

SALMONELLA ANTIGENS

(WIDAL ANTIGENS)

INTRODUCTION:

The infection is by ingestion of contaminated material like food, water, milk etc. The organisms (typhoid bacteria) 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.

PRINCIPLE

A patient suffering from typhoid fever develops antibodies specific to the infecting organisms. Widal is a test for presence of these antibodies in significant concentration. The bacterial suspension (antigen) is mixed with patient's serum in various dilutions. Appearance of agglutination in highest dilutions determines the titer of the serum.

SAMPLE:

Fresh serum should be used. In case of any delay, serum should be stored at 2-8°C. The sample should not be inactivated.

STORAGE AND STABILITY:

All reagents are stable at 2-8°C till the expiry date mentioned on the individual label.

PRECAUTIONS:

1. Bring all the reagents to room temperature before use.
2. Serum should not be inactivated.
3. Use clean and dry glasswares.
4. Include positive and negative control sera for greater proficiency in interpretation of results.
5. Shake antigen vial well before use.
6. Serum should be clear.

PROCEDURE:

A. Rapid Slide Test (Widal Screening Test):

1. Use clean and dry glass slide.
2. Place one drop of undiluted serum in the respective circles. (1-4)
3. Add one drop of antigen O, H, A (H), B (H) in circles 1, 2, 3, 4 respectively.
4. Mix the contents of each circle with separate stick and spread to fill the whole circle area.
5. Rock the slide for one minute and observe for agglutination.
6. If agglutination is visible within one minute, then proceed for quantitative estimation.

B. Quantitative Widal (Tube) Test:

1. Take two sets of 8 clean dry Tubes (10 x 75 mm/ widal tubes)
2. 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 ml.	1.9	1.0	1.0	1.0	1.0	1.0	1.0	1.0
Patient's Serum (Undiluted) ml.	0.1	-	-	-	-	-	-	-
Transfer Diluted Serum ml	-	1.0	1.0	1.0	1.0	1.0	1.0	-
Appropriate Antigen Drop	1	1	1	1	1	1	1	1

Arrow indicates 1 ml. mixture from the tube is transferred to the next tube & mixed.

3. Mix well and incubate at 37°C for 16-20 hours and observe for agglutination.
4. Follow above procedure for all four antigens.
5. Titre is the highest dilution of serum showing clear cut agglutination.

C. Quantitative Slide Test:

Clean the glass slide supplied in the kit. Proceed as follows.

Circle	Serum Volume	Appropriate Antigen Drop		Equivalent Titre
1	0.08 ml	1 Drop	Mix & rotate for one min. & observe agglutination	1:20
2	0.04 ml	1 Drop		1:40
3	0.02 ml	1 Drop		1:80
4	0.01 ml	1 Drop		1:160
5	0.005 ml	1 Drop		1:320

Repeat above procedure for visible agglutination observed in rapid slide screening test (which gives visible agglutination-step 6 in procedure A)

NOTE:

'O' Antigen shows granular agglutination.
All 'H' Antigens show floccular appearance.
Saline control suspension does not show agglutination and is a specimen for negative test result.

INTERPRETATION:

Agglutination titre greater than 1 : 80 is suggestive of infection.

QUALITY CONTROL PROCEDURE:

The use of positive, negative and saline controls are recommended along with serum specimen.

PRESENTATION:

Antigen	Pack Size (4x5 ml)
S. Typhi 'O'	1 x 5 ml
S. Typhi 'H'	1 x 5 ml
S. Para Typhi 'A (H)'	1 x 5 ml
S. Para Typhi 'B (H)'	1 x 5 ml
Positive control	1 x 0.5 ml

HOW TO OPEN DROPPER BOTTLE:

