**Swemed** DIAGNOSTICS

# WIDAL TEST

**INTRODUCTION:**
Enteric fever specific agglutinins (antibodies) are detected in patients after 15 days of fever. BCG vaccinated patient's serum may show elevated titre of all three 'H' agglutinins. Stained salmonella antigens are used to detect and identify specific antibodies in serum samples from patients suffering from enteric fever.

**PRINCIPLE:**
Bacterial suspension which carry antigen will agglutinate on exposure to antibodies to salmonella organisms.

**SAMPLE :**
Fresh seurm is preferred. In case of any delay performing the test, serum should be stored at $2^0$ - $8^0$ C.

**STORAGE & STABILITY OF REAGENTS:**
All reagents are ready to use and stable at $2^0$ – $8^0$ C till the expiry date.

**REAGENTS:**
1. Antigen suspension, S. typhi O.
2. Antigen suspension, S. typhi H.
3. Antigen suspension, S. paratyphi 'AH'.
4. Antigen suspension, S. paratyphi 'BH'.
5. Polyspecific positive control
6. Glass Slides with 6 reaction circles and Mixing sticks.

Bring all reagents to Room Temperature before testing. Shake well antigens before actual use.

**PROCEDURE:**
**SLIDE TEST**
1. Place one drop of positive control on one reaction circles of the slide.
2. Pipette one drop of Isotonic saline on the next reaction cirlcle. (-ve Control)
3. Pipette one drop of the patient serum tobe tested onto the remaining four reaction circles.
4. Add one drop of Widal TEST antigen suspension 'H' to the first two reaction circles. (PC & NC)
5. Add one drop each of 'O', 'H', 'AH' and 'BH' antigens to the remaining four reaction circles.
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 within one minute.

**SEMI QUANTITATIVE METHOD :**
1. Pipette one drop of isotonic saline into the first reaction circle and then place 5, 10, 20, 40, 80 ul of the test smple on the remaining circles.
2. Add to each reaction circle, a drop of the antigen which showed agglutination with the test sample in the screening method.
3. Using separate mixing sticks, mix th e contents of each circle uniformly over the reaction circles
4. Rock the slide gently back and forth, observe for agglutination macroscopically within one minute

| **STANDARD TUBE TEST METHOD** |
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| 1. Take 4 sets of 8 Kann tubes/test tubes and label them 1 to 8 for O, H, AH and BH antibody detection. |
| 2. Pipette into the tube No.1 of all sets 1.9 ml of isotonic saline. |
| 3.To each of the remaining tubes (2 to 8) add 1.0 ml of isotonic saline. |
| 4.To the tube No.1 tube in each row add 0.1 ml of the serum sample to be tested and mix well. |
| 5.Transfer 1.0 ml of the diluted serum from tube no.1 to tube no.2 and mix well. |
| 6. Transfer 1.0 ml of the diluted 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. Tube No.8 in all the sets, serves as a saline control. Now the dilution of the serum sample achieved in each set is as follows:<br>Tube No. : 1 2 3 4 5 6 7 8 (control)<br>Dilutions 1:20 1:40 1:80 1:160 1:320 1:640 1:1280 - |
| 9. To all the tubes (1 to 8) of each set add one drop of the respective WIDALTEST antigen suspension (O, H, AH and BH) from the reagent vials and mix well |
| 10. Cover the tubes and incubate at $37^0$ C overnight (approximately 18 hours) |
| 11.Dislodge the sedimented button gently and observe for agglutination |

**INTERPRETATION OF RESULTS:**
**SLIDE TEST METHOD**
Agglutination is a positive test result and if the positive reaction is observed with 20 ul of test sample, it indicates presence of clinically significant levels of the corresponding antibody in the patient serum.
No agglutination is a negative test result and indicates absence of clinically significant levels of the corresponding antibody in the patient serum.

**QUANTITATIVE METHOD**

The titre of the patient serum using Widal test antigen suspensions is the highest dilution of the serum sample that gives a visible agglutination. The sample which shows the titre of 1:80 or more should be considered as clinically significant.

**REFERENCES:**
Cruickshank, R(1962) Medical Microbiology, 12th Ed, P:4003.

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