Agglutination test

Agglutination tests detect antibody or antigen and involve agglutination of bacteria, red cells, or antigen- or antibody-coated latex particles. Such tests rely on the bivalent nature of antibodies, which can cross-link particulate antigens

* Agglutination/Hemagglutination - When the antigen is particulate the reaction of an antibody with the antigen can be detected by agglutination (clumping) of the antigen. When the antigen is an erythrocyte the term hemagglutination is used. The term agglutinin is used to describe antibodies that agglutinate particulate antigens. When the antigen is an erythrocyte the term hemagglutination is often used. All antibodies can theoretically agglutinate particulate antigens but IgM due to its high valence is particularly good agglutinin.

a) Qualitative agglutination test - Agglutination tests can be used in a qualitative manner to assay for the presence of an antigen or an antibody. The antibody is mixed with the particulate antigen and a positive test is indicated by the agglutination of the particulate antigen.

e.g. A patients red blood cells mixed with antibody to a blood group antigen to determine a persons blood type.

b) Quantitative agglutination test - Agglutination tests can also be used to quantitate the level of antibodies to particulate antigens. In this test one makes serial dilutions of a sample to be tested for antibody and then adds a fixed number of red blood cells or bacteria or other such particulate antigen and determines the maximum dilution which gives agglutination.

The maximum dilution that gives visible agglutination is called the titer.

Prozone effect - On occasion one observes that when the concentration of antibody is high (i.e. lower dilutions) there is no agglutination and then as the sample is diluted agglutination occurs. The lack of agglutination at high concentrations of antibodies is called the prozone effect.

Lack of agglutination in the prozone is due to antibody excess resulting in very small complexes which do not clump to form visible agglutination.

Applications of agglutination tests

1) Determination of blood types or antibodies to blood group antigens.

2) To assess bacterial infections such as widal test

Widal test

Clinical significance:

Widal test is for detection and semi-quantitation of salmonella antibodies, which are increased in typhoid fever.

Principle of the Widal test:

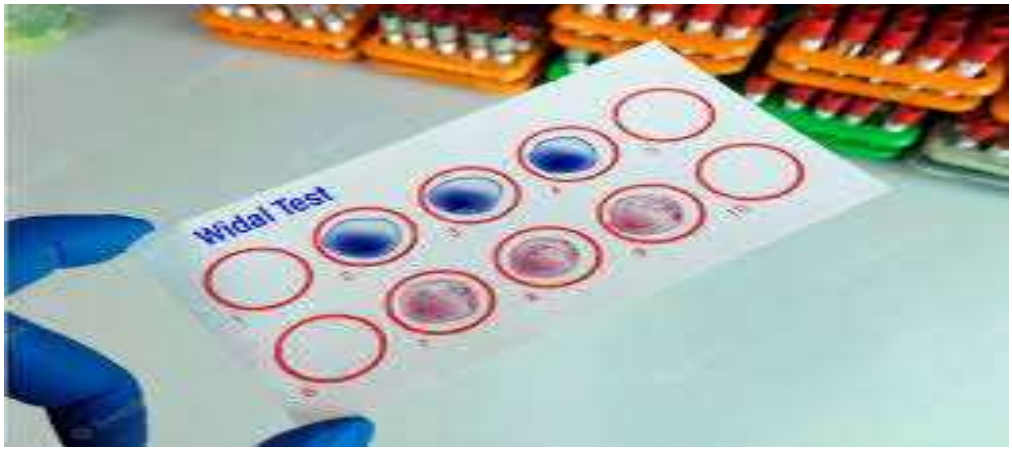
- The stained antigens are standardized suspensions of killed bacteria prepared for the detection and semi-quantitation of Salmonella antibodies in serum.
- Dilutions of patient's serum, in tubes or in microtiter plates, are tested against suspensions of somatic (O) antigen and flagellar (H) antigen of each organism likely to be encountered in the patient's environment.
- If sufficient salmonella antibodies are present in the serum of the patient, they will agglutinate bacterial suspensions which carry the homologous antigens.

Widal test- slide agglutination Test:

- This test is only used as a rapid screening of urgent specimens.
- The titration method should always follow for negative as well as positive results encountered with the slide method.

