

## HiPer<sup>®</sup> Widal Test Teaching Kit (Tube Test)

**Product Code: HTI018**

**Number of experiments that can be performed: 10**

**Duration of Experiment: 2 Days**

Day 1- Dilution of test sample and protocol

Day 2- Observation

### **Storage Instructions:**

- The kit is stable for 12 months from the date of manufacture
  - Store all the Antigen solutions, Test Sample at 2-8 °C

## Index

<b>Sr. No.</b>	<b>Contents</b>	<b>Page No.</b>
1	Aim	3
2	Introduction	3
3	Principle	3
4	Kit Contents	4
5	Materials Required But Not Provided	4
6	Storage	4
7	Important Instructions	4
8	Procedure	5
9	Observation and Result	5
10	Interpretation	5
11	Troubleshooting Guide	6

### Aim:

To detect the titre value of antibodies present in test serum due to the infection of *Salmonella* genus causing enteric or Typhoid Fever by using quantitative tube agglutination test.

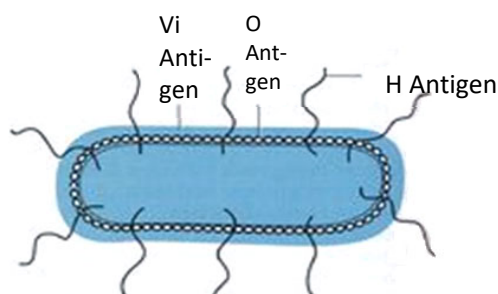
### Introduction:

Widal test is a serological method to diagnose enteric fever or typhoid which is caused by the infection with pathogenic microorganisms like *Salmonella typhi*, *Salmonella paratyphi* A, B and C. This method of diagnostic test is based upon a visible agglutination reaction either in a test tube or on a slide between antibodies of patient serum and antigens specifically prepared from *Salmonella* sp.

### Principle:

Enteric fever or typhoid is a life threatening disease which usually occurs due to the infection of pathogenic microorganisms, e.g. *Salmonella typhi*, *Salmonella paratyphi* A, B and C. These microorganisms are transmitted to human body through food and drinks contaminated with fecal matter. Early diagnosis and treatment for this fever are essential to avoid serious clinical complications. During the course of infection antibodies are produced against *Salmonella* antigens. Widal test, a serological method for the detection of *Salmonella* sp., was developed by F Widal in 1896. During this test a visible agglutination is formed due to the reaction in a test tube or on a slide between antibodies present in the infected person's blood sample and specific antigens of *S. typhi* and *S. paratyphi*. The tube agglutination test is a quantitative method which is used for the determination of titre values of antibodies present in the patient serum.

The organisms causing enteric fever possess two major antigens namely somatic antigen, O and a flagellar antigen, H along with another surface antigen, Vi. During infection antibodies are produced in patient's sera against *Salmonella typhi* O and H and *Salmonella paratyphi* AH and BH antigens. During infection antibodies are produced in patient's sera against these antigens. Antigens specifically prepared from this organism are used in the agglutination test to detect the presence of antibodies in patients' sera which are elucidated in response to infection by these bacteria. There are some agglutinins that are produced in the patient's serum during the fever period, which react with somatic antigen O of *Salmonella typhi*, A or B of *Salmonella paratyphi* and then with flagellar antigen H which is common in most of the *Salmonella* species. In this test four specific antigen suspensions are used e.g. H, *Salmonella typhi* (O antigen), *Salmonella paratyphi* - A and *Salmonella paratyphi* - B. If agglutination occurs with O antigen then it is considered positive for *Salmonella typhi*. If agglutination occurs in A or B antigen then it is confirmed as positive for *Salmonella paratyphi*. Agglutination will occur in H antigen circle for all the cases of antigens like O, A, and B. *Salmonella* species are characterized by three antigens present on the cell, as shown in figure 1.



**Fig 1: Antigenic structure of Salmonella**

**O Antigen:** This is a somatic antigen and is present on the outer membrane of the cell. Its specificity is determined by the nature of the repeating units in the outer O-polysaccharide chain. Somatic antigens are heat stable, alcohol resistant and forms compact and granular clumps when mixed with O antisera.

