
Agglutination Reactions

2- widal Test

Widal test is a serological test widely used for diagnosis of enteric fever. This test was introduced by Widal in 1896, for diagnosis of typhoid and paratyphoid fever caused by *Salmonella* species, the infection occurs by ingestion of contaminated water and food.

Salmonella

-non capsulated, it is 'motile 'Gram negative bacilli ' Short bacilli having 1400 serotypes. (Figure 1).

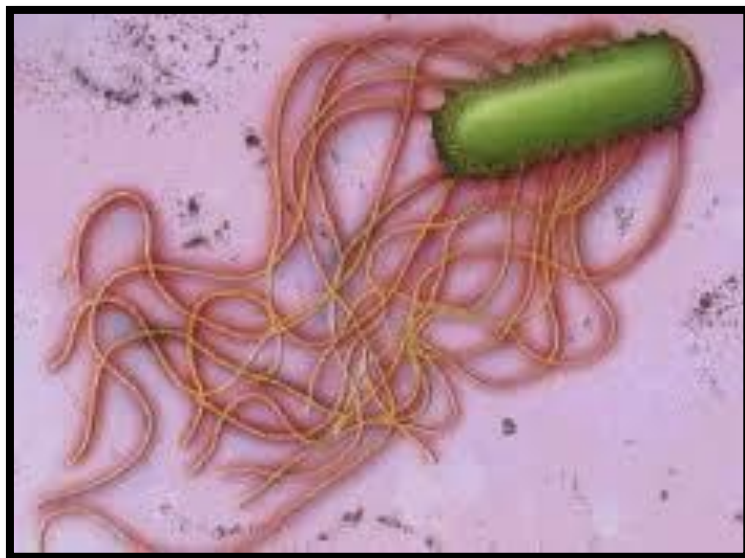


Figure: (1) salmonella

Salmonella Species

- **Salmonella Typhi (enteric fever).**
- **Salmonella Paratyphi (A,B,C) (enteric fever).**
- **Salmonella Typhimurium (food poisoning).**
- **Salmonella Enteritidis (enterocolitis).**
- **Salmonella Choleraesuis (septicaemia).**

Principal of Widal test:

• Widal test for typhoid and paratyphoid fever is an agglutination test. The typhoid bacillus causes two types of agglutinins to be produced. The agglutinins are called:

- Flagella (H) agglutinins.
- Somatic (O) agglutinins.

• The patient serum is tested for those O and H antibodies against the antigen suspensions.

Widal Test has Antigen include:

1- Salmonella Typhi (O) Antigen.
Salmonella Typhi(H) Antigen.

2- Salmonella Paratyphi A.....(O) Antigen.
Salmonella Paratyphi A(H) Antigen.

3- Salmonella Paratyphi B.....(O) Antigen.
Salmonella Paratyphi B(H) Antigen.

4- Salmonella Paratyphi C.....(O) Antigen.
Salmonella Paratyphi C(H) Antigen.

Methods of Widal tests: Slide and Tube method.

Procedures Slide method

•Specimen: serum/plasma

- Take clean slide
- Add a drop of serum, which is obtained, from non hemolyzed blood.
- Add a drop of antigen suspension.
- Mix well antigen suspension and serum for (24 min).
- Look for agglutination. (Figure 2 A).

Tube method Used to confirm slide test method.

-Modification of tube test method by Welch and Mickle at 1936.

•Procedure

•Sample: serum/plasma

- For each antigen arrange 10 small test tubes in a rack.
- Place 0.9 ml of saline in the 1st tube and 0.5ml in the remaining 9 tubes.
- Add 0.1ml of fresh cell free serum to the 1st tube.
- Mix and transfer 0.5 ml to tube 2,3,4,5,6,7,8, and tube 9.
- From tube 9 discard 0.5 ml.
- Tube 10 will contain only saline and will serve as a negative control.
- Mix antigens well and add 0.5 ml to each tube. Mix by gently shaking the tubes.
- the final dilutions are :1:20,1:40,1:80,1:160 .ect and Incubate at 37 c for 24 hrs.

- Read the negative control at the end of the incubation period. It should have no agglutination. Read the test one row of antigen at a time. For reading a white light shining vertically above the tube is best and using a black background.
- shake the tubes gently . The H type of agglutination is easily broken up and may be missed . The agglutination is more granular

- Report the highest dilution with definite clump.

Reporting of Widal Reactions

•The Widal test is reported by giving the titer for both O and H antibodies. The antibody titer is taken as the highest dilution of serum in which agglutination occurs. The type of agglutination seen with O reactions is granular while that seen with H reactions. Both slide and tube tests are more easily read against a dark background.

If no agglutination occurs the test should be reported as:

S. typhi O titer less than in 20 (O 1:20).

S. typhi H titer less than in 20 (H 1:20).

Interpretation of the Widal Reaction

•A Negative test does not necessarily mean the patient is not infected. Reaction occurs in infected patients about 50% during the 1st week, 80% in the 2nd week, 90-95% in the 3rd or 4th week.

-Positive reactions with O antigen occur earlier in the disease than the reaction with the H antigen. H antigen reactions may remain sometimes for years.

-A positive reaction occurs after typhoid vaccination and lasts for 1-5 years. In endemic regions, natural agglutinins may be present in the serum.

