HiPer® Widal Test Teaching Kit (Slide Test)

Product Code: HTI017

Number of experiments that can be performed: 20

Duration of Experiment: 1hour

Storage Instructions:

- The kit is stable for 12 months from the date of manufacture
- Store all the antigen solutions, Test sample, positive and negative control at 2-8°C
- Other kit contents can be stored at room temperature (15-25°C)

ISO 9001-2015 CERTIFIED
WHO GMP CERTIFIED
ISO 13485-2016 CERTIFIED

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Index

Aim:

To detect the presence of *Salmonella* genus which causes enteric or Typhoid Fever by using qualitative slide agglutination test.

Introduction:

Widal test is a serological method to diagnose enteric fever or typhoid which is caused by the infection with pathogenic microorganisms like *Salmonella typhi*, *Salmonella paratyphi* A, B and C. This method of diagnostic test is based upon a visible agglutination reaction either in a test tube or on a slide between antibodies of patient serum and antigens specifically prepared from *Salmonella sp*.

Principle:

Enteric fever or typhoid is a life threatening disease which usually occurs due to the infection of pathogenic microorganisms, e.g. *Salmonella typhi*, *Salmonella paratyphi* A, B and C. These microorganisms are transmitted to human body through food and drinks contaminated with fecal matter. Early diagnosis and treatment for this fever are essential to avoid serious clinical complications. During the course of infection antibodies are produced against Salmonella antigens. Widal test, a serological method for the detection of *Salmonella sp*., was developed by F Widal in 1896. During this test a visible agglutination is formed due to the reaction in a test tube or on a slide between antibodies present in the infected person's blood sample and specific antigens of *S. typhi* and *S. paratyphi*. For the slide agglutination test, stained *Salmonella* antigens are used to detect the presence of specific agglutinin in the patient's serum. The slide agglutination test is used as a primary screening procedure.

The organisms causing enteric fever possesses two major antigens namely somatic antigen, O and a flagellar antigen, H along with another surface antigen, Vi. During infection antibodies are produced in patient's sera against *Salmonella typhi* O and H and *Salmonella paratyphi* AH and BH antigens. During infection antibodies are produced in patient's sera against these antigens. Antigens specifically prepared from this organism are used in the agglutination test to detect the presence of antibodies in patients' sera which are elucidated in response to infection by these bacteria. There are some agglutinins that are produced in the patient's serum during the fever period, which react with somatic antigen O of *Salmonella typhi*, A or B of *Salmonella paratyphi* and then with flagellar antigen H which is common in most of the *Salmonella* species. In this test four specific antigen suspensions are used e.g. *Salmonella typhi (* H antigen), *Salmonella typhi* (O antigen), *Salmonella paratyphi* - A and *Salmonella paratyphi* - B. If agglutination occurs with O antigen then it is considered positive for *Salmonella typhi*. If agglutination occurs in A or B antigen then it is confirmed as positive for *Salmonella paratyphi*. Agglutination will occur in H antigen circle for all the cases of antigens like O, A, and B. *Salmonella* species are characterized by three antigens present on the cell, as shown in figure 1.

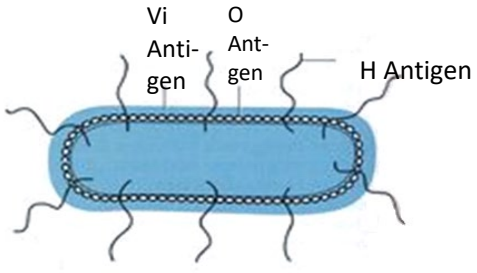


Fig 1: Antigenic structure of Salmonella

O Antigen: This is a somatic antigen and is present on the outer membrane of the cell. Its specificity is determined by the nature of the repeating units in the outer O-polysaccharide chain. Somatic antigens are heat stable, alcohol resistant and forms compact and granular clumps when mixed with O antisera.

