Ana-Chek® WIDAL TEST (4x5 ml)

METHOD – IMMUNO AGGLUTINATION
PRODUCT CODE – AB06

ANAMOL
THE ORIGINAL MAKERS

INSTRUCTIONS FOR USE

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

Ana-Chek® 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. It is simple, rapid and convenient screening test. The slide test is standardized in such a way that they can be used for either slide or tube technique. In doubtful cases, it is recommended to perform the tube technique for obtaining conclusive results. 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 titre for all antigens.

REAGENTS

R1 - *S. typhi* 'O' Antigen	: 5 ml.
R2 - *S. paratyphi* '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

Store the reagent at 2-8 ^{0}C. DO NOT FREEZE. The reagent is light sensitive. The shelf life of the reagent is as per the expiry date mentioned on the reagent vial labels. Discard the reagent if they become contaminated or do not demonstrate correct activity with controls. Do not interchange the reagents from other batches.

PRECAUTIONS & HANDLING

The reagents/samples should be handled by qualified personnel only. Bring all the reagents and samples to room temperature before use. Shake all the antigens thoroughly before use. Avoid using turbid, contaminated or inactivated serum. Discard reagent/sample as per good laboratory practices and local regulatory requirements. Read the instructions given on the labels and instructions for use carefully before using the kit. The kit is intended for in-vitro diagnostic use only. Don't freeze the reagent. Do not shake the reagent vigorously. Contamination of the reagent should be avoided.

SPECIMEN COLLECTION & STORAGE

Use fresh serum obtained by centrifugation of clotted blood. Samples may be stored at 2-8 ^{0}C for 48 hours before performing the test. For longer period of time, serum must be stored in frozen condition. Haematic, lipemic or contaminated serum must be discarded.

MATERIALS REQUIRED BUT NOT PROVIDED

Test tubes with stand, Micropipette with tips, Stop watch, Incubation chamber.

PROCEDURE

A. RAPID SCREENING SLIDE TEST:

1. On a slide with multiple circles, place 50 µl of test serum in each of the four circles and 50 µl of Positive Control in the last circle respectively.
2. Add one drop each of 'O', 'H', 'AH' and 'BH' antigens in each of the four circles respectively. Add one drop of any one antigen in the last circle with positive 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 validates test results. No agglutination up to one minute is a negative test (normal saline), 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 titre.

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 Titre
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. SEMI-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
Appropriate Antigen	One Drop	One Drop	One Drop	One Drop	One Drop	One Drop

3. Mix well after each addition and incubate at 50 ^{0}C for 4 hours.
4. Observation for agglutination. The highest dilution for Serum which shows clear–cut agglutination indicates the antibody titre.

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 therefore 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 course, 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.

NOTE

1. Sera from normal individual may show agglutination up to 1:40 dilution.
2. Agglutination titre greater than 1:80 is considered significant and usually suggestive of infection.
3. Many populations or communities can show high levels of residual antibodies, often in excess of 1:80 - 1:160 due to previous infection.
4. For the test to be clinically significant, a rise in titre must be demonstrated instead of just high titre for one of the tests.
5. Widal is only screening test. For confirmation, further tests are required.
6. 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.
7. As with all diagnostic procedures, the Physician should evaluate data obtained by use of this kit in light of other clinical information.
8. For accuracy of results, the procedure has to be followed meticulously.

BIBLIOGRAPHY

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Symbol	Explanation	Symbol	Explanation
	Manufactured By		In Vitro Diagnostic Use
	Lot Number		Read Instructions Before Use
	Catalogue Number		Storage Temperature
	Manufacturing Date		Number of Tests / Volume
	Expiry Date		Do Not Reuse
	Protect from Sunlight		Keep Dry

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GMP
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