



The WIDAL TEST.

Principle, Procedure and Interpretation.

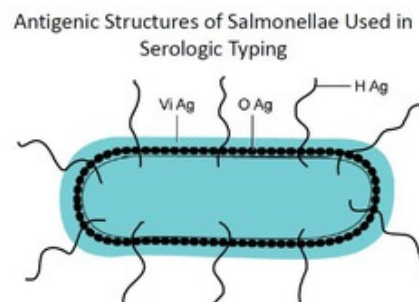
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WIDAL TEST

Introduction of Widal test

This test was developed by Georges Fernand Widal in 1896 and helps to detect presence of salmonella antibodies in a patient's serum.

The Widal test is one method that may be used to help make a presumptive diagnosis of enteric fever, Enteric fever is a life-threatening illness caused by infection with the bacterium *Salmonella enterica* serotype Typhi (*S. typhi*), usually transmitted through food and drinks contaminated with fecal matter. It is associated with symptoms that include high fever, fatigue, headache, abdominal pain, diarrhea or constipation, weight loss, and a rash known as "rose spots." Early diagnosis and treatment are important because serious complications, including severe intestinal bleeding or perforation, can develop within a few weeks.



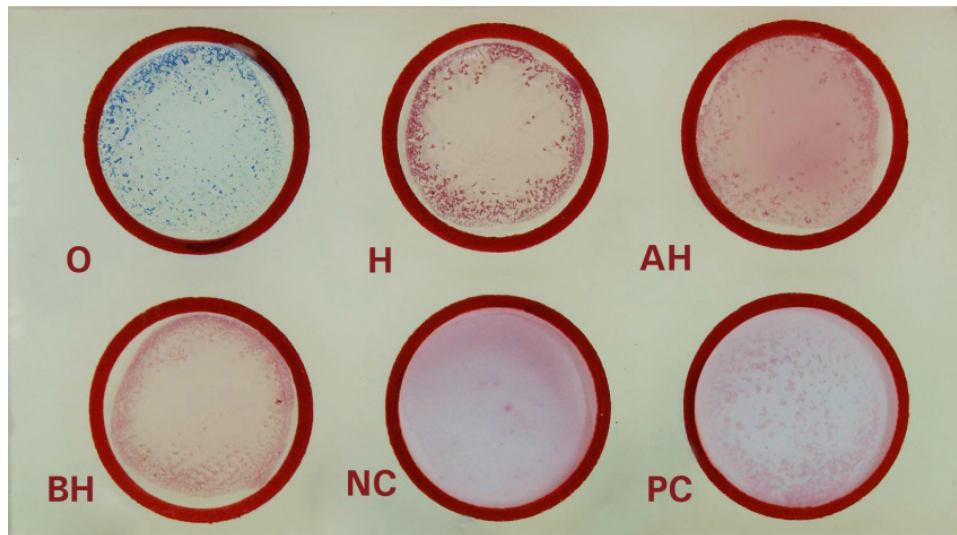
The organisms causing enteric fever possess two major antigens namely somatic antigen (O) and a flagellar antigen (H) along with another surface antigen,

During infection with typhoid or paratyphoid bacilli, antibodies against flagellar antigen of *S. typhi* (H), *S. paratyphi* A (AH), *S. paratyphi* B (BH) and Somatic Antigen of *S. typhi* (O) usually become detectable in blood 6 days after the onset of infection.

- Positive result is indicated by the presence of agglutination,
- Absence of agglutination indicates a negative result.

The paratyphoid O antigens are not employed as they cross react with the typhoid O antigen.

- If agglutination occurs with O antigen then it is considered positive for *Salmonella S.typhi*.
- If agglutination occurs in A or B antigen then it is confirmed as positive for *Salmonella S.paratyphi*. Agglutination will occur in H antigen for all the cases of antigens like O, A, and B.



PROCEDURE OF WIDAL TEST

The Widal test can be conducted in two ways:

- **Slide agglutination Widal test**
 1. Qualitative Slide Test
 2. Quantitative Slide Test
- **Tube agglutination Widal test**
 Tube agglutination has more accuracy as compared to the slide agglutination technique. However, A slide widal test is more popular among diagnostic laboratories as it gives rapid results.

