WIDAL

(Slide Test Method)

Determination of Widal

CLINICALSIGNIFICANCE

Enteric fever occurs when pathogenic microorganisms like S. typhi, S. parathypiA, S. parathypi B infect the human body. During the course of disease, the body responds to this antigenic stimulation by producing antibodies whose titre rises slowly in early stages, to maxima and then slowly falls till it is undetectable. However in endemic areas, antibody titresupto 1:60 may be detectable in normal population. Antibodies to Salmonella organism 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 WIDAL antigen suspension.

PRINCIPLE OF THE METHOD

The Widal is a slide test for the measurement of typhoid fever in human serum.

When a patient's serum containing antibodies to S. typhi and S. paratyphi is mixed with the respective antigen, agglutination takes place which indicates the presence of antibodies to a particular antigen (O, H, AH, or BH).

REAGENT PROVIDED

1. Reagent Pack
2. Antigen Suspension S. typhi 'O'
3. Antigen Suspension S. typhi 'H'
4. Antigen Suspension S. parathyphi 'AH'
5. Antigen Suspension S. parathyphi 'BH'
6. Polyspecific positive control (Goat)

ACCESSORIES PACK

1. Glass slide with 6 reaction circles.
2. Mixing Sticks
3. Disposable sample dispensing pipettes with rubber teats

WORKING REAGENT PREPARATION

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

STORAGE AND STABILITY

All the components of the kit are stable until the expiration date on the label when stored at 2-8°C and contaminations are prevented during their use. Do not use reagents over the expiration date.**Do not freeze the reagents.**

SAMPLES

Fresh serum samples should be used. The samples can be stores at 2-8°C, if not immediately used.

ASSAY GUIDELINES

PROCEDURE FOR SLIDE METHOD:

A. Rapid Slide Test (Screening Test)

1. Clean the glass slide provided.
2. Place 1 drop of serum in each of the first four circles labeled 1 to 4.
3. Add one drop of antigen O, H, AH, BH, in circles 1, 2, 3, 4 respectively.
4. Mix the contents in each circle with separate sticks and spread it to fill the whole area of the circle.
5. Rock the slide gently for one minute and observe for agglutination. If agglutination is visible within one minute proceed to determine the titre of the particular antibody by quantitative slide test or quantitative tube test.

B. Quantitative Slide Test

1. On consecutive circles of a clean slide labeled 1 to 5, add 80µl, 40µl, 20µl, 10µl and 5µl of the test serum.
2. Add one drop of appropriate antigen suspension in each circle.
3. Mix well the serum and the antigen
4. Rotate the slide by hand for about a minute and observe for the agglutination.
5. The highest dilution of the serum which gives clear agglutination is the antibody titre. The serum volumes in the quantitative slide test correspond approximately to the tube titre as shown below.

Serum Volume	80µl	40µl	20µl	10µl	5µl
Titre	1:20	1:40	1:80	1:160	1:320

C. Confirmatory Quantitative Slide Test

1. Take a set of 8 clean dry tubes (10x75mm)
2. Dilute each serum sample as follows:

Test Tube	1	2	3	4	5	6	7	8
Serum Dilution	1:20	1:40	1:80	1:160	1:320	1:640	1:1280	Saline Control
Normal Saline	1.9 ml	1 ml	1 ml	1 ml	1 ml	1 ml	1 ml	1 ml
Patient's Serum								
(undivided)	0.1 ml	-	-	-	-	-	-	-
Transfer		-	-	-	-	-	-	
Diluted Serum	-	1 ml	1 ml	1 ml	1 ml	1 ml	1 ml	
Appropriate								
Antigen	1 drop	1 drop	1 drop	1 drop	1 drop	1 drop	1 drop	1 drop

3. Mix well and then incubate at 37°C for 16 to 20 hrs. and observe for agglutination.
4. Note the highest dilution showing clearly visible agglutination with naked eye.
5. Saline control should remain unchanged which indicates a negative test.

KEE DIAGNOSTICS

INTERPRETATION OF RESULTS:

Agglutination titre 1:80 or more is significant. An increase in titre 4 to 5 days after the first test is suggestive of an active infection.

PROCEDURE OF TUBE TEST:

A. Serum Dilution

1. Take 5 small test tubes and number them.
2. Add normal saline as indicated in the table below in all the five tubes.
3. Add 0.5 ml of the test serum to the first tube, mix and transfer 3.5 ml to the next tube (Tube no. 2). Transfer successively to tube no. 5 as indicated in the table.

Tube No.	1	2	3	4	5
Saline (ml)	7.0	3.5	3.5	3.5	3.5
Serum (ml)	0.5				
Mix and Transfer	3.5	3.5	3.5	3.5	3.5 Discard
Dilution	1:15	1:30	1:60	1:120	1:240

B. Titre Estimation

1. Arrange four rows of 6 small tubes in a test rack horizontally.
2. Add 0.5 ml of serum dilutions in the 5 test tubes in the sequence, i.e. 1:15 to 1: 240 in each row.
3. Addd isotonic saline in the 6th tube in each row.
4. Then add 0.05 ml of S. Typhi-O, S. Typhi-H, S. Paratyphi-AH, S. Paratyphi-BH antigens separatelt to each row horizontally.
5. Shake the tubes well and incubate at 37°C overnight for 16-20 hrs.
6. Observe for agglutination, 'O' antigen shows granular agglutination and 'H' antigen gives floccular appearance.

The highest dilution which gives the agglutination is the Titre.
Saline control should remain unchanged.

Test Tube	1	2	3	4	5	6
Initial Serum Dilution	1:20	1:40	1:80	1:160	1:320	-
Vol. of Serum Dilution (ml)	0.5	0.5	0.5	0.5	0.5	-
Saline (ml)	-	-	-	-	-	0.5
Appropriate Antiigen (ml)	0.05	0.05	0.05	0.05	0.05	0.05
Final Dilution	1:30	1:60	1:120	1:240	1:480	Control

INTERPRETATION OF RESULTS:

Agglutination Titre of 1:120 or more is significant. An increase in Titre, 4 to 5 days after the first test is suggestive of an active infection.

NOTES

1. Individuals vaccinated with TAB vaccine may show moderate titre against all three 'H' antigens.
2. Persons who suffered with enteric fever in the past may show moderate titre when suffering from other unrelated illness. Such anamanesic appearance of agglutination can be identified. If the patient is tested again after few days. Only a rising titre is indicative of true infection. The use of positive, negative and saline controls are recommended along with serum specimen.

REFERENCES

1. Felix A, Brit Med. J. 11, 597 (1942)
2. Protel, R.L. et al, Lancet, 11.330 (1971)
3. Cruicks, R. Medical Microbiology.
4. 12th Edition P.403 (1982)

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