Procedure: -

1. Allow all reagents and samples to reach room temperature before use.
2. Place 80 µL of undiluted serum in a 3 cm diameter circle on a white tile (slide).
3. Add one drop of the appropriate well-shaken suspension using the dropper provided.
4. Mix by stirring for a few seconds and spread to fill the whole circle.
5. Rotate slowly and read agglutination at one minute.
6. The reaction is roughly equivalent to that obtained in a tube agglutination test with a serum dilution of 1 in 20.
7. *If agglutination is visible within one minute, confirm with Tube Agglutination Test, by which a significant titer should be obtained.*



Widal test - Tube Agglutination:

Procedure: -

1. Allow all reagents and samples to reach room temperature before use.
2. Make one row of serum dilutions for each antigen to be tested (8 rows for 8 antigens).
3. The total number of tubes will be 32; label each tube in each row with the appropriate antigen and dilution.
   Example:
   Row One: typhi O 1:20, typhi O 1:40, typhi O 1:80, etc.
   Row Two: typhi H 1:20, typhi H 1:40, typhi H 1:80, etc.
4. Two types of tubes are generally used for the test, a narrow tube with a conical bottom (Dreyer's tube) for H agglutination, and a short round bottomed tube (felix tube) for O agglutination Label tube 8 of each row "Suspension Control".
5. Using normal saline (0.85%) as a diluent, pipette 1.9 mL to the first tube of each row and 1.0 mL in each of the rest of tubes.
6. Pipette 0.1 mL of patient's serum to the first tube (1:20) of each row. Mix the contents of tube 1 and transfer 1.0 mL to tube 2. Repeat for each tube, up to tube 7 (discard 1.0 mL from tube 7) as shown in the given scheme.
7. Add one drop of the appropriate well-shaken suspension to each tube of a given row, including the suspension control tube.
8. Mix all tubes thoroughly and cover tightly with parafilm.
9. Incubate all tubes at 37°C for 24 hours. (For urgent results, incubate O suspensions at 50°C for four hours and H suspensions at 50°C for two hours, but after reading preliminary results, reincubate all tubes at 37°C overnight and reread).
10. At the end of the incubation period, examine each tube for agglutination under bright light.
11. Read each tube in a row against the suspension control tube and referring to **(Results of Widal Test - Tube Agglutination)** below.
12. If test is positive at a dilution of 1:1280, repeat the test on further dilutions of the serum: 1:2560, 1:5120, etc.
13. As a positive control for each suspension, a dilution series of the appropriate Salmonella Positive Control Serum may be included.

Tube No.	1	2	3	4	5	6	7	8
Saline	1.9 mL	1.0 mL	1.0 mL	1.0 mL	1.0 mL	1.0 mL	1.0 mL	1.0 mL
Serum	0.1 mL	–	–	–	–	–	–	–
	→ *1.0 ml serial dilution* →							–
	Mix & transfer 1.0 mL to 2	Mix & transfer 1.0 mL to 3	Mix & transfer 1.0 mL to 4	Mix & transfer 1.0 mL to 5	Mix & transfer 1.0 mL to 6	Mix & transfer 1.0 mL to 7	Mix & discard 1.0 mL	0.0 mL
Final Titer	**1:20**	**1:40**	**1:80**	**1:160**	**1:320**	**1:640**	**1:1280**	**Suspension Control**

Reading the Results of Widal Test - Tube Agglutination:

A. Antibodies against H and O antigens usually appear by 7th–10th day of the illness and increase steadily till the third or the fourth week, after which it declines gradually. Hence, the blood collected before 7–10 days will be negative for antibodies.
B. Demonstration of a fourfold or more rise in titer of antibodies in a paired sample, one sample collected in the first week and second sample is collected in the third week, is more useful than demonstration of antibodies in a single serum.
C. An elevated level of antibodies may be present in sera of patients suffering from enteric fever in past and in sera of individuals with inapparent infection or vaccination against the enteric fever. Therefore, the mere demonstration of antibodies in the Widal test should need not be considered to be suggestive of the enteric fever.
D. Serum from an individual vaccinated with TAB vac- cine may show high titers of antibodies to *S.* Typhi and *S.* Paratyphi A and B. However, in

case of infection, high titers of antibodies will be seen only against the infecting species.

E. Patients treated early with antibiotics, may show a poor antibody response.
F. Hold each tube under bright light and flick it without shaking.
G. In a positive O reaction, there is an obvious granular agglutination.
H. In a positive H reaction, there is a floccular appearance.
I. In a negative reaction, the appearance of the suspension should be unchanged and show a typical swirl when the tube is flicked without visible agglutination. The tube should not be shaken.
J. In the suspension control tube, the appearance of the suspension should be unchanged and show a typical swirl when the tube is flicked without visible agglutination. The tube should not be shaken.
K. The titer in each positive case is the dilution of the serum in the last tube showing agglutination.
L. Example: S.paratyphi B-O 1:640 and S.paratyphi B-H 1:320
M. A high proportion of normal individuals can show positive reactions; therefore titers of less than 1:80 are of doubtful significance.
N. If test is positive at a dilution of 1:1280, repeat the test on further dilutions of the serum: 1:2560, 1:5120, etc.