Reagents

Test kit contains the following reagents

Reagent 1: *S.typhi* (H)

Reagent 2: *S.typhi* (O)

Reagent 3: *S.paratyphi* A (H)

Reagent 4: *S.paratyphi* B (H)

Reagent 5: Positive control

Specimen

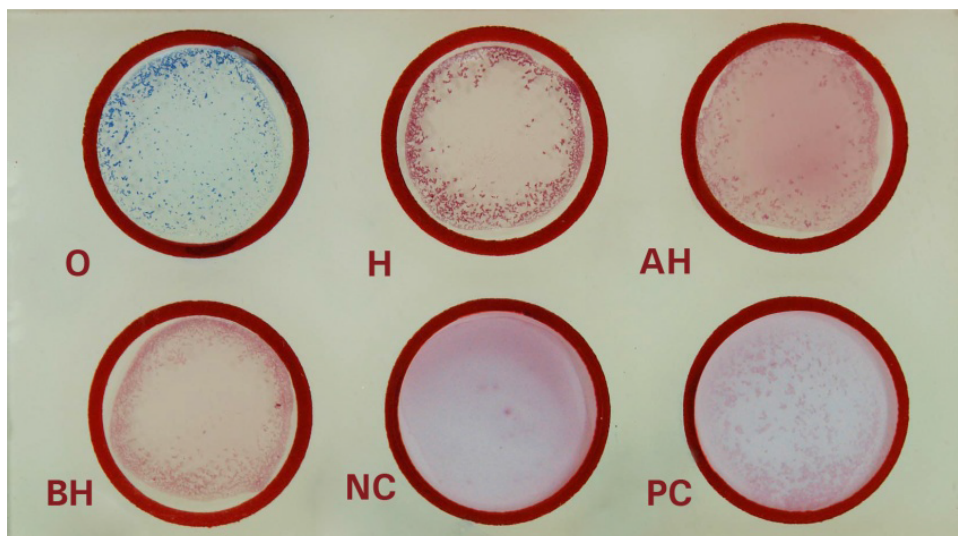
Fresh serum (patient) free from contamination should be used. In case of delay in testing, store the sera samples at 2-8°C in a refrigerator.

Note:

- Specimen is used undiluted.
- Do not use hemolyzed specimen.
- Do not heat or inactivate the specimen.

A. Qualitative slide test for screening

- 1- Bring all reagents to room temperature and mix well.
- 2- Add 1 drop of test sample (serum) (25µl) into each reaction circle labeled as O, H, AH, BH according to given antigen solution.
- 3- Add 1 drop of positive control (25µl) into the circle marked as PC and 1 drop of negative control (25µl) into the reaction circle marked as NC.
- 4- Add antigen solutions of Salmonella typhi 'O', Salmonella typhi 'H', Salmonella paratyphi 'AH' and Salmonella paratyphi 'BH' to circles labeled as O, H, AH, BH respectively in which test samples has been added.
- 5- Mix it thoroughly with the aid of applicator stick and rotate the slide gently.
- 6- Observe for agglutination.



- **Positive Test:** Agglutination within a minute
- **Negative Test:** No agglutination

B. S.Quantitative slide test

This is performed for the samples, which showed positive agglutination during qualitative test.

Procedure:

1. Bring all reagents to room temperature and mix well.
2. Dispense one drop of saline into the first reaction circle and then place 5, 10, 20, 40, 80ul of the test sample (serum) on the remaining circles.
3. Add a drop of the antigen, which showed agglutination with the test sample in the screening (qualitative) method, to each circle.
4. Mix the contents of each circle with the aid of applicator stick and rotate the slide gently.
5. Observe for agglutination.
- 6.

Circle no.	Serum volume	Appropriate antigen	Titre
1	80ul	1 drop	1:20
2	40ul	1 drop	1:40
3	20ul	1 drop	1:80
4	10ul	1 drop	1:160
5	5ul	1 drop	1:320



Interpretation :

