WIDAL

SALMONELLA STAINED SUSPENSION

INTENDED USE

Vitro rapid slide test for the qualitative and semi-quantitative determination of specific antibodies present in serum against Salmonella typhi O & H, Salmonella paratyphi AH, BH, CH, AO, BO & CO antigens.

METHOD

Slide agglutination test.

BACKGROUND

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 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 Vitro 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.

ASSAY PRINCIPLE

When the colored, smooth, attenuated VITRO 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.

REAGENTS

Vitro widal contains ready to use concentrated, vitally stained, smooth antigen suspensions of the bacilli;

S. typhi 'O', S. typhi 'H', S. paratyphi 'AO', S. paratyphi 'BO', S. paratyphi 'CO', S. paratyphi 'AH', S. paratyphi 'BH', S. paratyphi 'CH',

A polyspecific positive control reactive with these antigens.

Each batch of reagents undergoes rigorous quality control at various stages of manufacture for its specificity and performance.

REAGENT STORAGE AND STABILITY

Store the reagents at 2-8°C. DO NOT FREEZE.

The shelf life of reagents is as per the expiry date mentioned on the reagent vial labels.

SPECIMEN

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

PROCEDURE

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

Slide Screen Method

1. Place one drop of positive control onto a reaction circle of the glass slide.
2. Place one drop of isotonic saline onto the next reaction circle of the glass slide.
3. Place one drop of patient serum to be tested onto each of the required number of reaction circles.
4. Add one drop of appropriate VITRO antigen suspension to the reaction circles containing Positive control & isotonic saline.
5. Add one drop of appropriate VITRO antigen suspensions to the reaction circles containing the patient 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 glass 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 isotonic saline.
3. To each of the remaining tubes (2 to 8) add 1 ml of isotonic saline.
4. To tube No. 1 of all sets add 0.1 ml of serum sample to be tested and mix well.
5. Transfer 1.0 ml of the diluted serum sample from tube No. 1 to tube No.2 and mix well.
6. Transfer 1.0 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 VITRO 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.

Medical Device Safety Services
MDSS GmbH
Burckhardtstr. 1
30163 Hannover, Germany
Tel.: +49 511 6262 8630
Fax: +49 511 6262 8633

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INTERPRETATION OF RESULTS

Slide Screen Method

Agglutination is a positive test result and indicates presence of the corresponding antibody in the patient serum. No agglutination is a negative test result and indicates absence of the corresponding antibody in the patient serum.

Slide Semi-Quantitative Method

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.

Quantitative Method

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

PROCEDURAL NOTES

1. Positive results obtained in the slide test should be confirmed with the tube test to establish whether the titers are diagnostically significant or not.
2. TAB vaccinated patients may show a high titer of antibodies to each of the antigens.
3. 'O' being a somatic antigen brings about a coarse, compact, granular agglutination whereas 'H' being a flagellar antigen brings about larger, loose, flocculent agglutination.
4. While the 'O' antigen is species specific, the 'H' antigen is specific to the serotype.
5. Turbid and contaminated sera should not be used for testing.
6. Generally antibody titers 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.
7. It is recommended that results of the tests should be correlated with clinical findings to arrive at the final diagnosis.
8. Since techniques and standardization vary from lab to lab one tube difference in tube titers can be expected.

   The performance of the reagents should be validated occasionally using the positive control provided. Good physiological saline may be used as a negative control

WARNING & PRECAUTION

1. In vitro diagnostic reagent for laboratory and professional use only. Not for medicinal use.
2. The *S. typhi 'O'*, *S. paratyphi 'CO'* reagents contain 0.5% Phenol, *S typhi 'H', S. paratyphi 'AH', S. paratyphi 'BH'*, *S. paratyphi 'CH'* reagents contain 0.3% Formaldehyde and *S. paratyphi 'AO', S. paratyphi 'BO'* reagents contain 0.7% Ethanol as preservative. Avoid contact with skin and mucosa. On disposal flush with large quantities of water.
3. Only a clean and dry glass slide must be used. Clean the glass slide with distilled water and wipe dry.

BIBLIOGRAPHY

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5. Hull R., Hirsh H., Jay R., et al., Different intensities of oral anticoagulant therapy in the treatment of proximal-vein thrombosis. N. Engl. J. Med. 1982; 307: 1676-81.
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SYMBOL DECLARATION

	Manufacturer
	Consult instructions for use
LOT	Batch code (Lot #)
REF	Catalog number
	Temperature limitation
IVD	In vitro diagnostic medical device
	Use by
	Caution. Consult instructions
	Keep away from light

ORDERING INFORMATION

REF	Product	SIZE
5011	Salmonella typhi O	1 X 5 ml
5021	Salmonella typhi H	1 X 5 ml
5031	Salmonella paratyphi AH	1 X 5 ml
5041	Salmonella paratyphi BH	1 X 5 ml
5051	Salmonella paratyphi CH	1 X 5 ml
5061	Salmonella paratyphi AO	1 X 5 ml
5071	Salmonella paratyphi BO	1 X 5 ml
5081	Salmonella paratyphi CO	1 X 5 ml
	Vitro widal kit containing	
	Salmonella typhi O	5 X 5 ml
	Salmonella paratyphi BO	5 X 5 ml
	Vitro widal kit containing	
	Salmonella typhi O	1 X 5 ml
	Salmonella typhi H	1 X 5 ml
	Salmonella paratyphi A	1 X 5 ml
	Salmonella paratyphi B	1 X 5 ml
	Polyspecific positive control	1 X 0.5 ml
	Vitro widal kit containing	
	Salmonella typhi O	1 X 5 ml
	Salmonella typhi H	1 X 5 ml
	Salmonella paratyphi AH	1 X 5 ml
	Salmonella paratyphi BH	1 X 5 ml
	Salmonella paratyphi CH	1 X 5 ml
	Salmonella paratyphi AO	1 X 5 ml
	Salmonella paratyphi BO	1 X 5 ml
	Salmonella paratyphi CO	1 X 5 ml
	Polyspecific positive control	1 X 0.5 ml

Manufactured in Egypt by:
Vitro Scient
www. vitroscient.com

Technical Support:
+202 26439699
info@vitroscient.com

orders:
+202 26439698
order@vitroscient.com

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