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# SN Widal Test Teaching Kit

Solution Based

Innovation Speaks

# SN Widal Test Teaching Kit (Slide Test)

**Product Code: SNLS –TK216**

**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$^{o}$C
- Other kit contents can be stored at room temperature (15-25$^{o}$C)



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## Aim:

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

## Introduction:

The Widal test is a serological method used to diagnose typhoid and paratyphoid fever. It detects agglutination between Salmonella O and H antigens and antibodies in the patient's serum. A significant rise in antibody titers suggests active infection. The test can be performed using slide (qualitative) or tube (quantitative) methods. It is simple and widely used but may give false positives in endemic areas or vaccinated individuals.

## Principle:

Typhoid, also known as enteric fever, is a life-threatening disease caused by *Salmonella typhi* and *Salmonella paratyphi A*, B, and C. It typically spreads through ingestion of food or water contaminated with fecal matter, especially in areas with poor hygiene and sanitation. Upon entering the human body, these bacteria trigger an immune response that leads to the production of specific antibodies. The major antigens involved in this immune reaction are the somatic (O), flagellar (H), and the surface (Vi) antigens. During infection, the patient's serum contains agglutinins (antibodies) that react with these specific antigens. To detect these antibodies, the Widal test is performed, which is a serological agglutination test developed by F. Widal in 1896. This test is used to identify the presence of antibodies against Salmonella antigens in the patient's serum. The Widal test can be performed using either the tube agglutination method or the rapid slide agglutination method. In the slide method, stained *Salmonella* antigen suspensions are mixed with the patient's serum to observe visible agglutination. Agglutination indicates the presence of specific antibodies and helps confirm the infection. The test uses four types of antigen suspensions:

*S. typhi* O antigen
*S. typhi* H antigen
*S. paratyphi* A antigen
*S. paratyphi* B antigen

Agglutination with O antigen suggests infection with Salmonella typhi. Reactions with A or B antigens indicate infection with S. paratyphi A or B, respectively. The H antigen is common in most *Salmonella* species and may show agglutination in multiple types of infections. This test serves as a quick and cost-effective screening tool, especially in regions where typhoid is endemic. However, confirmation through culture methods or molecular diagnostics is often recommended for accuracy.

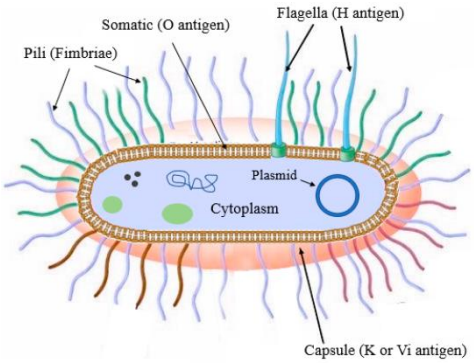



**Fig 1: Antigenic structure of Salmonella**

**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. | Materials Provided | Quantity | Storage |
|---|---|---|---|
| | | 20 expts | |
| 1 | *Salmonella typhi* 'O' Antigen | 1.6 ml | 2-8$^0$C |
| 2 | *Salmonella typhi* 'H' Antigen | 1.6 ml | 2-8$^0$C |
| 3 | *Salmonella paratyphi* 'AH' Antigen | 1.6 ml | 2-8$^0$C |
| 4 | *Salmonella paratyphi* 'BH' Antigen | 1.6 ml | 2-8$^0$C |
| 5 | Positive control | 0.6 ml | 2-8$^0$C |
| 6 | Negative control | 0.6 ml | 2-8$^0$C |
| 7 | Test Serum Sample | 2.4 ml | 2-8$^0$C |
| 8 | Slide for Widal | 1 No. | RT |
| 9 | Disposable Mixing Sticks | 120 Nos. | RT |

## Materials Required But Not Provided:

Micropipettes, Tips, Gloves and Masks.

## Storage:

SN 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$^o$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.**

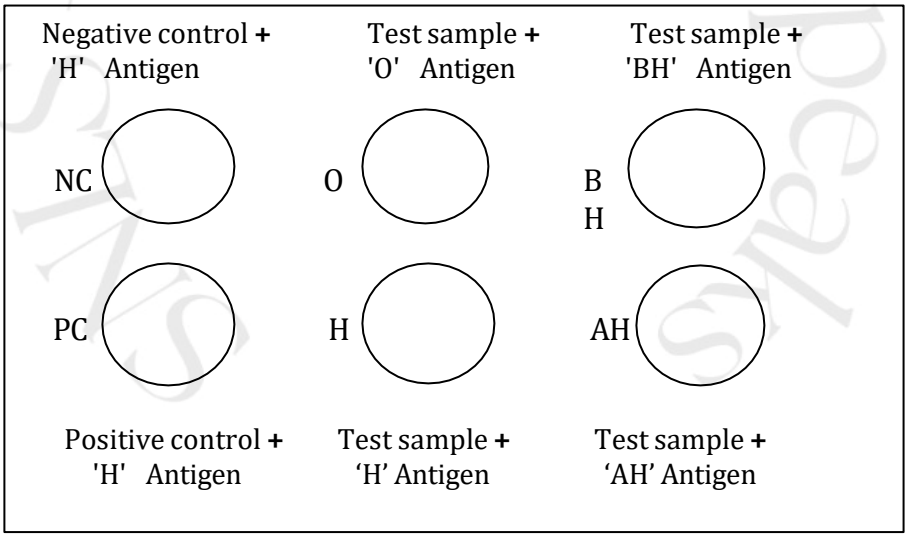



**Fig 2: Diagrammatic representation of Widal Slide test**

## Observation and Result:

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

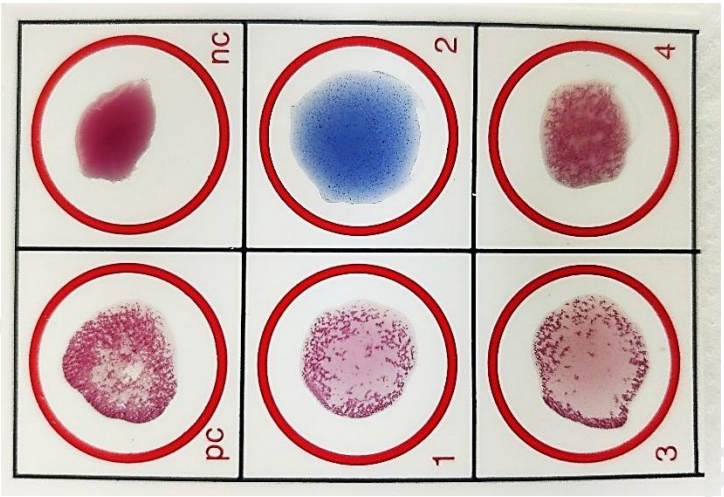



**Fig 3: Agglutination reaction for different antigen solutions with Test sample**

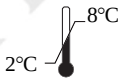
**Well labeled as 'pc':** Positive control + *Salmonella typhi* 'H' Antigen
**Well labeled as 'nc':** Negative control + *Salmonella typhi* 'H' Antigen
**Well No. 1:** Test Sample + *Salmonella typhi* 'H' Antigen
**Well No. 2:** Test Sample + *Salmonella typhi* 'O' Antigen
**Well No. 3:** Test Sample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




Storage temperature

Do not use if package is damaged





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