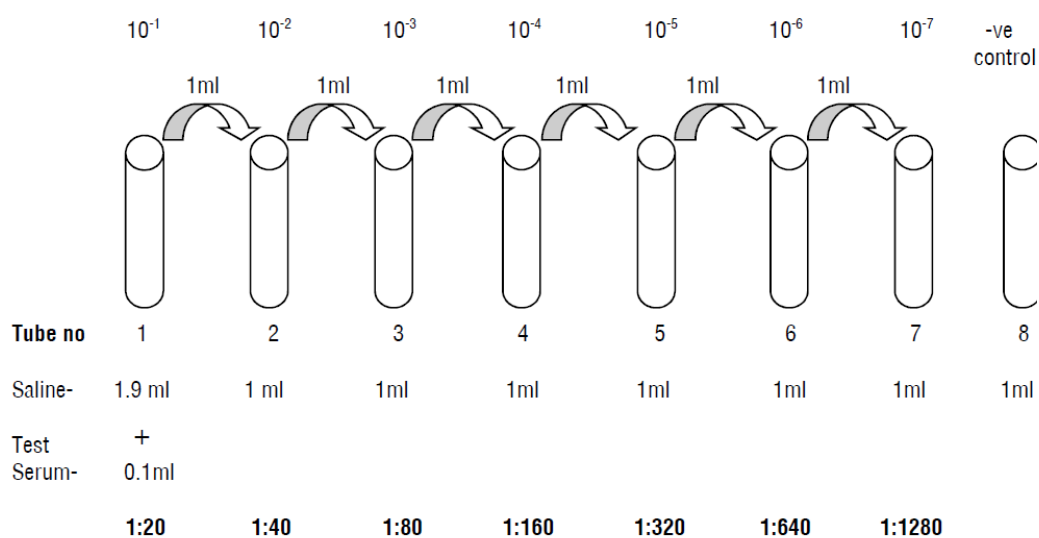
The antibody titre of the test sample its highest dilution that gives a visible agglutination. Agglutinin titre greater than 1:80 is considered as significant infection and low titres indicate absence of infection.

C. Quantitative tube test

Procedure:

1. Bring all reagents to room temperature and mix well.
2. Prepare 4 sets of test tubes for individual antigen. Each set contains 1- 8 tubes.
3. Add 1.9 ml of 0.85% sterile saline to tube no.1 of each antigen set.
4. To tube no.2-8 of all sets add 1ml of physiological saline.
5. To tube No.1 of all sets add 0.1 ml of test sample to be tested and mix well.
6. Transfer 1ml of the diluted serum sample from tube No.1 to tube No. 2 and mix well.
7. Transfer 1ml of the diluted serum sample from tube No.2 to tube No.3 and mix well. Continue this serial dilution till tube No.7 in each set of antigen.
8. Discard 1.0ml of the diluted serum from tube No.7 of each set.
9. So the dilutions of the serum sample from tube No.1 to 7 respectively in each antigen set are 1:20, 1:40, 1:80, 1:160, 1: 320, 1:640, 1: 1280.
10. Tube no.8 is negative control with 0.85% sterile saline.

Tube	1	2	3	4	5	6	7	8
Serum dilution	1:20	1:40	1:80	1:160	1:320	1:640	1:1280	Saline control
Normal saline	1.9 ml	1.0ml	1.0ml	1.0ml	1.0ml	1.0ml	1.0ml	1.0ml
Patient serum	0.1 ml							
Transfer diluted serum	1ml	1ml	1ml	1ml	1ml	1ml	1ml (discard 1ml)	
Appropriate antigen	1 drop	1 drop	1 drop	1 drop	1 drop	1 drop	1 drop	1 drop



- 11- To one set i.e. from tube no.1- 8 add 50 μ l of Salmonella typhi 'O' antigen.
- 12- In second set i.e. from tube no.1- 8 add 50 μ l of Salmonella typhi 'H' antigen.
- 13- Respectively for third and fourth sets, add Salmonella paratyphi 'AH' and Salmonella paratyphi 'BH' to all tubes from 1-8.
- 14- Mix well, cover and incubate these tubes overnight at 37 degree

Celcius (approximately 18 hours).

- 15- After incubation dislodge the sediment and observe for agglutination.

The antibody titre of the test sample is its highest dilution that gives a visible agglutination. Agglutinin titre greater than 1:80 is considered as significant infection and low titres indicate absence of infection.

- Saline control should remain unchanged as it is a negative control.

LIMITATIONS OF WIDAL TEST

1. Tests done within 7 days of illness and after 4 weeks are usually negative.
2. The local titre of the place should be known for the results interpreted correctly.
3. This test (Quantitative) is highly time consumable.
4. Previous typhoid vaccination may contribute to elevated agglutinins in the non-infected population.
5. Other infections of non-enteric salmonella infection such as Typhus, Immunological disorders, chronic liver disease may cause false positive reaction.
6. Cross reaction between malaria parasites and salmonella antigens may cause false positive Widal agglutination test
7. Showed false positive in the area of Typhoid infections and researches showed 65% false positive for normal people.

TYPHOID FEVER VERSUS PARATYPHOID FEVER

Typhoid is caused by a bacteria called Salmonella typhi	Paratyphoid fever is caused by a bacteria called known Salmonella paratyphi
Signs and symptoms are comparatively severe and debilitating	Signs and symptoms are not as severe and debilitating
A vaccine is available to protect against typhoid	There is no mode of immunological prevention

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→ You can Watch the Test Online

<https://www.youtube.com/watch?v=hRzOwSTkF0s&t=2s>