. If the inflammation remains aggravated, antibody-mediated immune reaction is activated and different types of immune cells are engaged to resolve the disease. The immune system is composed of cellular and humoral elements. The cellular component includes mast cells, neutrophils, macrophages, T and B lymphocytes, and plasma cells. The humoral component includes complement, lyzozyme, interferon, antibodies, and cytokines. All work cooperatively to eliminate immunogenic foreign substances from the body.

Immunoassays

To aid in the diagnosis of disease caused by infectious microorganisms, immunoassays have been developed. These biochemical and serological techniques are based on the detection and quantitation of antibodies generated against an infectious agent, a microbe, or non-microbial antigen.

Because antibodies can be produced against any type of macromolecule, antibody-based techniques are useful in identifying molecules in solution or in cells. A blood

sample is collected from the patient during the acute phase of the disease when antibody levels are high. Serum is then isolated and the concentration of antibodies is measured through various methods. Most assays rely on the formation of large immune complexes when an antibody binds to a specific antigen which can be detected in solution or in gels. Recent methods employ pure antibodies or antigens that have been immobilized on a platform and that can be measured using an indicator molecule. These methods provide high sensitivity and specificity and have become standard techniques in diagnostic immunology.

Serology is the study of blood serum and other bodily fluids for the identification of antibodies.

Serology is the branch of immunology that studies the antigen — antibody reaction in vitro.

There are many types of immunological test -

1-Agglutination test.
2- Precipitation test
3-Complement test.
4-Immunoflorescence test.
5-Enzyme-linked ImmunosorbantAssay(ELISA) test.
6- Neutralization test.
7- Haemagglutination test &haemgglutination inhibition test.

1-<u>Agglutination test</u>:-

Agglutination tests are based on the presence of agglutinating antibodies in patient sera that can react with specific antigens to form visible clumps. In the agglutination tests, the antibody - antigen reaction can be either a direct or passive agglutination reaction.

Agglutination is commonly used as a method of identifying specific bacterial antigens, and in turn, the identity of such bacteria. Because the clumping reaction occurs quickly and is easy to produce, agglutination is an important technique in the diagnosis of infections, the identification of microorganisms, and in blood typing.

The difference between agglutination and coagulation, Agglutination means clumping of RBCs together due to antigen-antibody reaction.

Blood coagulation, or clotting, is the process of converting blood into a semisolid jelly-like substance , forming a Blood clot starts when there is an injury to the body.

Agglutination reactions are classified as :- direct, indirect (passive) and reverse passive agglutination reactions.

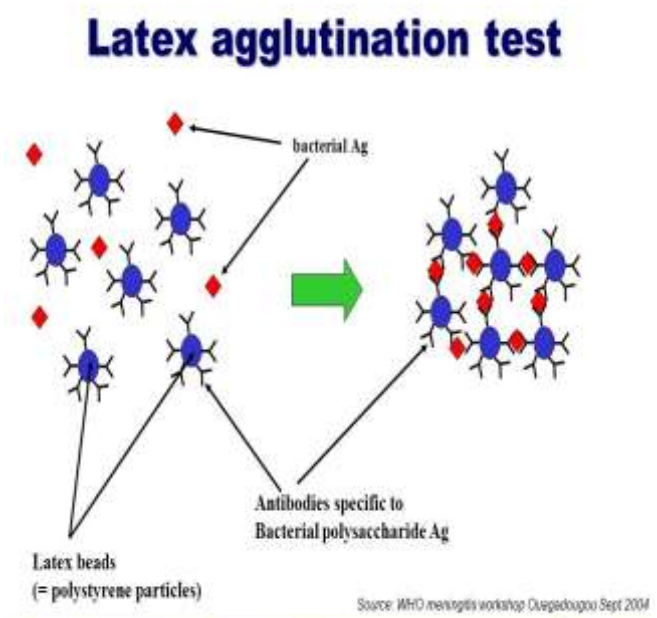
Direct Agglutination Test refers to the assays in which the antigen directly agglutinates with the antibody.