**Vi antigens:** This is a virulence antigen which is a capsular polysaccharide that overlays the O antigen. This capsule is not necessary for infection but it increases the infectivity by making it less detectable by the body's immune system. It is heat labile and can be detected using Vi antisera. Vi antigen can interfere with O antigen testing.

**H Antigens:** This is a heat labile flagellar antigen which is inactivated both by boiling and alcohol. H antigens rapidly form fluffy clumps when treated with the corresponding antisera. H antigen induces rapid formation of corresponding antibodies as it is strongly immunogenic.

Widal Test Teaching Kit (Tube Test) utilizes the principle for visible agglutination of Salmonella antigen suspension upon reaction with anti-salmonella antibodies present in test serum.

### Kit Contents:

**Table 1: Enlists the materials provided in this kit with their quantity and recommended storage**

Sr. No.	Product Code	Materials Provided	Quantity	Storage
			10 expts	
1	TKC347	<i>Salmonella typhi</i> 'O' Antigen	4.4 ml	2-8 <sup>0</sup> C
2	TKC348	<i>Salmonella typhi</i> 'H' Antigen	4.4 ml	2-8 <sup>0</sup> C
3	TKC349	<i>Salmonella paratyphi</i> 'AH' Antigen	4.4 ml	2-8 <sup>0</sup> C
4	TKC350	<i>Salmonella paratyphi</i> 'BH' Antigen	4.4 ml	2-8 <sup>0</sup> C
5	TKC354	Test Sample	4.4 ml	2-8 <sup>0</sup> C

### Materials Required But Not Provided:

**Glass wares:** Sterile test tubes with caps.

**Reagent:** 0.85% sterile saline.

**Other requirements:** Incubator, Micropipettes, Tips.

### Storage:

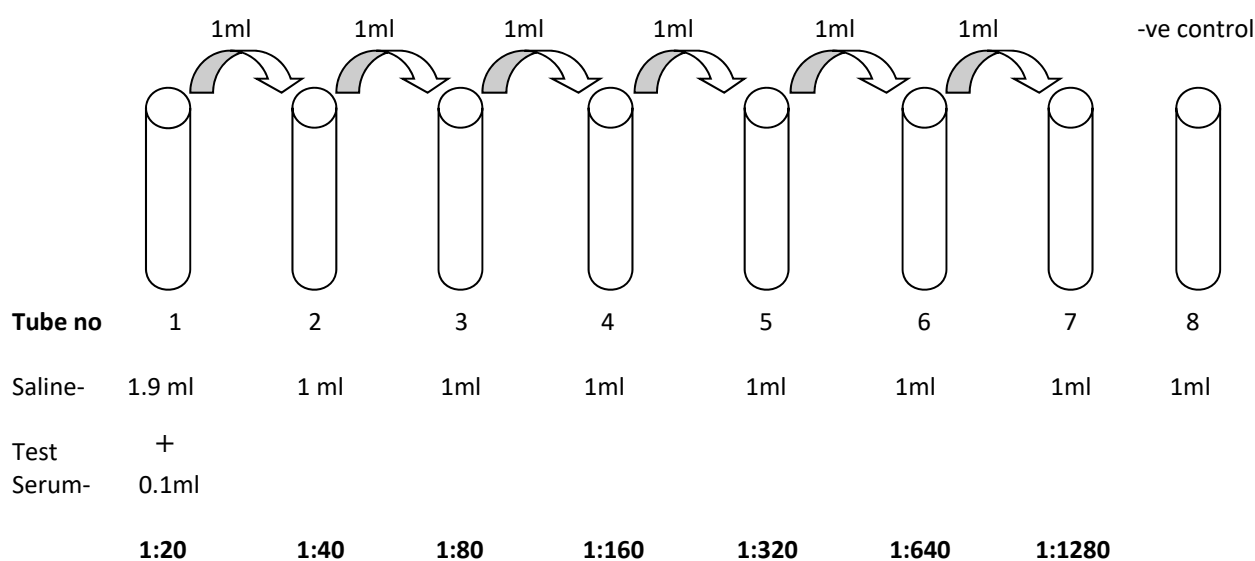
HiPer® Widal Test Teaching Kit (Tube Test) is stable for 12 months from the date of manufacture without showing any reduction in performance. On receipt, store the Antigen solutions and Test sample at 2-8°C.

