

Practical Microbiology
Lab (1) . Second course

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Serology Test(Antigen and Antibody Detection test)

A laboratory test that checks for the presence of antibodies or other substances in a blood sample.

Antibodies are proteins made by the body's immune system in response to a foreign substance or microorganism, such as a virus. Serology tests look for certain antibodies to see whether a person has been exposed to or infected with a virus or other infectious agent.

Sometimes antibodies are made against the body's own tissues. In that case, serology tests are used to look for those antibodies and help diagnose certain immune system disorders, such as autoimmune disorders and immunodeficiency disorders.

Serological tests have a long history and have been used successfully for the diagnosis of many infectious diseases (e.g., **HIV, syphilis, and viral hepatitis**).

When A person is exposed to foreign macromolecules called antigens (Ag) , the immunologic system produces proteins called antibodies (Ab) which react specifically with the antigens responsible for their synthesis .

Blood serum that contains the specific antibody called antiserum . Serology is the study of antigen- antibody reaction in vitro.

Serological reactions provide methods for the diagnosis of disease and for the identification and quantitation of antigens (bacteria and proteins) and antibodies.

Antigen-antibody interactions are affected by various environmental factors, such as PH , salt concentration and temperature . The forces that hold antigen-antibody complexes together are their ionic attractions.

Serological Tests Procedure involving Direct Determination of Antigen-Antibody Reactions: include the following test

1.Agglutination Test:

These test is used to detect antibodies (agglutinins) and identify cellular antigens (insoluble antigens) . When cells interact with the antibodies clumps aggregate into masses visible with the naked eye.

When antibodies combines with bacteria in vivo opsonization occurs .

Antibodies , have at least two combing sites (bivalent) and antigens have multiple sites result in antigen-antibody lattice formation that can build up into large complexes. Contain two types direct and indirect(fig.1 and 2).

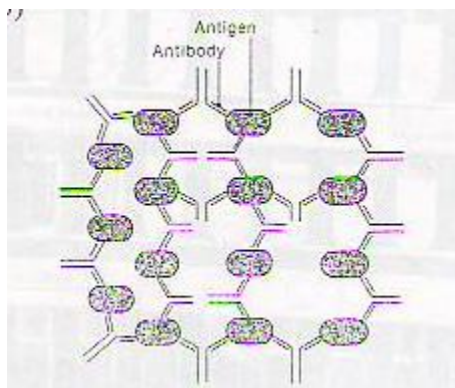


Figure (1) Antigen- Antibody

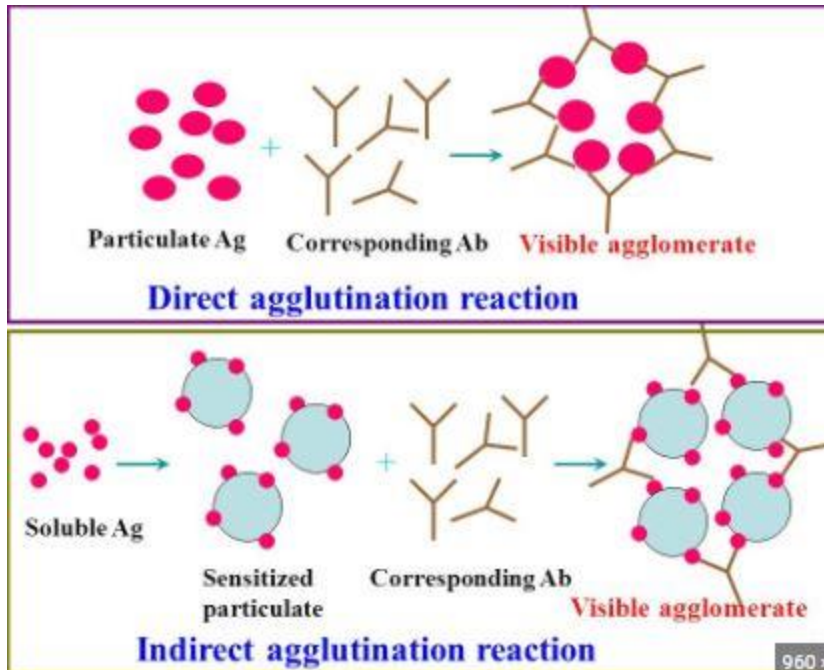


Fig .2(Agglutination Test)