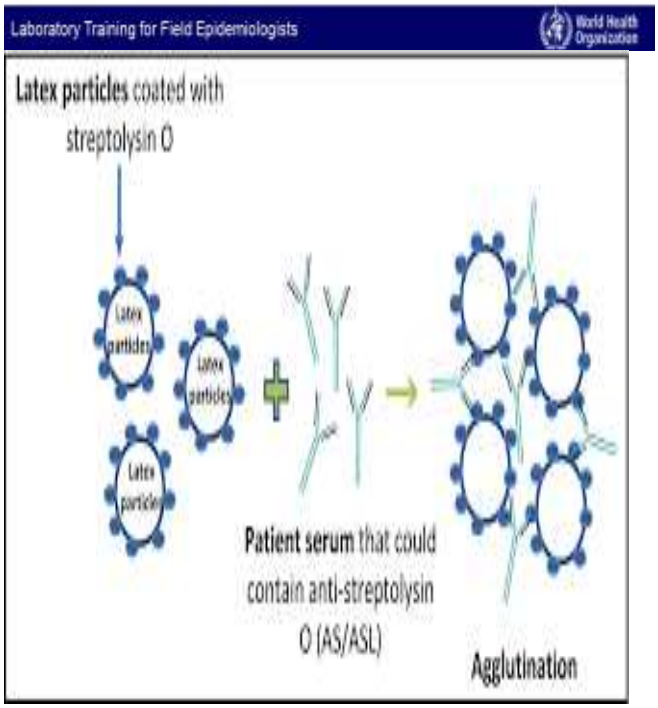
Indirect or passive agglutination involves coating of antigen on the surface of a carrier molecule (e.g. RBC, latex), such that the antibody binds to the coated antigen and agglutination takes place on the surface of the carrier molecule. They are also referred to as ‘ particle agglutination test.’

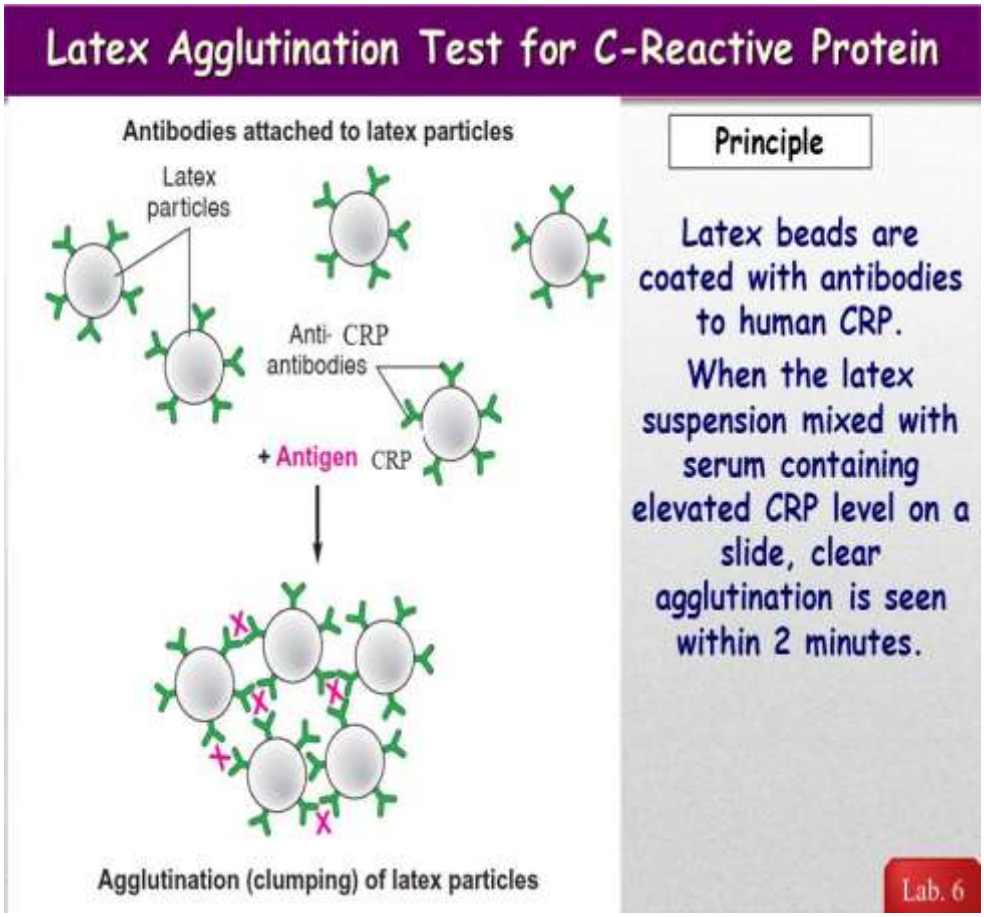
Reverse passive agglutination test is a special type of particle agglutination test in which the antibody is coated on a carrier molecule which detects antigen in the patient’s serum.

