PATHOZYME DIAGNOSTICS

RAPICHEK WIDAL ANTIGENS

(SLIDE AGGLUTINATION)

CAT No.: WAS

Reagent Kit For Qualitative & Quantitative Determination Of Salmonella Antibodies In Serum.

DIAGNOSTIC SIGNIFICANCE:

The infection is by ingestion of contaminated material like food, water, milk etc. The organisms (typhoid bacteria) pass through small intestine via lymphatics to mesenteric glands and then invade the blood stream. The specific agglutinins appear in serum of a patient suffering from enteric fever after 6 to 8 days of fever. 'Widal' Test is for identification of fever (P.U.O.) as enteric as well as one of the screening test for potential carriers of the disease.

PRINCIPLE:

A patient suffering from typhoid fever develops antibodies specific to the infecting organisms. Widal is a test for presence of these antibodies in significant concentration. The bacterial suspension (antigen) is mixed with patient's serum in various dilutions. Appearance of agglutination in highest dilutions determines the titer of the serum.

SPECIMEN COLLECTION:

Fresh serum should be used. In case of any delay, serum should be stored at 2-8^{0}C. The sample should not be inactivated.

KIT PRESENTATION:

PACK SIZE	2 + 2 X 5 ml	4 X 5 ml
S. Typhi 'O'	2 X 5 ml	5 ml
S. Typhi 'H'	2 X 5 ml	5 ml
S. Para Typhi 'A (H)'	-	5 ml
S. Para Typhi 'B (H)'	-	5 ml
Positive control	1 Vial	1 Vial
Glass Slide	1 Nos	1 Nos

REAGENT STORAGE & STABILITY:

All reagents included in the kit are stable at 2-8^{0}C until the expiry date stated on the label.

PRECAUTION:

1. Bring all the reagents to room temperature before use.
2. Serum should not be inactivated.
3. Use clean and dry glassware.
4. Include positive and negative control sera for greater proficiency in interpretation of results.
5. Shake antigen vial well before use.
6. Serum should be clear.

PROCEDURE:

(A) Rapid Slide Test (Widal Screening/Qualitative Test):

1. Use clean and dry glass slide.
2. Place one drop of undiluted serum in each of the first four circles (1-4).
3. Add one drop of antigen O, H, A(H). B(H) in circles 1, 2, 3, 4 respectively.
4. Mix the contents of each circle with separate stick and spread to fill the whole circle area.
5. Rock the slide for one minute and observe for agglutination.
6. If agglutination is visible within one minute, then proceed for quantitative estimation.

(B) Quantitative Slide Test:

Clean the glass slide supplied in the kit proceed as follows.

Circle No.	Serum Volume (ml)	Appropriate Antigen drop		Titre
1	0.08	1 Drop	Mix and rotate for one minute and observe agglutination	1:20
2	0.04	1 Drop		1:40
3	0.02	1 Drop		1:80
4	0.01	1 Drop		1:160
5	0.005	1 Drop		1:320

Repeat above procedure for visible agglutination observed in rapid slide screening test (which gives visible agglutination-Step 6 in procedure A).

INTERPRETATION:

O' Antigen shows granular agglutination.
All 'H' Antigens show floccular appearance.
Saline control suspension does not show agglutination and is a specimen for negative test result.
Agglutination titre greater than 1: 80 is suggestive of infection.

NOTE:

1. Do not read result beyond 1 minute.
2. Improper mixing and drying of reagents may lead to erroneous results.
3. Use a clean and dry glass slide only. Clean the glass slide with distilled water and wipe it dry.
4. While dispensing reagent, hold glass dropper vertically to get uniform drop size.
5. Include positive and negative controls for greater proficiency of result interpretation. Use normal saline as negative control.
6. Care should be taken to empty the dropper after use in order to avoid the possibilities of false positive results.
7. Do not interchange the bottle droppers.
8. Avoid performing the test directly under the fan.

PROCEDURE LIMITATIONS:

Rapid slide tests or quantitative slide tests are non-specific type of tests. The positive result should be further confirmed by tube test and other microbiological investigations.
In non-vaccinated population, the titre of 1:80 between 7th or 10th day of fever is of diagnostic value and the same titre increases gradually during subsequent period.
In vaccinated population, the question of anamnestic response should always be borne in mind and 'H' titre should not be taken into account for the purpose of diagnosis unless there is a rising titre of 'H' during subsequent period.

REFERENCE:

1. Felix, A., Brit. Med. Jr., 11, 1942; 597.
2. Protell, R.I. Et al., Lancet, 11, 1971; 330.
3. N. C. Dey, Medical Bacteriology, 6th edition, 1970; 259.

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