Examples on agglutination tests as following:

A. *Widal test* :

A reaction involving the agglutination of typhoid bacilli (Salmonella bacteria) with serum (containing typhoid antibodies), for patient having typhoid fever .see figure 3



Fig.(3) : Widal Test

B. VDRT test (*Venereal Disease Research Laboratory*)

In this , agglutination occur between syphilitic regain (Ab) and a particulate suspension of sterols coated with the cardiolipin hapten (Ag).(Figure- 4)

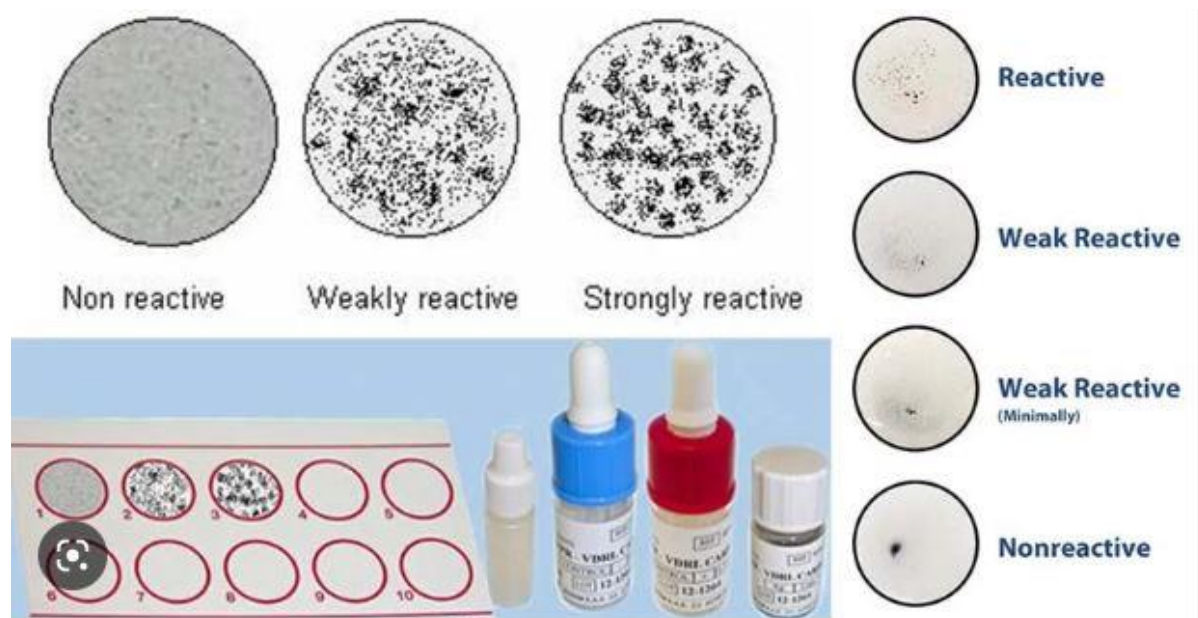


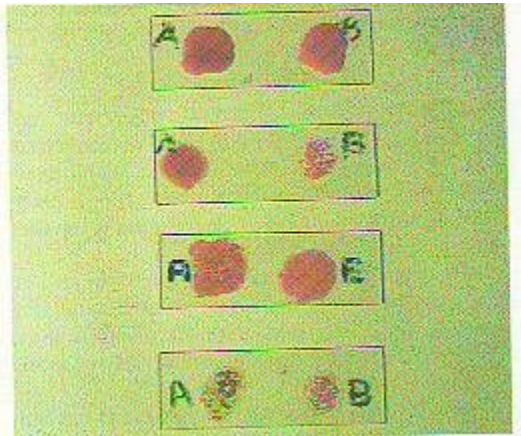
Fig.4; VDRT Test

c-.Hemagglutination : (Blood Groups) (fig. 5)

Is the agglutination of red blood cells by antibodies individuals with blood types A,B, or AB have antigens on the surface of their red blood cells .