Latex Agglutination Test (LAT) for Antibody Detection: A Passive Agglutination Test with Antigen bound to the surface of latex beads (particles).

Latex Agglutination Test (LAT) for Antigen Detection: A Reverse Passive Agglutination Test with Antibody bound to the surface of latex beads.


Latex agglutination test
bacterial Ag
Antibodies specific to
Bacterial polysaccharide Ag
Latex beads
(= polystyrene particles)
Source: WHO meningitis workshop Ouagadougou Sept 2004


Laboratory Training for Field Epidemiologists
World Health Organization
Latex particles coated with streptolysin O
Latex particles
Latex particles
Latex particles
Patient serum that could contain anti-streptolysin O (AS/ASL)
Agglutination



The application of agglutination test in clinical medicine :-

1- In determine a person's ABO blood group for transfusion.

2- To detect the presence relative amount of specific Ab in patients serum.

3- widely used for rapid diagnosis of several disease such as: -

a- widal test — typhoid fever (salmonellosis)

b- Rose bengal — Malta fever (Brucellosis)

c- VDRL (veniral Disease References Lab) for syphilis (*Treponemapallidum)*

d- ASOT (Anti — streptolysin O test).

❖ **Widal Test:-**

The **Widal test** is one method that may be **used** to help make a presumptive diagnosis of enteric fever, also known as typhoid fever.

Typhoid is a bacterial infection that can lead to a high fever, diarrhea, and vomiting. It can be fatal. It is caused by the bacteria **Salmonella typhi**. The infection is often passed on through contaminated food and drinking water, and it is more prevalent in places where handwashing is less frequent.

- Widal Test is an agglutination test which detects the presence of serum agglutinins (H and O) in patients serum with typhoid and paratyphoid fever.
- When facilities for culturing are not available, the Widal test is the reliable and can be of value in the diagnosis of typhoid fevers in endemic areas.
- The patient's serum is tested for O and H antibodies (agglutinins).

Salmonella antibody starts appearing in serum at the end of first week and rise sharply during the 3rd week of endemic fever. In acute typhoid fever, O agglutinins can usually be detected 6–8 days after the onset of fever and H agglutinins after 10–12 days.

The idea is to detect the antibodies to salmonella in patient's serum.This test demonstrates the presence of somatic (O) and flagellar (H) agglutinins to Salmonella typhi in the patient's serum using suspensions of O and H antigens.Antigens of S. paratyphi A, S. paratyphi B, S. paratyphi C are included in most commercial kits.We can do by using :Slide agglutination method.Tube agglutination method.

Slide agglutination method:

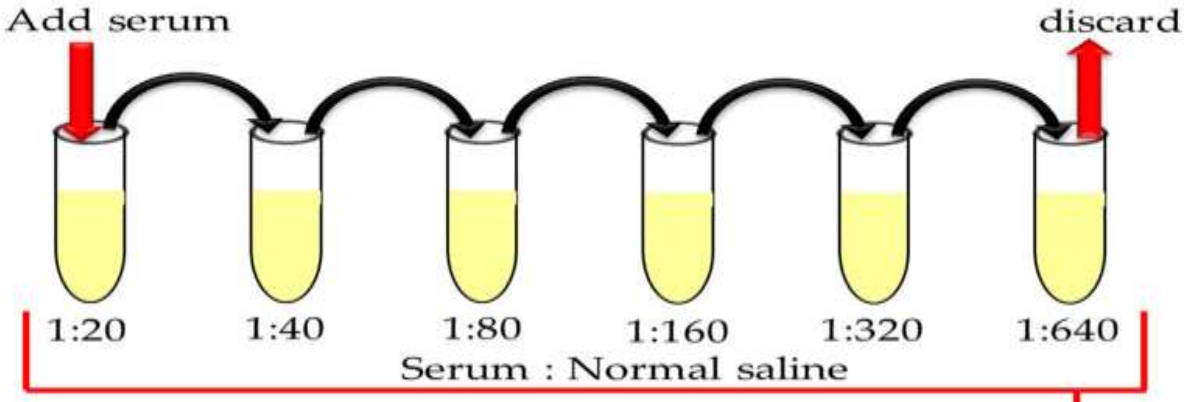
Slide Widal test is more popular among diagnostic laboratories as it gives rapid results. Qualitative test:Dispense 0.08 of undiluted patient serum sample onto a raw of circles.Add one drop of each O or H antigen suspension to the serum (shake

