

WIDAL SLIDE TEST ('O', 'H', 'AH', 'BH' Antigen, +ve Control)

Salmonella Antigens

(Slide Agglutination)

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Ref. IS-SAW.078, 4x5 ml

INTENDED USE

This diagnostic reagent kit is used 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 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.

PRESENTATION

All the reagents to be stored at 2-8°C	
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

PRECAUTION

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.

REAGENT STORAGE AND STABILITY

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

SPECIMEN COLLECTION

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

TEST PROCEDURE

A. Rapid Slide Test (Widal Screening Test)

1. Place one drop of undiluted serum in the respective circles. (1-4)
2. Add one drop of antigen O, H, A (H), B (H) in circles 1, 2, 3, 4 respectively.
3. Mix the contents of each circle with separate stick and spread to fill the whole circle area.

4. Rock the slide for one minute and observe for agglutination.
5. 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	Serum Dilution	Normal Saline ml	Patient's Serum (Undiluted) ml	Transfer Diluted Serum ml	Appropriate Antigen Drop
1	1:20	1.9	0.1	-	1
2	1:40	1.0	-	1.0	1
3	1:80	1.0	-	1.0	1
4	1:160	1.0	-	1.0	1
5	1:320	1.0	-	1.0	1
6	1:640	1.0	-	1.0	1
7	1:1280	1.0	-	1.0	1
8	Saline Control	1.0	-	-	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)

TEST RESULTS

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.

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.

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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