

# WIDAL TUBE TEST (‘O’, ‘H’, ‘AH’, ‘BH’ Antigen)

## Salmonella Antigens

(Tube Agglutination)

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**Ref.** IS-SAW.78P, 4x50 ml

### INTENDED USE

This diagnostic reagent kit is use for detection of specific antibodies present in response to the stimulation by specific antigen of Salmonella (group).

### INTRODUCTION

The infection is by ingestion of contaminated material like food, water, milk etc. The organisms (typhoid bacterial) pass through small intestine via lymphatics to mesenteric glands and then invade the blood stream. The specific agglutinins appear in serum of a patient suffering from enteric fever after 6 to 8 days of fever.

‘Widal’ Test is for identification of fever (Pirexia of unknown origin, P.U.O) as enteric as well as one of the screening test for potential carriers of the disease.

### DIAGNOSTIC SIGNIFICANCE

The organism (*Salmonella typhosa*) is responsible for producing the disease in mankind which is known as enteric fever or typhoid fever, which is characterized generally by very high consistent fever, loss of appetite, transitory bacteraemia, round or oval shaped ulcer with smooth peritoneal surface of payer’s patches and solitary lymphoid follicles or ileum etc. The organism possess ‘O’ antigen on the cell wall and ‘H’ antigen on it’s flagella, against which the host body produces immunespecific antibodies, to counteract the effect of corresponding antigens. On the other hand the paratyphoid fever caused by *Salmonella paratyphi A* or *paratyphi B* is characterized by milder course of disease. These organisms also possess somatic ‘O’ and flagellar antigen which is termed as ‘A(H); and ‘B(H)’ respectively. The other organisms of *Salmonella* species like (*S. Typhimurium*) responsible for causing food poisoning or Arisona group causing fetal infection do have similar antigenic properties.

### PRINCIPLE

The killed bacterial suspension of *Salmonella* carries specific ‘O’ and ‘H’ antigen which will react with immuno specific antibodies which may be present in patient serum and agglutinate the antigen, and produce agglutinin or clumps in the tube.

### PRESENTATION

All the reagents to be stored at 2-8°C	
Antigen	Pack size (4x50 ml)
S. Typhi ‘O’	1x50 ml
S. Typhi ‘H’	1x50 ml
S. Para Typhi A ‘(H)’	1x50 ml
S. Para Typhi B ‘(H)’	1x50 ml

### REAGENT STORAGE AND STABILITY

All reagents are stable till expiry date mentioned on the label when stored at 2-8°C away from direct light.

### SPECIMEN COLLECTION

Fresh serum sample is preferred. In case of any delay the sample should be stored at 2-8°C away from direct light. However the test is to be performed within in 24 hours of collection of sample.

### TEST PROCEDURE

1. For each serum sample under test, arrange four rows of 6 tubes each (3"x3/8") in a rack.

2. Prepare master dilution by taking 5 tubes (5"x5/8") in another rack. Place 7 ml of normal saline (0.85% Sodium Chloride) in the first tube and 3.5 ml in each of the remaining four tubes. Add 0.5 ml of serum to the first tube and mix well. Continue successive transfer of 3.5 ml from quantities till the last tube is reached. This will give final dilution of 1:30, 60, 120, 240 & 480.
3. Transfer 0.5 ml quantities from the master dilution tubes of each tube of the corresponding vertical row in test rack. Place 0.5 ml of normal saline to each of the tubes in the Last (i.e.6th) row to serve as controls.
4. To each of the six tube in the first, second, third and fourth horizontal row, add 0.5 ml of S. Typhi ‘O’, S. Typhi ‘H’, S.paratyphi A‘(H)’ and S.paratyphi B‘(H)’ antigens respectively.
5. Shake the rack well to mix and incubate at 37°C for over night (16-20 hours).

In addition to the pattern of sedimented organisms, the decrease in opacity of the supernatant as compared to the Saline control tube must be observed and taken into account while judging the degree of agglutination.

### TEST RESULTS

Note the highest dilution in which there is evidence of agglutination as observed by naked eye or a hand lens.

With ‘H’ antigen the pattern of agglutination is floccular cottonwool type is positive whereas with ‘O’ antigen it is fine granular and mat type at the bottom of the tube is positive.

Sera from normal individuals may agglutinate these antigens, level of ‘normal’ agglutinin varies in different countries and different communities.

Agglutination of 1:120 and more are significant and rise in the titres on repetition of the test after a few days will confirm the diagnosis of enteric fever.

### LIMITATIONS FOR INTERFERENCE

Quantitative tube test is non-specific type of test. In case of doubt, the results should be further confirmed by microbiological tests, microscopy, histopathology etc.

### NOTE

1. Use clean glassware free from dust or debris.
2. Care should be taken to maintain the temp. of 37°C throughout the period of incubation.
3. Agglutination titres of 1:120 and above are typically found in cases of enteric fever. The specific organism responsible is determined by noting the ‘H’ agglutination titre.
4. Anamnestic Reaction: Persons who have suffered from enteric infection in past or who had received TAB vaccine may show appearance of agglutinins in moderate titre when suffering from other unrelated illness. Such Anamnestic appearance of agglutinins can be differentiated from true infection by demonstrating the marked rise in the titre when the test is repeated after a few days.
5. TAB Vaccination: A moderated rise in titre of all three ‘H’ agglutinins simultaneously against all antigens in suggestive of recent TAB Vaccination. A careful history - taking will easily clarify the point.

### REFERENCES

1. Felix A. (1942) Brit Med. Jr. 11,597.
2. Protell r.l.et.al. (1971) Lancet, 11,330. S Medical Bacteriology. N.C. Dey (1970) 259-284.



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