the reagent bottle before use) .Mix well using a stirring stick gently rotate the slide for one minute.Appearance of agglutination gives qualitative positive results.

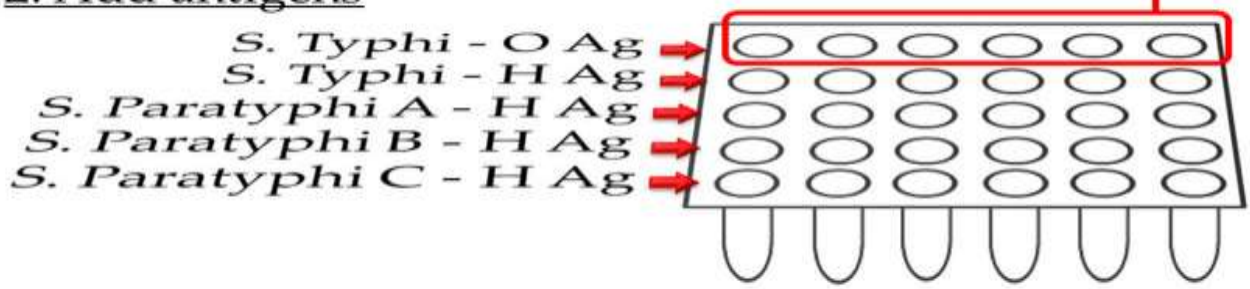
Tube agglutination method:

- Take 4 sets of 8 Kahn tubes/test tubes and label them 1 to 8 for O, H, AH and BH antibody detection.
- Pipette into the tube No.1 of all sets 1.9 ml of isotonic saline.
- To each of the remaining tubes (2 to 8) add 1.0 ml of isotonic saline.
- To the tube No.1 tube in each row add 0.1 ml of the serum sample to be tested and mix well.
- Transfer 1.0 ml of the diluted serum from tube no.1 to tube no.2 and mix well.
- Transfer 1.0 ml of the diluted sample from tube no.2 to tube no.3 and mix well. Continue this serial dilution till tube no.7 in each set.
- Discard 1.0 ml of the diluted serum from tube No.7 of each set.
- Tube No.8 in all the sets, serves as a saline control. Now the dilution of the serum sample achieved in each set is as follows: Tube No. : 1 2 3 4 5 6 7 8 (control) Dilutions 1:20 1:40 1:80 1:160 1:320 1:640 1:1280.
- To all the tubes (1 to 8) of each set add one drop of the respective WIDALTEST antigen suspension (O, H, AH and BH) from the reagent vials and mix well.