Vi antigens: This is a virulence antigen which is a capsular polysaccharide that overlays the O antigen. This capsule is not necessary for infection but it increases the infectivity by making it less detectable by the body's immune system. It is heat labile and can be detected using Vi antisera. Vi antigen can interfere with O antigen testing.

H Antigens: This is a heat labile flagellar antigen which is inactivated both by boiling and alcohol. H antigens rapidly form fluffy clumps when treated with the corresponding antisera. H antigen induces rapid formation of corresponding antibodies as it is strongly immunogenic.

Widal Test Teaching Kit (Slide Test) utilizes the principle for rapid slide agglutination to detect the presence of *Salmonellatyphi* and *paratyphi*. The kit gives direct results in form of visible agglutination.

Kit Contents:

Table 1: Enlists the materials provided in this kit with their quantity and recommended storage

Sr. No.	Product Code	Materials Provided	Quantity	Storage
			20 expts	
1	TKC347	*Salmonella typhi* 'O' Antigen	1.6 ml	2-8^{0}C
2	TKC348	*Salmonella typhi* 'H' Antigen	1.6 ml	2-8^{0}C
3	TKC349	*Salmonella paratyphi* 'AH' Antigen	1.6 ml	2-8^{0}C
4	TKC350	*Salmonella paratyphi* 'BH' Antigen	1.6 ml	2-8^{0}C
5	TKC351	Positive control	0.6 ml	2-8^{0}C
6	TKC352	Negative control	0.6 ml	2-8^{0}C
7	TKC383	Test Serum Sample	2.4 ml	2-8^{0}C
8	TKC353	Slide for Widal	1 No.	RT
9	TKC125	Disposable Mixing Sticks	120 Nos.	RT

Materials Required But Not Provided:

Micropipettes, Tips, Gloves and Masks.

Storage:

HiPer® Widal Test Teaching Kit (Slide Test) is stable for 12 months from the date of manufacture without showing any reduction in performance. On receipt, store the Antigen solutions, Positive control and Negative control at 2-8°C.

Important Instructions:

1. Widal Slide Test Kit should be used according to the kit instructions.
2. Allow all reagents to reach room temperature before use.
3. Do not dilute any of the kit reagents.
4. Do not inter mix the reagents.
5. Do not freeze any of the kit reagents.
6. Ensure the slide is clean and dry prior to use.
7. Wear masks and gloves while handling the reagents.

Procedure:

1. Before starting the experiment, bring all reagents to room temperature and mix well.
2. Mark the circles of slides as PC (Positive control), NC (Negative control), O, H, AH, BH as per antigen solutions used for testing (as shown in figure 2).
3. Add a drop (25 µl) of positive control into the circle marked as PC of given slide.
4. Then add 25 µl of negative control into the reaction circle marked as NC.
5. Add 25 µl of test sample into each reaction circle labeled as O, H, AH, BH according to given antigen solution.
6. Add 25 µl of Antigen solution of *Salmonella typhi* 'H' into PC and NC circle each. Mix well using new mixing stick for each circle.
7. To circles labeled as O, H, AH, BH in which test samples have been added, add antigen solutions of *Salmonella typhi* 'O', *Salmonella typhi* 'H', *Salmonella paratyphi* 'AH' and *Salmonella paratyphi* 'BH', respectively.
8. Mix the content of each reaction circle uniformly with separate mixing stick.
9. Rock the slide gently (approximately for one minute) and observe for agglutination.

Note: After usage, wash the slide with appropriate disinfectant and let it dry. The slide is reusable.

