SPECTRUM

The Creative Approach to Bioscience

Widal Test (Salmonella Ab)

REF: 718 000	50 Test	2 x 2.5	ml
REF: 718 001	50 Test	4 x 2.5	ml
REF: 718 002	100 Test	4 x 5	ml
REF: 718 003	100 Test	2 x 5	ml (O,H)
REF: 718 004	100 Test	2 x 5	ml (O,BO)
REF: 718 005	100 Test	8 x 5	ml
REF: 718 006	100 Test	6 x 5	ml
REF: 718 002-O	100 Test	1 x 5	ml
REF: 718 002-H	100 Test	1 x 5	ml
REF: 718 002-AH	100 Test	1 x 5	ml
REF: 718 002-BH	100 Test	1 x 5	ml
REF: 718 002-AO	100 Test	1 x 5	ml
REF: 718 002-BO	100 Test	1 x 5	ml

Intended Use

Spectrum Widal set is intended for the detection of anti-salmonella antibodies in human serum.

Background

Ecteric 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 rise slowly in early stages, to a maximum 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 Spectrum salmonella 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, killed salmonella antigen suspensions are mixed/incubated with patient serum, anti-salmonella antibody 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

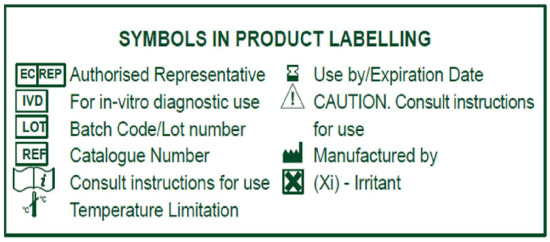
Spectrum widal kit contains ready to use concentrated, smooth antigen suspensions of the bacilli; S.typhi "O", S.typhi "H", S.paratyphi "AH", S.paratyphi "BH" , S.paratyphi "AO" , S.paratyphi "BO" , S.paratyphi "CO" ,S.paratyphi "CH" ,Negative control and polyspecific positive control reactive with these antigens. Each batch of reagents undergoes rigorous quality control at various stages of manufacture for its specificity and performance.

Reagent Storage and Stability

1. Store the reagents at 2 – 8^{O}C (Do not freeze).
2. the shelf life of reagent is as per the expiry date mentioned on the reagent vial labels.

Note

1. In vitro diagnostic reagent for laboratory and professional use only. Not for medicinal use.
2. The S. typhi "O" reagent contains 0.5% phenol, S.typhi "H". S.paratyphi "AH", S. paratyphi "BH", S. paratyphi "AO" , S. paratyphi "BO" ,S. paratyphi "CO" and S. paratyphi "CH" reagents contain 0.3% formaldehyde as preservatives. 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.



Specimen Collection and Storage

1. No special preparation of the patient is required prior to sample collection by approved techniques. Do not use haemolysed samples.
2. Clean and dry glassware free from detergents must be used for sample collection.
3. Don't heat inactivate the serum.
4. Freshly collected serum is preferable.Store sample at 2 – 8^{O}C in case of delay in testing for up to 72 hours.

Available Salmonella Antigens and Controls

1. S.typhi "O" Antigen suspension.
2. S.typhi "H" Antigen suspension.
3. S.paratyphi "AH" Antigen suspension.
4. S.paratyphi "BH" Antigen suspension.
5. S.paratyphi "AO" Antigen suspension.
6. S.paratyphi "BO" Antigen suspension.
7. S.paratyphi "CO" Antigen suspension.
8. S.paratyphi "CH" Antigen suspension.
9. Negative control.
10. Polyspecific positive control (Goat).

Additional material required

Slide test method: stop watch, variable micropipettes and mixing sticks.
Quantitative method: Time, Kahn Tubes / test tubes, pipettes (0.1 ml, 1 ml), isotonic saline, incubator (37^{O}C), test tube rack.

Procedure

(a) Bring reagents to room temperature before testing.
(b) 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 and onto another circle.
2. Place one drop one drop of negative control onto the next reaction circle of the glass slide.
3. Place one drop (50 µl) of patient serum to be tested onto each of the required number of reaction circle.
4. Add one drop of appropriate Spectrum Salmonella antigen suspension to the reaction circles containing positive control and negative control .
5. Add one drop of appropriate Spectrum Salmonella 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 80µl, 40µl, 20µl, 10µl and 5µl 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 and 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 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 all respective well mixed Spectrum Salmonella antigen suspensions from the reagent vials and mix well
10. Cover and incubate at 37^{O}C overnight (approximately 18 hours).
11. Dislodge the sedimented button gently and observe for agglutination.

Interpretation of the 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 Spectrum Salmonella antigen suspensions is the highest dilution of the serum sample that gives a visible agglutination.

Performance Characteristics

All the performance characteristics are found in the corresponding Technical Report and available on request

Remarks

1. Positive results obtained in the slide test should be confirmed with the tube test to establish whether the titres are diagnostically significant or not.
2. TAB vaccinated patients may show a high titre 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, flocculant 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 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.
7. Its 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 titres can be expected.
9. The performance of the reagents should be validated occasionally using the positive control provided. Good physiological saline may be used as a negative control.

ORDERING INFORMATION

CATALOG NO.	CONTENTS
718 000	1 x 2.5 ml Widal O 1 x 2.5 ml Widal H
718 001	1 x 2.5 ml Widal O 1 x 2.5 ml Widal H 1 x 2.5 ml Widal AH 1 x 2.5 ml Widal BH Polyspecific positive control
718 002	1 x 5 ml Widal O 1 x 5 ml Widal H 1 x 5 ml Widal AH 1 x 5 ml Widal BH Polyspecific positive control
718 003	1 x 5 ml Widal O 1 x 5 ml Widal H
718 004	1 x 5 ml Widal O 1 x 5 ml Widal BO
718 005	1 x 5 ml Widal O 1 x 5 ml Widal H 1 x 5 ml Widal AH 1 x 5 ml Widal BH 1 x 5 ml Widal AO 1 x 5 ml Widal BO 1 x 5 ml Widal CO 1 x 5 ml Widal CH Polyspecific positive control Negative control
718 006	1 x 5 ml Widal O 1 x 5 ml Widal H 1 x 5 ml Widal AH 1 x 5 ml Widal BH 1 x 5 ml Widal AO 1 x 5 ml Widal BO Polyspecific positive control Negative control
718 002-O 718 002-H 718 002-AH 718 002-BH 718 002-AO 718 002-BO	1 x 5 ml Widal O 1 x 5 ml Widal H 1 x 5 ml Widal AH 1 x 5 ml Widal BH 1 x 5 ml Widal AO 1 x 5 ml Widal BO

References

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

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