An individual having blood type – O lacks these antigens .

These surface antigens are referred as agglutinogens (isoantigens), and their antibodies called agglutinins (isoantibodies).













ABO BLOOD GROUP SYSTEM				
GROUP	A	B	AB	O
RED BLOOD CELL TYPE				
ANTIGENS PRESENT	 Antigen A	 Antigen B	 Antigen AB	✗ None
ANTIBODIES PRESENT	 Anti - B	 Anti - A	✗ None	 Anti - A + Anti - B

Figure . (5): Blood Grouping

Agglutination reactions are determined by two ways :

➤ **Microscopic- slide method : (fig.6)**

This is a screening test to determine if Ab present in the serum.

Procedure

Take glass slide and divide it in half with a wax pencil –label the left side A and the right side B.

To area A, add 1 drop of antigen suspension with Pasteur pipette and , with other pipette add 1 drop of antiserum . Mix gently with a toothpick-Discard the toothpick.

To area B, add 1 drop of antigen suspension and 1 drop of normal serum . Mix gently with a toothpick.

Observe area B as a control to show no agglutination , should be observed within 5 minutes .

Report the result .

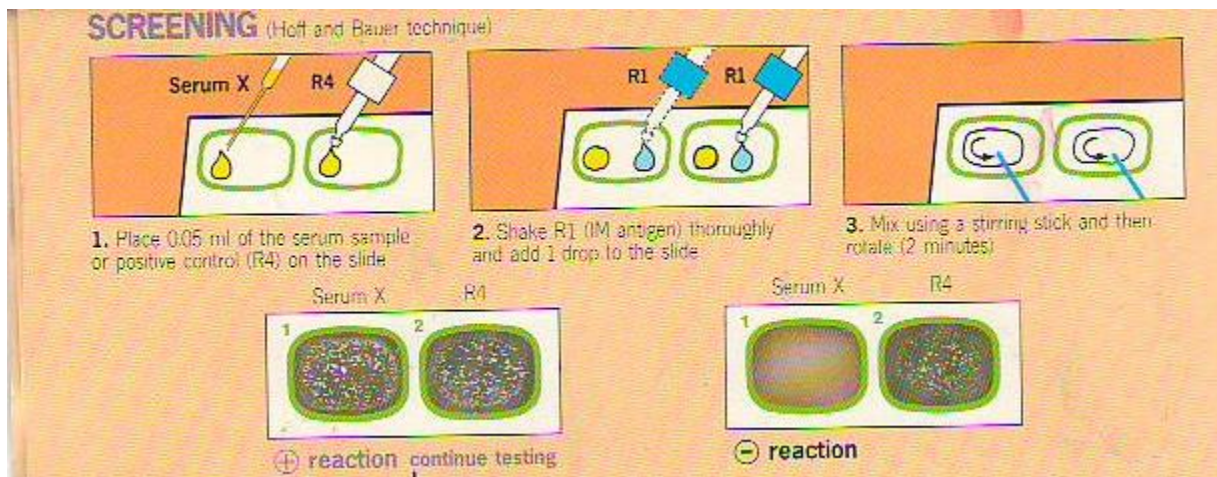


Figure (6) : Slide Agglutination Test

➤ **Tube Agglutination Methode :(Determination of Titer) (fig.7)**

This method employed in identifying bacteria and designed to estimate the amount of antibody (titer) present in the serum .

The titer of the antiserum is an indication of its antibody level . It is defined as the reciprocal of the last or highest dilution showing agglutination .