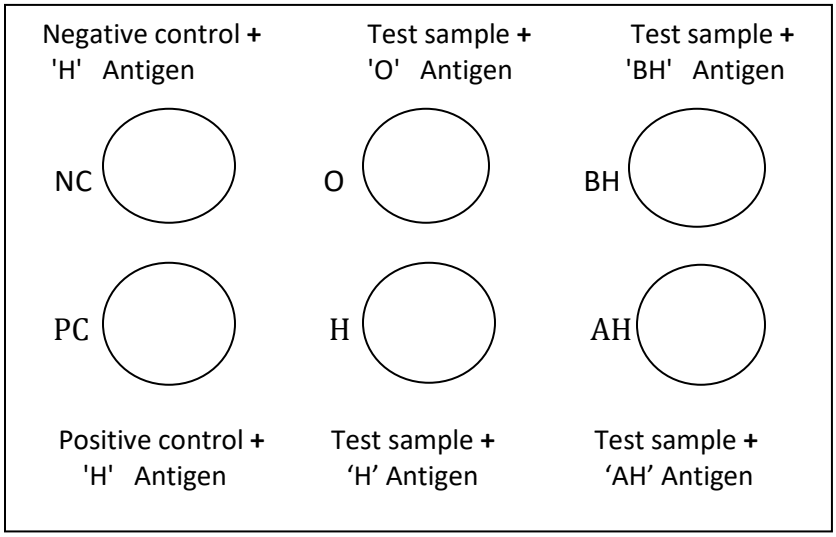


Fig 2: Diagrammatic representation of Widal Slide test

Observation and Result:

After mixing the test sample with Antigen Solution, Positive control, Negative control separately observe for the agglutination reaction.

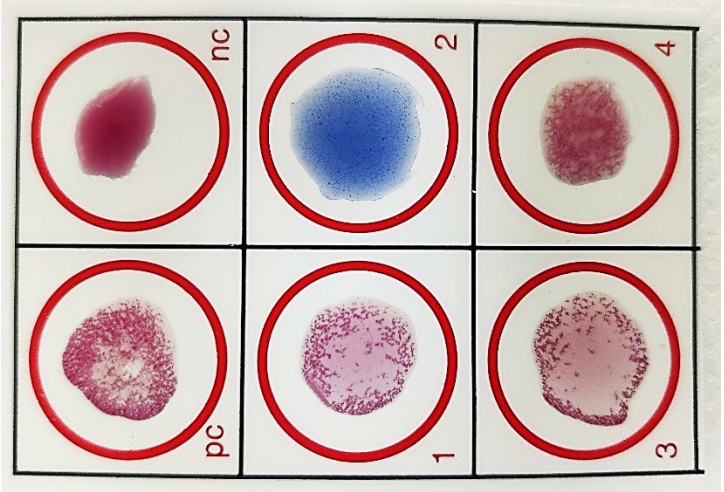


Fig 3: Agglutination reaction for different antigen solutions with Test sample

Well labeled as 'pc': Positive control + *Salmonella typhi* 'H' Antigen
Well labeled as 'nc': Negative control + *Salmonella typhi* 'H' Antigen
Well No. 1: Test Sample + *Salmonella typhi* 'H' Antigen
Well No. 2: Test Sample + *Salmonella typhi* 'O' Antigen
Well No. 3: Test Sample + *Salmonella typhi* 'AH' Antigen
Well No. 4: Test Sample + *Salmonella typhi* 'BH' Antigen

Interpretation:

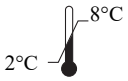
During the infection of *Salmonella*, the human body responds to the antigenic stimulus and as a result corresponding antibody are produced. When the test sample is treated with colored and attenuated Salmonella antigen suspensions, the antibodies present in the sample react with the antigen suspension to give clearly visible agglutination which can be seen through naked eye. Therefore, formation of agglutination indicates positive result which means the presence of anti-salmonella antibodies in the test sample. No agglutination is a negative test result which indicates the absence of anti-salmonella antibodies.

Troubleshooting Guide:

Sr. No.	Problem	Possible Cause	Solution
1	False positive result	The reagents were mixed with each other	Ensure that the reagents are added properly onto the respective well without mixing with other reagent.
		Incubated for a longer time	The results should be read within the time period mentioned in the brochure
2	No agglutination observed	The reagents are not stored under proper conditions	Ensure that the antigen solutions, positive and negative control are stored in refrigerator (2-8°C)

Technical Assistance:

At HiMedia we pride ourselves on the quality and availability of our technical support. For any kind of technical assistance mail at mb@himedialabs.com



Storage temperature

Do not use if package is damaged

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