

WIDAL TEST:

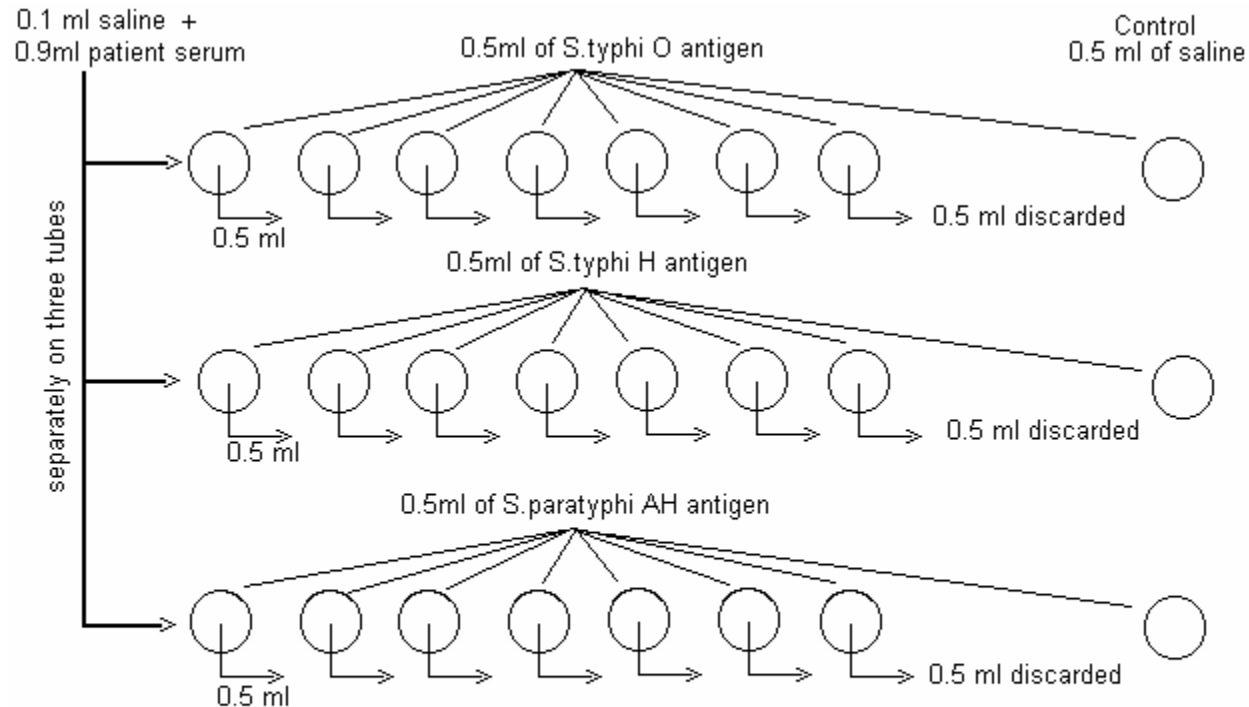
Widal test is a tube agglutination test employed in the serological diagnosis of enteric fever. The test is named after Georges Fernand Isidore Widal, a French physician and bacteriologist, born March 9, 1862, Algeria; died January 14, 1929, Paris.

Principle: Patients' suffering from enteric fever would possess antibodies in their sera which can react and agglutinate serial doubling dilutions of killed, coloured Salmonella antigens in a tube agglutination test.

Requirements: Widal rack, round-bottomed Felix tubes, conical-bottomed Dreyer's tubes, water bath, doubly diluted patient serum in three-four rows, Killed coloured suspensions of *S.typhi* O antigen, *S.typhi* H antigen, *S.paratyphi* AH antigen and optionally *S.paratyphi* BH antigen.

Preparation of antigens: *Salmonella typhi* 901 strain is used to prepare *S.typhi* O and *S.typhi* H antigens. O antigens for *S.paratyphi* A and *S.paratyphi* B are not taken as they cross-react with *S.typhi* O antigen. H antigen suspension is prepared by treating overnight broth culture or saline suspension of Salmonella with 0.1% formalin. For preparing O antigen suspension, Salmonella are grown on phenol agar (1:800) to inhibit flagella. The growth is then emulsified in small volume of saline, mixed with 20 times its volume of alcohol, heated at 40°C to 50°C for 30 minutes and centrifuged. The antigens are treated with chloroform (preservative) and appropriate dyes are added for easy identification of antigens.

