ACCUCARE
WIDAL ANTIGENS
FOR SLIDE & TUBE TESTS

Qualitative / Semi-quantitative determination of S.Typhi/S.P.Typhi in Serum
Only for *In Vitro Diagnostic* Use

ORDER INFORMATION

REF	CONT
WIS 3	8 x 5 ml

PRINCIPLE

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

REAGENT COMPOSITION

Widal contains ready to use concentrated, 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' and / or polyspecific positive control reactive with these antigens.

SAMPLE COLLECTION AND PRESERVATION

1. No special preparation of the patient is required prior to sample collection by approval 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 inactive the serum.
4. Though freshly collected serum is preferable, store samples at 2-8°C in case of delay in testing, for up to 72 hours.

ADDITIONAL MATERIAL REQUIRED

Slide test method: Stop watch, Variable Micropipettes.
Quantitative method: Timer, Kahn tubes / test tubes, pipettes (0.1 ml, 1 ml), Physiological saline, incubator (37°C), Test tube rack.

REAGENT STORAGE AND STABILITY

All reagents are stable up to the expiry date mentioned on the label when stored at 2-8℃. Do not freeze.

ASSAY PROCEDURE
PROCEDURE FOR SLIDE SCREEN METHOD:

1. Place one drop of positive control onto a reaction circle of the glass slide.
2. Place 50 µl of physiological saline on to the next reaction circle of the glass slide.
3. Place one drop of patient's serum to be tested on to each of the required number of reaction circles.
4. Add one drop of appropriated 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 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 titre 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 in to 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 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 sets , serves as a saline control.
9. To all 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).
11. Dislodge the sedimented button gently and observe for agglutination

INTERPRETATION OF RESULTS:

Slide Screen Method:

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.

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.

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Quantitative method

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

REMARKS

1. Positive results obtained in the slide test should be confirmed with the test tube 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. Similarly, an amnestic response to other vaccines and unrelated fevers in case of patients who have had prior infection or immunization may give a false result.
3. Agglutinins usually appear by the end of the first week of infection, blood sample taken earlier may give a negative result.
4. A rising titre is more significant than a single high titre. It is there fore necessary to evaluate two or more serum samples taken at 4-6 days. Intervals after the onset of the disease.
5. 'O' being a somatic antigen brings about a coarse, compact, granular agglutination whereas 'H' being a flagellar antigen brings about larger, loose, flocculant agglutination.
6. While the 'O' antigen is species specific, the 'H' antigen is specific to the serotype.
7. 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 tests.
8. 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.
9. False positive results are likely if the test is read more than one minute after mixing on the slide test.
10. Any deviation in test procedure could result in variable results.
11. Since techniques and standardization vary from lab to lab on tube difference in tube titres can be expected.
12. Use a separate disposable tip for each sample to prevent cross contamination.
13. After usage the antigen suspension should be immediately recapped and replaced at 2-8°C.
14. It is recommended that results of the tests should be correlated with clinical findings to arrive at the final diagnosis.
15. The performance of the reagents should be validated occasionally using the positive control provided. Good physiological saline may be used as a negative control.

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.
4. Reproducibility of Widal antigen suspensions is 100% (+/- one double dilution).

NOTE:

1. In vitro diagnostic reagent for laboratory and professional use only. Not for medicinal use.
2. The S. Typhi 'O', S. paratyphi 'CO' reagents contains 0.5% pheono, 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 along with 0.1% Sodium azide as preservatives. Avoid contact with skin and mucosa. Do not breathe vapour. In case of contact with eyes, rinse immediately with plenty of water and seek medical advice.
3. The reagent can be damaged due to microbial contamination or on exposure to extreme temperatures. It is recommended that the performance of the reagent to be verified with the positive and negative controls.
4. Shake the reagent vials well before use to disperse the antigen suspension uniformly and improve test readability.
5. Only clean and dry glass slides/tubes must be used. Clean the glass slide/tube with distilled water and dry.
6. It is necessary to use calibrated dropper provided I the reagent vial to dispense a reagent drop.
7. Widal antigen suspensions are not from human sources hence contamination due to HBsAg and HIV is practically excluded.
8. Accessories provided with the kit only must be used for optimum results.
9. Do not use damaged or leaking reagents.

BIBLIOGRAPHY

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2. Felix. (1942), Brit, Med.J., 11, 597.
3. Data on file: LAB-CARE DIAGNOSTICS (I) PVT. LTD.

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