



GENESIS

Instructions For Use

Widal Reagent (Agglutination Method)

Product	Cat no.	TEST
Widal Test (with Positive Control)	5101 101	100 Test
s.typhi (O)	5101 201	100 Test
s.typhi (H)	5101 301	100 Test
s.paratyphi (AH)	5101 401	100 Test
s.paratyphi (BH)	5101 501	100 Test

INTENDED USE

Widal Reagent (Slide and tube Agglutination Test) is intended for the detection of anti-salmonella antibodies in human serum.

SUMMARY

Enteric fever occurs when pathogenic microorganisms like *S. typhi*, *S. paratyphi A*, *S. 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 widal antigen suspensions. 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.

REAGENT

Widal contains ready to use concentrated, smooth antigen suspensions of the bacilli:

	Reagents
1	s.typhi (O)
2	s.typhi (H)
3	s.paratyphi (AH)
4	s.paratyphi (BH)
5	Positive Control (Polyspecific positive control reactive with these antigens)

REAGENT STORAGE AND STABILITY

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

ADDITIONAL MATERIAL REQUIRED

Slide test method: Stop watch, Variable Micropipettes.

Quantitative method: Timer, Kahn tubes / test tubes, Pipettes (0.1ml, 1ml), Physiological saline, Incubator (37°C), Test tube rack.

PRINCIPLE

When the coloured, smooth, attenuated Widal antigen suspensions are mixed / incubated with patient serum, anti-salmonella antibodies present in the patient serum react with the antigen suspensions to give agglutination.

Agglutination is a positive test result, indicating presence of anti-salmonella antibodies in the patient serum. No agglutination is a negative test result indicating absence of anti-salmonella antibodies.

SAMPLE COLLECTION AND STORAGE

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.

Symbols in Product Labeling			
	Authorized Representative		Expiration date
	For in-vitro diagnostic use		CAUTION, consult instructions for use
	Catalogue number		Manufactured by
	Lot number		Temperature Limit
	Consult instructions for use		

TEST PROCEDURE

Bring reagents and samples to room temperature before testing. Shake and mix antigens well before dispensing.

Slide Screen Method

1. Place one drop of positive control onto a reaction circle of the slide.
2. Place 40 µl of physiological saline onto the next reaction circle of the slide.
3. Place one drop of patient's serum to be tested onto each of the required number of reaction circles.
4. Add one drop of appropriate Widal antigen suspension to the reaction circles containing Positive control & physiological saline.
5. Add one drop of appropriate Widal antigen suspensions to the reaction circles containing the patient's serum.
6. Mix contents of each circle uniformly over the entire circle with separate mixing sticks.
7. Rock the slide gently back and forth, and observe for agglutination macroscopically at **one minute**.

Slide Semi-Quantitative Method

1. 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, 1:160, & 1:320 respectively.
2. Follow step No. 5-7 of slide screen method.

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

Quantitative Method

Tube-test Procedure

1. Take appropriate number of sets (as required; one set for each antigen suspension) of 8 Kahn tubes / test tubes and label them 1 to 8.
2. Pipette into tube No. 1 of all sets 1.9 ml of physiological saline.
3. To each of the remaining tubes (2 to 8) add 1 ml of physiological saline.
4. To tube No. 1 of all sets add 0.1 ml of serum sample to be tested and mix well.
5. Transfer 1 ml of the diluted serum sample from tube No. 1 to tube No. 2 and mix well.
6. 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.
7. Discard 1.0 ml of the diluted serum from tube No.7 of each set.
8. Now the dilutions 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.
9. To all the tubes (1 to 8) of each set add one drop of the respective well-mixed Widal antigen suspensions from the reagent vials and mix well.
10. Cover and incubate at 37°C overnight (approximately 18 hours).

Dislodge the sedimented button gently and observe for agglutination

PERFORMANCE CHARACTERISTICS

1. The positive control antisera should produce 1+ or greater agglutination at 1: 80 in the slide and tube test when tested with the Widal antigen suspensions.
2. The negative control should show no agglutination with any of the Widal antigen suspensions.
3. Generally accepted performance characteristic of this type of test is 70% specificity and sensitivity.

BIBLIOGRAPHY

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

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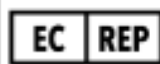


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