Procedure:(e.g. widal tube agglutination test for typhoid fever).

- Label nine clean test tubes with the proper dilutions place the tubes in a test tube rack .
- Pipette 0.9 ml of 0.85 % saline into tube no. 1 and 0.5 ml into the other eight tubes.
- Add 0.1 ml of salmonella antiserum to tube no. 1 mix by drawing the solution and blowing it out . Do not form bubbles . Do this many times.
- Transfer 0.5 ml with the same pipette to the second tube . Mix and continue transferring 0.5 ml aliquots through tube no. 8.
- Add 0.5 ml of salmonella O- antigen or heated **S. typhi** culture to each dilution tube (no.2-9). Mix by gently shaking the test tube rack to agitate all tubes .
- Incubate the tubes in a 37°C water bath for 1 hour.
- Check tube no. 9 (control) for nonspecific agglutination, then check tubes no. 1-8 for specific agglutination . Observe the bottom of each tube for agglutination if the tube shows agglutination, observes a compact mass on the bottom with a clear supernate . Controls are very important , because some bacterial suspensions agglutinate spontaneously.

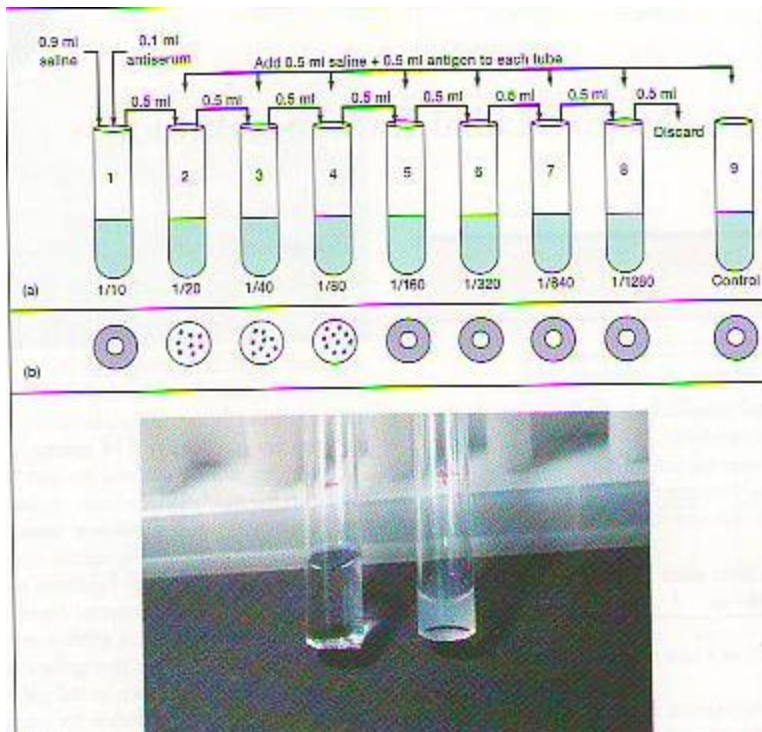
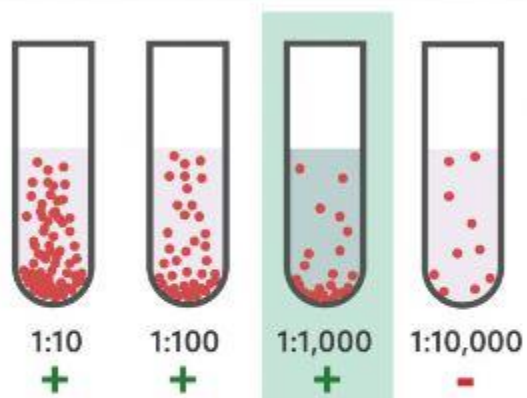


Figure (7) : Titer Determination



After 4 dilutions, 3 produce positive test reactions, the 4th does not. The titer is the highest dilution capable of producing a positive test result (the highlighted vial).

The End