

SeroMAX WIDAL

(Slide Agglutination Method)

ORDERING INFORMATION

Ref. No.	Pack Size	Antigen
AVWID8-40	8 x 5 ml	O, H, AH, BH, AO, BO, CO, CH

INTENDED USE :

SeroMAX WIDAL is an in-vitro diagnostic kit for the Qualitative and Semi-Quantitative determination of Antibodies to Salmonella typhi and Salmonella paratyphi in Human Serum.

PRODUCT FEATURES

- 1) Sera from normal individuals may show agglutination upto 1 : 40 dilution.
- 2) Agglutination Titre greater than 1 : 80 is considered to be significant and usually suggestive of infection.
- 3) SeroMAX WIDAL is only a Screening Test. For confirmation of results testing with SeroMAX WIDAL– T is recommended.
- 4) The correlation of test results with typical Clinical 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.
- 7) Bring all the reagents to room temperature before use.
- 8) Serum should not be inactivated.
- 9) Shake antigen vial well before use.
- 10) Avoid performing of the test directly under the fan.
- 11) In a non inoculated person the titre as high as 1 : 80 between 7th or 10th day of fever is of diagnostic value and the same titre increases gradually during subsequent period.
- 12) In an inoculated person the question of anamnestic response should always be born in mind and 'H' titre should not be taken into account for the purpose of diagnosis unless there is a rising titre of 'H' in subsequent period.

INTRODUCTION

Enteric fever occur when pathogenic microorganisms like *S. typhi*, *S. paratyphi A*, *paratyphi B*, *S. paratyphi C* infect the human body. During the course of disease, the body responds to this antigenic stimulus by producing antibodies whose titre rises slowly in early stages, to a maxima and then slowly falls till it is undetectable. Antibodies to Salmonella organisms may be detected in the patient serum from the second week after onset of infection. Information regarding the titres and whether or not they are rising or falling can be obtained by performing serological tests using Avecon antigen suspension. Usually tube titres of 1:80 and above are taken as diagnostically significant, however for endemic areas higher cut-offs may need to be established.

PRINCIPLE :

When the coloured, smooth suspension of attenuated WIDAL antigen suspensions are incubated with the patient serum, anti-Salmonella antibodies present in the patient's serum react with the antigen suspensions to produce agglutination.

Agglutination is a positive test result, indicating presence of Salmonella antibodies in the patient's serum. No agglutination is a negative test result indicating absence of Salmonella antibodies in the patient's serum.

REAGENT

SeroMAX WIDAL contains ready to use coloured, smooth antigen suspensions of the bacilli; *S. typhi* 'O', *S. typhi* 'H', *S. paratyphi* 'AH' *S. paratyphi* 'BH' *S. paratyphi* 'AO', *S. paratyphi* 'BO', *S. paratyphi* 'CH', *S. paratyphi* 'CO', and/or positive control reactive with these antigens. SeroMAX WIDAL reagents are versatile and standardized for use in a standard tube test procedure for the detection of *S. typhi* and *S. paratyphi* antibodies in the patient's serum. Each batch of reagents undergoes rigorous quality control at various stages of manufacture for its specificity and performance.

REAGENT STORAGE 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 label.

KIT CONTAINS

NaName of Reagent

<i>S. Typhi</i> 'O' Antigen	5 ml
<i>S. Typhi</i> 'H' Antigen	5 ml
<i>S. paratyphi</i> 'AH' Antigen	5 ml
<i>S. paratyphi</i> 'BH' Antigen	5 ml
<i>S. paratyphi</i> 'AO' Antigen	5 ml
<i>S. paratyphi</i> 'BO' Antigen	5 ml
<i>S. paratyphi</i> 'CO' Antigen	5 ml
<i>S. paratyphi</i> 'CH' Antigen	5 ml
Positive Control	0.7 ml
Negative Control	0.7 ml

Accessories :

Glass Slide	1 No.
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REAGENT RECONSTITUTION & STABILITY

Reagent are liquid stable no need for reconstitution. When the reagent is stored properly at 2-8°C & the contamination avoided, it is stable up to the expiry date mention on the label & kit box.

MATERIAL REQUIRED BUT NOT PROVIDED

Test tubes, Pasteur pipettes, Isotonic saline (0.9% NaCl Solution), Centrifuge, Timer, Mixing sticks, Sodium Hypochlorite (1%).

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.

SPECIMEN:

- (1). No special preparation of the patient is required prior to sample collection by approved techniques. Do not use haemolysed and turbid samples.
- (2). Clean and dry glassware free from detergents must be used for sample collection.
- (3). Do not heat inactivate the serum.
- (4). Though freshly collected serum is preferable, store samples at 2-8°C in case of delay in testing, for upto 72 hours.