1. Serial dilution

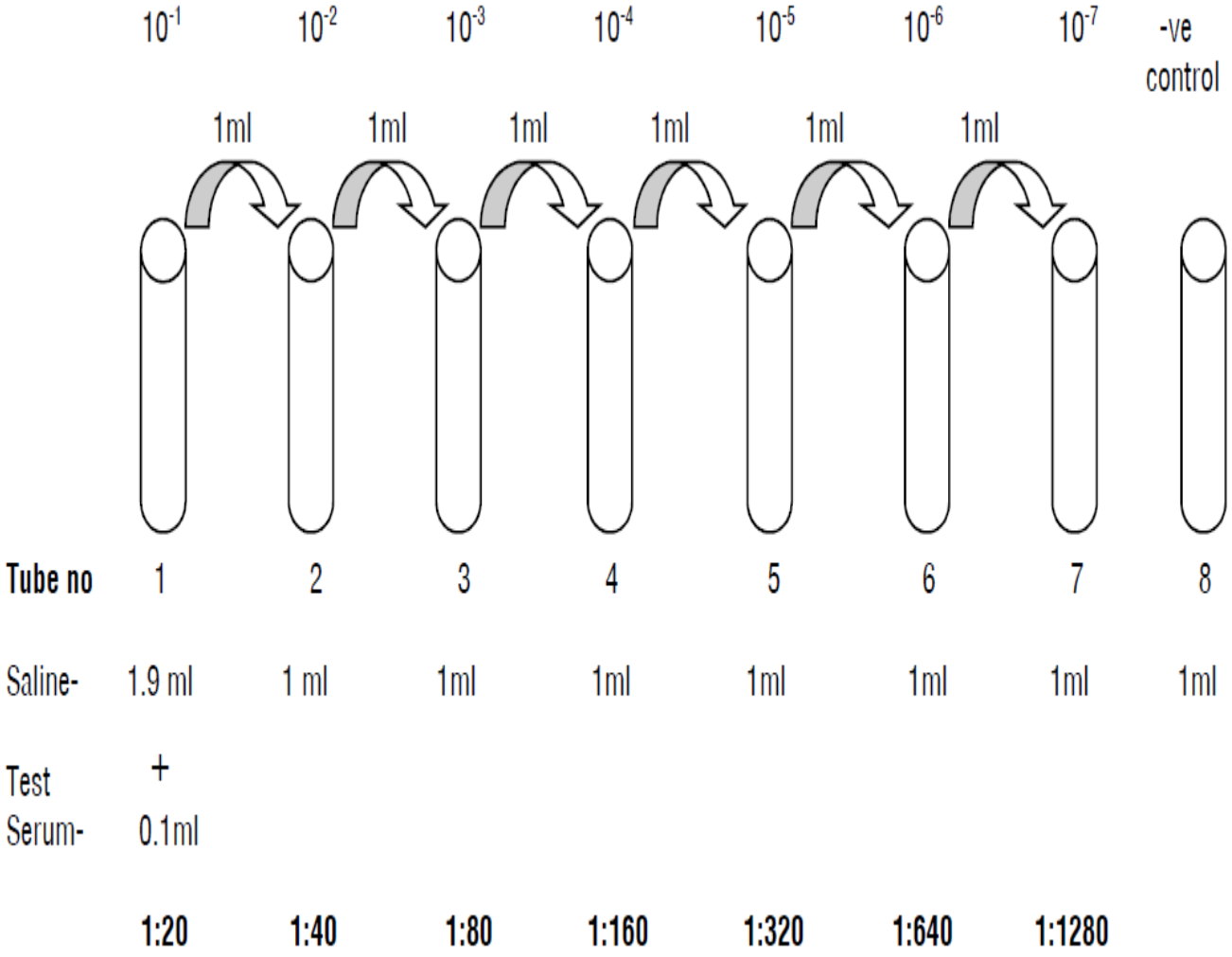


2. Add antigens



3. Mix properly, cover and incubate at 37° C overnight

4. Observe for agglutination and interpret the results



Result interpretation of Widal test:

Antibody titre greater than 1 : 80 is considered significant and usually suggests positive test for Salmonella infection.

The Widal test is positive

if "O" antigen titer is >1:160 = active infection.

If the "H" antigen titer is >1:160, it indicates past infection or in immunized persons.

The Widal test is an old serologic assay for detecting IgM and IgG antibodies to the O and H antigens of Salmonella. The test is unreliable, but is widely used in

developing countries because of its low cost. Newer serologic assays are somewhat more sensitive and specific than the Widal test, but are infrequently available.

When studying agglutination, there are a few terms that should be reviewed and understood:

- **Prozone** - excess antibody to the available amount of antigen (no agglutination is a result)
- **Zone of Equivalence** - optimal amounts of both antibody and antigen (results in agglutination)
- **Postzone** - excess antigen to the available antibody (no agglutination is a result)

The curve in the graph to the right depicts the formation of immune complexes as antibody concentration (y-axis) and antigen availability (x-axis) work in conjunction to form visible agglutination.