Procedure:



Initial dilution	1 in 10	1 in 20	1 in 40	1 in 80	1 in 160	1 in 320	1 in 640
Final dilution (after adding antigen)	1 in 20	1 in 40	1 in 80	1 in 160	1 in 320	1 in 640	1 in 1280

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Patient serum is doubly diluted by mixing and transferring from 1:10 to 1:640 in three-four rows. First row usually comprises of Felix tubes, where somatic *S.typhi* O antigen is added. For all the remaining rows, Dreyer's tubes are taken; where different flagellar H antigens are added. Each tube must contain 0.5ml of diluted serum. A test tube with only saline is kept in each row as control. All the tubes (including control) in a row are mixed with 0.5ml of antigen suspension. The first row is treated with *S.typhi* O antigen, the second row with *S.typhi* H antigen, the third row with *S.paratyphi* AH antigen and the fourth row with *S.paratyphi* BH antigen. Since infections by *S.paratyphi* B are rare, this antigen is usually omitted in the test. After all the tubes have been treated with specific antigen suspensions, the widal rack is placed in a thermostatically controlled water bath maintained at 37°C for overnight incubation. Another approach is to incubate the tubes at 50-55°C.

Reading the results: The control tubes must be examined first, where they should give no agglutination. The agglutination of O antigen appears as a "matt" or "carpet" at the bottom. Agglutination of H antigens appears loose, wooly or cottony. The highest dilution of serum that produces a positive agglutination is taken as titre. The titres for all the antigens are noted.

Slide widal test:

A slide widal test is more popular among diagnostic laboratories as it gives rapid results.

Qualitative test: One drop each of undiluted patients' serum samples for the four antigens are placed on the circled card and one drop of each of the four Salmonella antigens are added separately and gently rotated for one minute. Appearance of agglutination gives qualitative results. To know the titre for each of the antigens, the test is repeated with dilutions of serum.

Quantitative test: 80 µl, 40 µl, 20 µl, 10 µl and 5 µl of patient's serum each for the four antigens are placed on the circled card. To each series of serum specimen, one drop of specific antigen is added to each, mixed and rotated for one minute. Agglutination in each of these is noted. 80 µl corresponds to 1 in 20 dilution, 40 µl to 1 in 40, 20 µl to 1 in 80, 10 µl to 1 in 160 and 5 µl corresponds to 1 in 320 titre.

Interpretation of widal test:

- Timing of test is important, as antibodies begin to arise during end of first week. The titres increase during second, third and fourth week after which it gradually declines. The test may be negative in early part of first week.
- Single test is usually of not much value. A rise in titre between two sera specimens is more meaningful than a single test. If the first sample is taken late in the disease, a rise in titre may not be demonstrable. Instead, there may be a fall in titre.
- Baseline titre of the population must be known before attaching significance to the titres. The antibody levels of individuals in a population of a given area give the baseline titre. A titre of 100 or more for O antigen is considered significant and a titre in excess of 200 for H antigens is considered significant.
- Patients already treated with antibiotics may not show any rise in titre, instead there may be fall in titre. Patients treated with antibiotics in the early stages may not give positive results.
- Patients who have received vaccines against Salmonella may give false positive reactions. This can be differentiated from true infection by repeating the test after a week. True untreated infection results in rise in titre whereas vaccinated individuals don't demonstrate any rise in titre.
- Those individuals, who had suffered from enteric fever in the past, sometimes develop anti-Salmonella antibodies during an unrelated or closely related infection. This is termed anamnestic response and can be differentiated from true infection by lack of any rise in titre on repetition after a week.
- Antigen suspensions with fimbrial antigens may sometimes give false positive reactions due to sharing of fimbrial antigens by some Enterobacteriaceae members. Antigen suspension must be devoid of fimbrial antigens.

* Widal test is losing its relevance in Western and European nations but continues to be used in India.

Last edited on March 2009