TEST PROCEDURE :

1. RAPID SCREEN SLIDE TEST:-

- i) Place one drop of positive control onto a reaction circle of the slide.
- ii) Place 50 µl of physiological saline onto the next reaction circle of the slide.
- iii) Place one drop of patient's serum to be tested onto each of the required number of reaction circles.
- iv) Add one drop of appropriate Avecon antigen suspension to the reaction circles containing Positive control & physiological saline.
- v) Add one drop of appropriate Avecon antigen suspensions to the reaction circles containing the patient's serum.
- vi) Mix contents of each circle uniformly over the entire circle with separate mixing sticks.
- vii) Rock the slide gently back and forth, and observe for agglutination

macroscopically at one minute.

Interpretation of Result

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.

2. SLIDE SEMI-QUANTITATIVE METHOD

- i) Using a pipette place 80 µl, 40 µl, 20 µl, 10 µl, and 5 µl of patient serum to be tested on 5 different reaction circles on the slide. The corresponding titres obtained will be 1:20, 1:40, 1:80, 1:60, & 1:320 respectively.
- ii) Follow step No. 5-7 of slide screen method.

Note: This method is recommended for obtaining quick approximate titres only.

Interpretation of Result

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.

3. TUBE TECHNIQUE USING SLIDE ANTIGENS:

- Take appropriate number of set (as required; one set for each antigen suspension) of 8 Kahn tubes/ test tubes and label them 1 to 8.
- Pipette into tube No. 1 of all set 1.9 ml of physiological saline.
- To each of the remaining tubes (2 to 8) add 1 ml of physiological saline.
- To tube No. 1 of all sets add 0.1 ml of serum sample to be tested and mix well.
- Transfer 1 ml of the diluted serum sample from tube No. 1 to tube No. 2 and mix well.
- Transfer 1 ml of the diluted serum sample from tube No. 2 to tube No. 3 and mix well. Continue this serial dilution till tube No.7 in each set.
- Discard 1.0 ml of the diluted serum from tube No. 7 of each set.
- Now the dilution of the serum sample achieved from tube No. 1 to 7 respectively in each set is as follows: 1:20, 1:40, 1:80, 1:160, 1:320, 1:640, 1:1280. Tube No. 8 in all the sets, serves as a saline control.
- To all the tubes (1 to 8) of each set add one drop of the respective wellmixed Avecon antigen suspension from the reagent vials and mix well.
- Cover and incubate at 37 C overnight (approximately 18 hours).
- Dislodge the sedimented button gently and observe for agglutination.

Interpretation of Result

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

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	1.0 ml	1.0 ml	1.0 ml	1.0 ml
Diluted Serum	—	—	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
						Discard

REMARKS

- Positive result obtained in the slide test should be confirmed with the test to establish whether the titres are diagnostically significant or not.
- TAB vaccinated patient may show a high titre of antibodies to each of the antigens. Similarly, an amnesic response to other vaccines and unrelated fevers in case of patients who have had prior infection or immunization may give a false result.
- Agglutinins usually appear by the end of the first week of infection, blood sample taken earlier may give a negative result.
- A rising titre is more significant than a single titre. It is therefore necessary to evaluate two or more serum sample taken at 4-6 days intervals after the onset of the disease.
- 'O' being a somatic antigen bring about a coarse, compact, granular agglutination whereas 'h' being a flagellar antigen brings about larger, loose, flocculant agglutination.
- While the 'O' antigen is species specific, the 'H' antigen is specific to the serotype.
- 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 test.
- 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.
- False positive results are likely if the test is read more than one minute after mixing on the slide test.
- Any deviation in test procedure could result in variable result.

- Since techniques and standardization vary from lab one tube difference in tube titres can be expected.
- Use a separate disposable tip for each sample to prevent cross contamination.
- After usage the antigen suspension should be immediately recapped and replaced at 2-8 C
- It is recommended that result of the test should be correlated with clinical finding to arrive at the final diagnosis.
- The performance of the reagents should be validated occasionally using the positive control provided.

PERFORMANCE CHARACTERISTICS

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














WARRANTY

This product is designed to perform as described on the label and package insert. The manufacturer disclaims any implied warranty of use and sale for any other purpose.

REFERENCES:

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

Symbols Used on Pack

	Catalogue Number		Warning/Caution
	Batch No.		In vitro diagnostic device
	Manufacturing Date		Storage Limit
	Expiry Date		Consult instruction for use
	Manufacturer		Keep away from sunlight
	Keep Dry		Do not use if package is damaged
	Contains sufficient no. of test		