

COMBIWIDAL - OH

(SLIDE & Tube Test Method)

KIT NAME	KIT SIZE	CAT. NO
Combi Widal - OH	2+2 x 5 ml	SCWO04005M



INTRODUCTION

Salmonella typhi & Salmonella paratyphi are the causative agents of "Enteric Fever". In enteric fever, once the patient is on medication it becomes difficult to isolate the organisms. In serological tests the antibodies produced as a result of infection are detected by using the killed bacterial antigens. The antibodies from the patient's serum react with the corresponding antigens to cause clumping or agglutination.

METHOD PRINCIPLE

The antibodies present in the serum sample react with the corresponding bacterial antigens to give agglutination.

REAGENTS

Reagent Name	SCWO04005M
R1 S.typhi O antigen	2 x 5 ml
R2 S.typhi H antigen	2 x 5 ml
R3 Positive Control	1 vial

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 use 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

1. 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'

antigens in the first 2 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. Then proceed for semi-quantitative slide or tube technique for determination of antibody titre.

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, 0.02 ml, 0.04ml, & 0.08ml of test serum in the remaining five circles 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 two 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 is 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

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
Appropriate Antigen	One Drop	One Drop	One Drop	One Drop	One Drop	One Drop

1.0 ml Discard

- Mix well after each addition and incubated at 37°C for 18-20 hours.
- Observation for agglutination. The highest dilution for Serum which shows clear -cut agglutination indicates the antibody titre.

NOTE

- Sera from normal individual may show agglutination up to 1:40 dilution.
- Agglutination titre greater than 1:80 is considered significant and usually suggestive of infection.

- Widal is only screening test. For confirmation of results, testing with Widal-T is recommended.
- 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.
- As with all diagnostic procedures, the Physician should evaluate date obtained by use of this kit in light of other clinical information.
- For accuracy of results, the procedure has to be followed meticulously.
- Slide Test Method: Additional Material Required Stop Watch
Quantitative method: Timer, Test Tubes, Pipettes(0.1ml, 1.0ml) Saline, and Incubator(37°C).

LITERATURE

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



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