

### ORDER INFORMATION

Kit Size	Cat. No.
4 x 5 ml	WIS 1
2+2 x 5 ml	WIS 2
2 x 5 ml	WIS 10

### CLINICAL SIGNIFICANCE

Typhoid fever is an acute infectious disease characterized by definite lesions in Peyer's patches, mesenteric glands and spleen accompanied by fever, headache and abdominal symptoms. It is also called 'enteric' fever. Most frequent and dangerous complication in the late stages of this fever is intestinal haemorrhage and perforation.

The causative organism of the typhoid fever is a gram negative bacillus, Salmonella. In our country typhoid is commonly caused by Salmonella typhi, S. Paratyphi A and S. Paratyphi B. These organisms possess somatic (O) as well as flagellar 'H' antigens. The three serotypes have common 'O' antigen but possess different 'H' antigens. During infection with these bacteria, antibodies appear in the patient's, within 2 to 5 weeks. A rising titre is highly significant for diagnosis of an active infection.

The detection of these antibodies in suspected patients forms the basis of the 'Widal' test. The antigens used in Widal Kit are standardised, smooth suspensions of killed bacilli, which are stained for their easy identification in agglutination reactions. Two test procedures are employed. The rapid slide agglutination test is done as a screening test to establish the presence or absence of an homologous antibody and a confirmatory test is done by test tube procedure.

### PRINCIPLE

When a patient's serum containing antibodies to S. typhi and S. paratyphi is mixed with the respective antigen, agglutination takes place which indicates the presence of antibodies to a particular antigen (O, H, AH, or BH)

### SAFETY PRECAUTIONS AND WARNINGS

This reagent is for *in vitro* diagnostic use only.

### SAMPLE COLLECTION AND PRESERVATION

Fresh serum samples should be used.

### REAGENT PREPARATION AND STORAGE

The sample can be stored at 2-8°C if not immediately used.

### REAGENT STABILITY

All reagents are stable upto the expiry date mentioned on the label when stored at 2-8°C. Do not freeze.

### ASSAY PROCEDURE

#### PROCEDURE FOR SLIDE METHOD :

##### A. Rapid Slide test (Screening test)

- Clean the glass slide provided.
- Place 1 drop of serum in each of the first four circles labeled 1 to 4.
- Add one drop of antigen O, H, AH, BH, in circles 1, 2, 3, 4 respectively.
- Mix the contents in each circle with separate sticks and spread it to fill the whole area of the circle.
- Rock the slide gently for one minute and observe for agglutination. If agglutination is visible within one minute proceed to determine the titre of the particular antibody by quantitative slide test or quantitative tube test.

##### B. Quantitative slide test

- On consecutive circles of a clean slide labeled 1 to 5, add 80µl, 40µl, 20µl, 10µl, and 5µl of the test serum.
- Add one drop of appropriate antigen suspension in each circle.
- Mix well the serum and the antigen.
- Rotate the slide by hand for about a minute and observe for the agglutination.
- The highest dilution of the serum which gives clear agglutination is the antibody titre. The serum volumes in the quantitative slide test correspond approximately to the tube titre as shown below.

Serum Volume	80µl	40µl	20µl	10µl	5µl
Titre	1:20	1:40	1:80	1:160	1:320

##### C. Confirmatory Quantitative slide test

- Take a set of 8 clean dry tubes (10x75mm)
- Dilute each serum sample as follows :

Test 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	1.9ml	1ml	1ml	1ml	1ml	1ml	1ml	1ml
Patient's Serum (undivided)	0.1ml	--	--	--	--	--	--	--
Transfer		→	→	→	→	→	→	
Diluted Serum	--	1ml	1ml	1ml	1ml	1ml	1ml	
Appropriate								
Antigen	1dro p	1dro p	1dro p	1dro p	1dro p	1dro p	1dro p	1drop

- Mix well and then incubate at 37°C for 16 to 20 hrs. and observe for agglutination.
- Note the highest dilution showing clearly visible agglutination with naked eye.
- Saline control should remain unchanged which indicates a negative test.

### INTERPRETATION OF RESULTS :

Agglutination titre 1:80 or more is significant. An increase in titre, 4 to 5 days after the first test is suggestive of an active infection.

### PROCEDURE FOR TUBE TEST :

#### A. Serum Dilution

- Take 5 small Test Tubes and number them.
- Add normal Saline as indicated in the Table below in all the five tubes.
- Add 0.5 ml. of the Test Serum to the first tube, mix and Transfer 3.5 ml. to the next tube (Tube No. 2). Transfer successively to tube no. 5 as indicated in the table.

Tube No.	1	2	3	4	5
Saline (ml)	7.0	3.5	3.5	3.5	3.5
Serum (ml)	0.5	↑	↑	↑	↑
Mix and Transfer	3.5	3.5	3.5	3.5	3.5 Discard
Dilution	1:15	1:30	1:60	1:120	1:240

**B. Titre Estimation**

1. Arrange four rows of 6 small tubes in a test rack horizontally.
2. Add 0.5 ml of serum dilutions in the 5 test tubes in the sequence, i.e. 1:15 to 1:240 in each row.
3. Add isotonic saline in the 6th tube in each row.
4. Then add 0.05 ml of S. Typhi-O, S. Typhi-H, S.Paratyphi-AH, S. Paratyphi. BH antigens separately to each row horizontally.
5. Shake the tubes well and incubate at 37°C overnight for 16-20 hrs.
6. Observe for agglutination, 'O' antigen shows granular agglutination and 'H' antigen gives floccular appearance.

The highest dilution which gives the agglutination is the Titre.  
Saline control should remain unchanged.

Test Tube	1	2	3	4	5	6
Initial Serum Dilution	1:20	1:40	1:80	1:160	1:320	--
Vol. of Serum Dilution (ml)	0.5	0.5	0.5	0.5	0.5	--
Saline (ml)	--	--	--	--	--	0.5
Appropriate Antigen (ml)	0.05	0.05	0.05	0.05	0.05	0.05
Final Dilution	1:30	1:60	1:120	1:240	1:480	Control

**INTERPRETATION OF RESULTS:**

Agglutination Titre of 1:120 or more is significant. An increase in Titre, 4 to 5 days after the first test is suggestive of an active infection

**NOTES**

1. Individuals vaccinated with TAB vaccine may show moderate titre against all three 'H' antigens.
2. Persons who suffered with enteric fever in the past may show moderate titre when suffering from other unrelated illness. Such anamnesic appearance of agglutination can be identified. If the patient is tested again after few days. Only a rising titre is indicative of true infection.
3. The use of positive, negative and saline controls are recommended along with serum specimen.

**BIBLIOGRAPHY**

1. Felix A, Brit Med. J. 11, 597 (1942)
2. Protel, R.L. et al, Lancet, 11.330 (1971)
3. Cruicks, R. Medical Microbiology. 12th Edition P. 403 (1982)