WIDAL SLIDE TEST ('O','H','AH','BH','AO','BO','CO','CH', Antigen, +ve & -ve Control)

Salmonella Antigens

(Slide Agglutination)

IMMUNOPAK

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Ref. IS-SAW.78T, 8x5 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 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

Pack Size (8x5 ml)			
S. Typhi 'O'	1 x 5 ml	S. Paratyphi–'BO'	1 x 5 ml
S. Typhi 'H'	1 x 5 ml	S. Paratyphi–'CO'	1 x 5 ml
S. Paratyphi–'AH'	1 x 5 ml	S. Paratyphi–'CH'	1 x 5 ml
S. Paratyphi–'BH'	1 x 5 ml	+ve Control	1 x 1 ml
S. Paratyphi–'AO'	1 x 5 ml	-ve Control	1 x 1 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^{0}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^{0}C for upto 72 hours. The sample should not be inactivated. Do not use haemolysed & turbid sample.

TEST PROCEDURE

A. SLIDE SCREEN TEST

1. Place one drop of positive control onto a reaction circle of the glass slide.
2. Place one drop of patient's serum to be tested & one drop of physiological saline on to each of the required number of reaction circles.
3. Add one drop of appropriated Widal antigen suspension to the reaction circles containing positive control & patient's serum & physiological saline.
4. Mix contents of each circle uniformly over the entire circle with separate mixing sticks.
5. Rock the slide gently back and forth, and observe for agglutination macroscopically at one minute.
6. If agglutination is visible within in one minute with positive control & patient's serum then proceed for quantitative estimation.

B. Quantitative (Tube) Test

1. Take two sets of 8 clean dry Tubes (10 x 75 mm/ Widal tubes) & 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	-	- Discard 1.0 ml	1

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

2. Mix well and incubate at 37^{0}C for 16-20 hours, dislodge the sediment button gently and observe for agglutination.
3. Follow above procedure for all antigens.
4. Titre is the highest dilution of serum showing clear cut agglutination.

C. Slide Quantitative Test

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

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

Slide Screen Test

Agglutination is a positive test result and indicates presence of the corresponding antibody in the patient's serum.

No agglutination is a negative test result and indicates absence of the corresponding antibody in the patient serum.

Slide Quantitative Test

Agglutination is a positive test result. The titre of the patient serum corresponds to the visible agglutination in the test circle with the smallest amount of serum sample.

Quantitative (Tube) Test

The titre of the patient serum using Widal antigen suspensions is the highest dilution of the serum sample that gives a visible agglutination.

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REMARKS

1. Positive results obtained in the slide test should be confirmed with the test tube to establish whether the titres are diagnostically significant or not.
2. TAB vaccinated patients may show a high titre of antibodies to each of the antigens. Similarly, an amnestic response to other vaccines and unrelated fevers in case of patients who have had prior infection or immunization may give a false result.
3. Agglutinins usually appear by the end of the first week of infection, blood sample taken earlier may give a negative result.
4. A rising titre is more significant than a single high titre. It is there fore necessary to evaluate two or more serum samples taken at 4-6 days. Intervals after the onset of the disease.
5. 'O' being a somatic antigen brings about a coarse, compact, granular agglutination whereas 'H' being a flagellar antigen brings about larger, loose, flocculant agglutination.
6. While the 'O' antigen is species specific, the 'H' antigen is specific to the serotype.
7. Serological findings are not intended as a substitute for culture. An appropriate attempt should be made to recover and identify the etiologic organisms through various culture and biochemical tests.
8. Generally antibody titres of 1:80 or more are considered clinically and diagnostically significant. However the significant titre may vary from population to population and needs to be established for each area.
9. False positive results are likely if the test is read more than one minute after mixing on the slide test.
10. Any deviation in test procedure could result in variable results.
11. Since techniques and standardization vary from lab to lab on tube difference in tube titres can be expected.
12. Use a separate disposable tip for each sample to prevent cross contamination.
13. After usage the antigen suspension should be immediately recapped and replaced at 2-8^{0}C.
14. It is recommended that results of the tests should be correlated with clinical findings to arrive at the final diagnosis.
15. The performance of the reagents should be validated occasionally using the positive control provided. Good physiological saline may be used as a negative control.

PERFORMANCE CHARACTERISTICS

1. The positive control antisera should produce 1+ or greater agglutination at 1:80 in the slide and tube test when tested with the widal antigen suspensions.
2. The negative control should show no agglutination with any of the Widal antigen suspensions.
3. Generally accepted performance characteristic of this type of test is 70% specificity and sensitivity.
4. Reproducibility of Widal antigen suspensions is 100% (+/- one double dilution).

NOTE

1. In vitro diagnostic reagent for laboratory and professional use only. Not for medicinal use.
2. Avoid contact with skin and mucosa. Do not breathe vapour. In case of contact with eyes, rinse immediately with plenty of water and seek medical advice.
3. The reagent can be damaged due to microbial contamination or on exposure to extreme temperatures. It is recommended that the performance of the reagent be verified with the positive and negative controls.
4. Shake the reagent vials well before use to disperse the antigen suspension uniformly and improve test readability.
5. Only clean and dry glass slides/tubes must be used. Clean the glass slide/tube with distilled water and dry.
6. It is necessary to use calibrated dropper provided with the reagent vial to dispense a reagent drop.
7. Widal antigen suspensions are not from human sources hence contamination due to HBsAg and HIV is practically excluded.
8. Accessories provided with the kit only must be used for optimum results.
9. Do not use damaged or leaking reagents.

REFERENCES

1. Cruickshank R., (1982), Medical Microbiology, 12th Edition, 403.
2. Felix. (1942), Brit, Med.J., 11, 597.

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