### Important Instructions:

1. Widal Tube Test Kit should be used according to the kit instructions.
2. Allow all reagents to reach room temperature before use.
3. Do not dilute any of the kit reagents.
4. Do not intermix the reagents.
5. Do not freeze any of the kit reagents.
6. Wear masks and gloves while handling the reagents.

### Procedure:

1. Before starting the experiment, 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 1 ml 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 1 ml of the diluted serum sample from tube No. 1 to tube No. 2 and mix well.
7. Transfer 1 ml 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 1.0 ml 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.



11. To one set i.e. from tube no.1- 8 add 50 µl of Salmonella typhi 'O' antigen.
12. In second set i.e. from tube no.1- 8 add 50 µl 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°C (approximately 18 hours).
15. After incubation dislodge the sediment and observe for agglutination.

### Observation and Result:

After incubation dislodge the sedimented button of every tube very gently and observe for agglutination macroscopically. Note down the titre value of the antibody in the given test sample for all the antigens.

### Interpretation:

During the infection of Salmonella the human body responds to the antigenic stimulus and as a result corresponding antibodies are produced. When the test sample is treated with colored and attenuated Salmonella antigen suspensions, the antibodies present in the sample react with the antigen suspension to give clearly visible agglutination which can be seen through naked eye. The antibody titre of the test sample

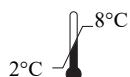
is its highest dilution that gives a visible agglutination. Agglutinin titre greater than 1:80 is considered as significant infection and low titres indicate absence of infection.

### Troubleshooting Guide:

Sr.No	Problem	Possible Cause	Solution
1	False positive result	The reagents were mixed with each other	Ensure that the reagents are added properly onto the respective well without spilling to the sides
		Incubated for a longer time	The results should be read within the time period mentioned in the brochure
2	No agglutination observed	The reagents are not stored under proper conditions	Ensure that the latex reagent, positive and negative control are stored in refrigerator (2-8°C)

### Technical Assistance:

At HiMedia we pride ourselves on the quality and availability of our technical support. For any kind of technical assistance mail at [mb@himedialabs.com](mailto:mb@himedialabs.com)



Storage temperature



Do not use if package is damaged



HiMedia Laboratories Private Limited,  
Reg. Off: Plot No. C-40, Road No. 21Y,  
MIDC, Wagle Industrial Area, Thane,  
(West) 400604, Maharashtra, INDIA.  
Web: [www.himedialabs.com](http://www.himedialabs.com)



12/2024

PIHTI018\_O/1221

HTI018-05

### Disclaimer :

User must ensure suitability of the product(s) in their application prior to use. Products conform solely to the information contained in this and other related HiMedia™ publications. The information contained in this publication is based on our research and development work and is to the best of our knowledge true and accurate. HiMedia™ Laboratories Pvt Ltd reserves the right to make changes to specifications and information related to the products at any time. Products are not intended for human or animal or therapeutic use but for laboratory, diagnostic, research or further manufacturing use only, unless otherwise specified. Statements contained herein should not be considered as a warranty of any kind, expressed or implied, and no liability is accepted for infringement of any patents.

HiMedia Laboratories Pvt. Ltd. Reg.office : Plot No. C-40, Road No. 21Y, MIDC, Wagle Industrial Area, Thane, (West) 400604, Maharashtra, INDIA.  
Customer Care No.: 00-91-22-6116 9797 Tel: 00-91-22-6147 1919, 6903 4800 Email: [techhelp@himedialabs.com](mailto:techhelp@himedialabs.com) Website: [www.himedialabs.com](http://www.himedialabs.com)