WIDAL TEST – 4x5 ml

METHOD - IMMUNO AGGLUTINATION
PACK SIZE - 4 x 5 ml (O, H, AH, BH)
PRODUCT CODE - AB02 *INSTRUCTIONS FOR USE*

ANAMOL
THE ORIGINAL MAKERS

INTENDED USE: Test for determination of antibodies against *Salmonella* antigens by Slide & Tube Test method.

INTRODUCTION

Salmonella typhi & *Salmonella paratyphi* are the causative agents of "Enteric Fever". The antigens of typhoid and paratyphoid consist of 2 distinct fractions – the stable somatic 'O' antigen and the labile flagellar 'H' antigen. The paratyphoid antigens are further classified into 'A' and 'B' species. In typhoid and paratyphoid, the 'H' antigen is type specific whereas the 'O' antigen is group specific.

METHOD PRINCIPLE

Widal antigens are the standardized smooth suspension of killed bacterial antigens of qualitative and semi-quantitative detection of *S. typhi* and *S. paratyphi* antibodies. An undiluted serum is used in slide test. This will react with immunospecific antibodies which may be present in patient serum and agglutinate the antigens to produce agglutination or clumps on the slide. The slide test are standardized in such a way that they can be used for either slide or tube technique. A marked rise in the titre to one serotype (above 1:80) suggests infection. Diagnostically, a rising antibody titre of at least four fold (two tube difference) is considered more significant than a single test. It is observed that individuals immunize with TAB vaccine may show a moderately high titer for all antigens.

REAGENTS

R1	- *S. typhi* 'O' Antigen	- 5 ml.
R2	- *S. typhi* 'H' Antigen	- 5 ml.
R3	- *S. paratyphi* 'AH' Antigen	- 5 ml.
R4	- *S. paratyphi* 'BH' Antigen	- 5 ml.
R5	- Positive Control	- 1 vial.

REAGENT STORAGE AND STABILITY

1. Store the reagent at 2-8°C. DO NOT FREEZE.
2. The reagent is light sensitive.
3. The shelf life of the reagent is as per the expiry date mentioned on the reagent vial labels.
4. Discard the reagent if they become contaminated or do not demonstrate correct activity with controls.
5. Do not interchange the reagents from other batches.

SEPCIMEN COLLECTION & STORAGE

1. Use fresh serum obtained by centrifugation of clotted blood.
2. Samples may be stored at 2-8 °C for 48 hours before performing the test. For longer period of time, serum must be stored in frozen condition.
3. Haematic, lipaemic or contaminated serum must be discarded.

PRECAUTIONS

1. Bring all the reagents and samples to room temperature before use.
2. Shake all the antigens thoroughly before use.
3. Avoid using turbid, contaminated or inactivated serum.

PROCEDURE

A. RAPID SCREENING SLIDE TEST:

1. On a slide with multiple circles, place 50 µl of test serum in each of the circles and 50 µl each of positive Control and Normal Saline in each of the last two circles respectively.
2. Add one drop each of 'O', 'H', 'AH', and 'BH' antigens in the first four circles respectively. Add one drop of any one antigen in the last two circles with positive control and negative control.
3. Mix the contents of each circle separately and spread it in the entire circle.
4. Rock the slide gently for One minute and observe for agglutination.

INTERPRETATION OF RESULTS

Agglutination with Positive Control and no agglutination with Normal Saline validate test results. No agglutination up to one minute is a negative test, and indicates the absence of corresponding antibodies.

Agglutination within one minute is a positive test, and indicates presence of corresponding antibodies. Then proceed for semi-quantitative slide or tube technique for determination of antibody titer.

NOTE: DO NOT OBSERVE RESULT AFTER ONE MINUTE.

B. SEMI-QUANTITATIVE SLIDE TEST:

1. Put one drop of normal saline in the first circle and 0.08 ml, 0.04 ml, 0.02 ml, 0.01 ml, & 0.005 ml of test serum in the subsequent five circles respectively. The corresponding titres will be 1:20, 1:40, 1:80, 1:160 and 1:320 respectively.
2. To each of the above circles, add one drop of the appropriate antigen, which gives agglutination in the screening slide test.
3. Mix the contents of each circle separately and spread it in the entire circle.
4. Rock the Slide gently for one minute & observe for agglutination.

INTERPRETATION OF RESULTS

The lowest volume of serum which shows clear agglutination indicates the cut-off level of the positive test and the corresponding antibody titre as per the tube technique as given below:

Serum Volume	Antibody Titer
0.08 ml	1:20
0.04 ml	1:40
0.02 ml	1:80
0.01 ml	1:160
0.005 ml	1:320

C. QUANTITATIVE METHOD:

Tube technique using slide antigens:

1. Perform the assay for all four antigens or for that which has given a positive result in the Screening Slide Test.
2. Take a set of six test tubes (10x75) for each antigen. Dilute the serum sample and set up the test as indicated in the table:

	Tube Number					
	1	2	3	4	5	6
Dilution	Saline Control	1:20	1:40	1:80	1:160	1:320
Normal Saline	1.0 ml	1.9 ml	1.0 ml	1.0 ml	1.0 ml	1.0 ml
Test Serum	-	0.1 ml	-	-	-	-
Diluted Serum from Previous Tube	-	-	1.0 ml	1.0 ml	1.0 ml	1.0 ml
Appropr-iate Antigen	One Drop	One Drop	One Drop	One Drop	One Drop	One Drop

3. Mix well after each addition and incubate at 37^{0}C for 18-20 hours.
4. Observation for agglutination. The highest dilution for Serum which shows clear–cut agglutination indicates the antibody titre.

NOTE

1. Sera from normal individual may show agglutination up to 1:40 dilution.
2. Agglutination titer greater than 1:80 is considered significant and usually suggestive of infection.
3. Widal is only screening test. For confirmation of results, testing with Widal-T is recommended.
4. The correlation of test results with typical clinical signs, symptoms and patient's history should be taken into account before arriving at the final diagnosis.
5. As with all diagnostic procedures, the Physician should evaluate data obtained by use of this kit in light of other clinical information.
6. For accuracy of results, the procedure has to be followed meticulously.

Additional material required for Slide Test Method: Stop Watch.

Additional material required for Quantitative method: Timer, Test Tubes, Pipettes (0.1ml, 1.0ml) Saline, and Incubator (37°C).

LITERATURE

1. Cruickshank, R. (1982) Medical Microbiology, 12th Edition, p 403.
2. Felix, A. (1942) Brit. Med. J., 11, 597.
3. Medical Bacteriology. N. C. Dey (1970), 259-284.
4. Protell R. I. *et.al* (1971) Lancet, 11, 330.

SYMBOLS: Read Instruction for use | IVD In Vitro Diagnostic Use Only | Manufactured by | Expiry Date | Storage Temperature

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