COMBIWIDAL - S

(SLIDE & Tube Test Method)

KIT NAME	KIT SIZE	CAT. NO
CombiWidal - S	4 X 5 ml	SCWS04005M

GENUINE BIOSYSTEM

INTRODUCTION

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

METHOD PRINCIPLE

CombiWidal- S antigens are the standardized smooth suspension of killed bacterial antigens of qualitative and semi-quantitative detection of S. typhi and S.paratyphi antibodies. The different floor to each antigen facilitates the differentiation of antigen and clear reading of agglutination. It also avoids the possible error or mixing and /or misinterpretation., As undiluted serum is used in Slide Test, it is a simple, rapid and convenient screening test, the slide test antigen are 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 sero type (above 1:80) suggests infection Diagnostically a rising antibody tire of at least four fold (two tube difference) is considered more significant than a single test. It is observed that individuals immunized with TAB vaccine may show a moderately high titre for all antigens.

KIT CONTENTS

Reagent Name	SCWS04005M
R1 S.typhi O Antigen	1 x 5 ml
R2 S.typhi H Antigen	1 x 5 ml
R3 S.typhi AH Antigen	1 x 5 ml
R4 S.typhi BH Antigen	1 x 5 ml
R5 Positive Control	1 No

WORKING REAGENT PREPARATION AND STABILITY

1. Store the reagent at 2-8°C. DO NOT FREEZE.
2. The shelf life of the reagent is as per the expiry date mentioned on the reagent vial labels.

SAMPLE COLLECTION & STORAGE:

1. No special preparation of the patients is required prior to sample collection by approved technique. Do not used haemolysed samples
2. Clean and dry glassware free from detergents must be used for sample collection.
3. Though freshly collected serum is preferable, store samples at 2-8°C in case of delay in testing for up to 72 hrs.

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.

ACCESSORIES

SLIDE - 1 No

PROCEDURE

I. Rapid Screening Slide Test:

1. On a slide with six circles, place 0.04 ml of test serum in each of the first four circles and 0.04 ml 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 and one drop of any one antigen in the remaining two circles.
3. Mix the contents of each circle separately and spread it in the entire circle.
4. Rock the slide gently for Two 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 two minute is a negative test, and indicates the absence of corresponding antibodies.

Agglutination with in two minute is a positive test, and indicates presence of corresponding antibodies. The proceed for semi-quantitative slide or tube technique for determination of antibody tire.

Do not observe result after one minute.

I. Semi-Quantitative Slide Test:

1. Put one drop of normal saline in the first circle and 0.005ml, 0.01ml, 0.02ml,0.04ml, & 0.08ml of test serum in the remaining five circles respectively. The corresponding titres obtained will be 1:320, 1:160,1:80,1:40,1:20 respectively.
2. To each of the above circles, add one drop of the appropriate antigen, which gives an 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 two minute & observe for agglutination.

NOTE:

This method is recommended for obtaining quick approximately titers only.

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 is given below:

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

QUANTITATIVE METHOD

II. 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 No.	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 Tube 2	-	-	1.0 ml	1.0 ml	1.0 ml	1.0 ml	1.0 ml Discard
Appropriate Antigen	One Drop	One Drop	One Drop	One Drop	One Drop	One Drop	

3. Mix well after each addition and incubated at 37°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 titre 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 date obtained by use of this kit in light of other clinical information.
6. For accuracy of results, the procedure has to be followed meticulously.

LITERATURE

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

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