Some persons will not produce antibodies because of other diseases. E.g. A gammaglobulinemia (absence of globulin in the Leukemia, and carcinoma (malignant tumor)

Taking antibiotics may cause a decrease in the titer.

Causes of positive widal result

-Chronic salmonellosis, associated with Schistosomiasis infection.

-Vaccination with typhoid vaccine

-Cross reaction with non typhoidal Salmonella.

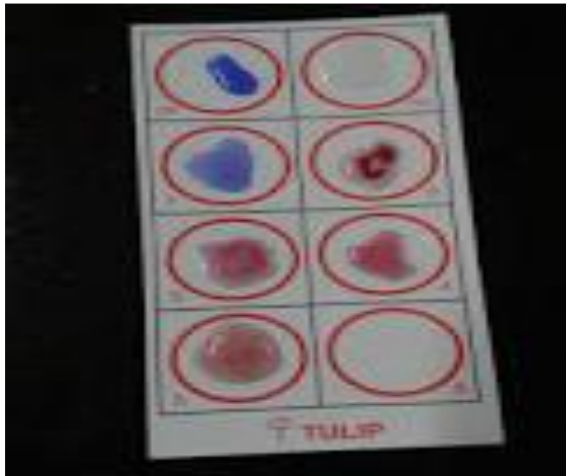
-infection with malaria or other enterobacteriaceae

-other diseases such as Chronic liver disease.

-Immunological disorder like rheumatic fever, and multiple myeloma.



(Figure 2 A): Widal tests kit



(Figure 2 B): Widal tests

3-pregnancy Test:

- It is simply a test that detects the presence of human chorionic gonadotrophin (HCG), a hormone which is present in the blood and urine of a pregnant woman and that is what is tested for in all pregnancy tests.

An indirect agglutination test detects the presence of HCG by reacting the serum or urine of the patient with anti HCG coated latex particles. As the HCG reacts with anti HCG coated latex particles, they form so many complexes and these complexes will form a lattice producing visible agglutination. It is "indirect" because the particles agglutinated, which in this case are the latex particles, are not the antibodies/antigens themselves, they only serve as "carriers" in order for the reaction to be visualized.

I don't think there is a direct agglutination test for pregnancy because in a direct agglutination test, the antigen is directly agglutinated by the antibody and since HCG is not a solid particle, this is not possible.

•Test contents:

- Instruction leaflet
- pregnancy test card
- anti HCG
- Plastic stike

Procedure of pregnancy Test:

1. Perform test at room temperature
- 2 . Add anti- HCG and serum or urine of a pregnant woman on the circle on card.
- 3 .Mix for 2 min.
- 4 .As soon as you have finished read and record the results .

Result of the test:

Presence of Agglutination → Positive . Figure (1)

No Agglutination→ Negative. Figure (1)

All pregnancy tests are based on the detection of HCG ,which is secreted by the placenta trophoblast after the fertilization of the ovum. HCG will appear in blood of pregnant women during 48 hr. while in urine appear after 2 weeks of pregnancy.

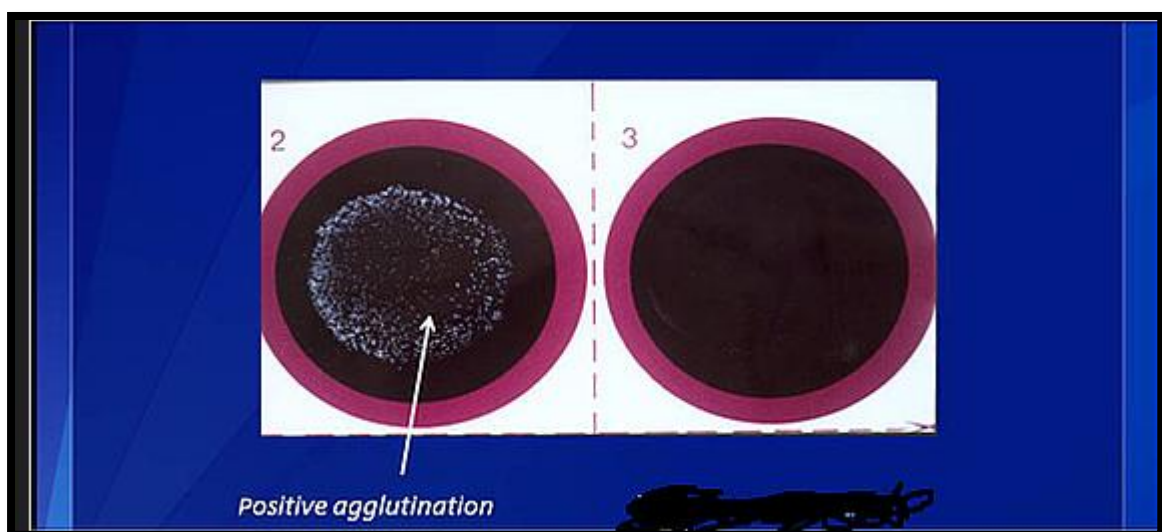


Figure (1): pregnancy Test

Abnormal finding:

-Increased levels

- 1- Pregnancy
- 2- Tumer
- 3 -Ectopic Pregnancy
- 4 -Choriocarcinoma of uterus ,testes and ovary.

-Decreased levels

- 1 - Dead fetus.
- 2- Incomplete abortion.