Febrile Antigen

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

CLINICAL SIGNIFICANCE

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 titer 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.
Febrile diseases diagnostic may be assessed either by microorganism isolation in blood, stools or urine, or by titration of specific antibodies, somatic (O) and flagellar (H). The detection of these antibodies forms the basis for the long-established Widal test. This test dictates that a serum with high levels of agglutinating antibodies to O and H >1/100 is indicative of the infection with these microorganism.

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.

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', in glycine buffer, pH 8.2. Preservative.

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.
All the kit components are ready to use, and will remain stable until the expiration date printed on the label, when stored tightly closed at 2-8ºC and contaminations are prevented during their use. Do not freeze: frozen reagents could change the functionality of the test. Mix reagents gently before use. Reagents deterioration: Presence of particles and turbidity.

SPECIMEN

Fresh serum. Stable 7 days at 2-8ºC or 3 months at –20ºC. Samples with presence of fibrin should be centrifuged. Do not use highly hemolized or lipemic samples.
No special preparation of the patient is required prior to specimen collection by approved techniques. Do not use hemolyzed serum.

PROCEDURE

Bring reagent and samples to room temperature before use.

Qualitative Method

1. Bring reagents to room temperature before testing. The sensitivity of the test may be reduced at low temperatures.
2. Shake and mix antigens well before dispensing.

Slide Screen Method

1. Place one drop (50ml) of positive control onto a reaction circle of the glass slide.
2. Place one drop (50ml) of isotonic saline onto the next reaction circle of the glass slide.
3. Place one drop (50ml) of patient serum to be tested onto each of the required number of reaction circles.
4. Add one drop (50ml) 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.**

Semi Quantitative Method
Using a micropipette, deliver 80, 40, 20, 10 and 5 µL of undiluted serum into separate circles of the slide test.
Place 1 drop (50 µL) of the antigen to each circle next to the sample to be tested.
Mix with a disposable stirrer and spread over the entire area enclosed by the circle.
Place the slide on a mechanical rotator at 80-100 r.p.m., for 1 minute.

Tube agglutination method

1. Prepare a row of tube test for each sample as follows:

Dilutions	1/20	1/40	1/80	1/160	1/320	1/640	----
Sample (ml)	100	----	----	----	----	----	
NaCl 9g/L (ml)	1.9	1.0	1.0	1.0	1.0	1.0	
	1ml	1ml	1 ml	1 ml	1 ml	1 ml	1 ml discard

2. Prepare 2 tubes for Positive and Negative control: 0.1 mL Control + 0.9 mL NaCl 9 g/L.
3. Add a drop (50µL) of antigen suspension to each tube.
4. Mix thoroughly and incubate tube test at 37ºC for 24 h.

INTERPRETATION OF RESULTS

Qualitative Method (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.

Tube agglutination method
Examine macroscopically the pattern of agglutination and compare the results with those given by all control tubes. Positive control should give partial or complete agglutination. Negative Control should not give visible clumping. Partial or complete agglutination with variable degree of clearing of the supernatant fluid is recorded as a positive. The serum titer is defined as the highest dilution showing a positive result.

Vitro Scient, Industrial Area, Basatin Al Ismailia, Belbis, Alsharkia, Egypt.
Tel. +20552642664; Email: info@vitroscient.com; Website: www.vitroscient.com

EC REP | **Medical Device Safety Services**
MDSS GmbH, Burckhardtstr. 1
30163 Hannover, Germany.

QUALITY CONTROL

Positive and Negative controls are recommended to monitor the performance of the procedure, as well as a comparative pattern for a better result interpretation. All result different from the negative control result, will be considered as a positive.

EXPECTED VALUES

Salmonellas: Titers 1/80 (O antibodies) and 1/160 (H antibodies) indicates recent infection.

The level of "normal" agglutinins to these organisms varies in different countries and different communities. It is recommended that each laboratory establish its own reference range.

PERFORMANCE EVALUATION

There is not a Reference Material for the sensitivity standardization of these reagents. For this reason, Vitro Scient adjust the sensitivity of their reagents against to specific antisera and commercial reagents of certified quality. Prozone effect: False negative results may be obtained with sera containing a high titer of antibodies. A dilution of these sera will give a positive result.

The generally accepted performance characteristics of this type of test is 70% sensitivity and specificity. Reproducibility of the Micropath Range of Reagents is 100% (+/- one double dilution).

INTERFERING SUBSTANCES

Bilirubin (20 mg/dL), hemoglobin (10 g/L), lipids (10 g/L), rheumatoid factors (300 IU/mL) do not interfere. Other substances may interfere[7]

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

NOTES

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.

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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
50101	Salmonella typhi O	100 tests
50201	Salmonella typhi H	100 tests
50301	Salmonella paratyphi AH	100 tests
50401	Salmonella paratyphi BH	100 tests
50501	Salmonella paratyphi CH	100 tests
50601	Salmonella paratyphi AO	100 tests
50701	Salmonella paratyphi BO	100 tests
50801	Salmonella paratyphi CO	100 tests
50901	**Vitro widal kit** containing	
	Salmonella typhi O	1 X 100 tests
	Salmonella typhi H	1 X 100 tests
	Salmonella paratyphi AH	1 X 100 tests
	Salmonella paratyphi BH	1 X 100 tests
	Polyspecific positive	1 X 0.5 ml
51201	**Vitro widal kit** containing	
	Salmonella typhi O	1 X 100 tests
	Salmonella typhi H	1 X 100 tests
	Salmonella paratyphi AH	1 X 100 tests
	Salmonella paratyphi BH	1 X 100 tests
	Salmonella paratyphi CH	1 X 100 tests
	Salmonella paratyphi AO	1 X 100 tests
	Salmonella paratyphi BO	1 X 100 tests
	Salmonella paratyphi CO	1 X 100 tests
	Polyspecific positive	1 X 0.5 ml

*All kit sizes are available with or without accessories which include:

- Positive Control.
- Negative Control.
- Slide.
- Mixing Straws.

Vitro Scient, Industrial Area, Basatin Al Ismailia, Belbis, Alsharkia, Egypt.
Tel. +20552642664; Email: info@vitroscient.com; Website: www.vitroscient.com

EC REP **Medical Device Safety Services**
MDSS GmbH, Burckhardtstr. 1
30163 Hannover, Germany.