ASTAM DIAGNOSTICS

PRODUCT INSERT

REF WIDL

PRATHAM®
WIDAL Test

INTENDED USE

PRATHAM® WIDAL Test is a latex slide agglutination test intended used for the qualitative and semi-quantitative detection of antibodies ('O', 'H', 'AH', 'BH') of S.typhi or S. Paratyphi in human serum. For professional use only.

INTRODUCTION

Enteric fever occurs when pathogenic microorganisms like S. typhi, S. paratyphi A and S. paratyphi B infect the human body. During the course of disease, the body responds to this antigenic stimulus by producing antibodies whose titre rises slowly in early stages, to a maxima and then slowly falls till it is undetectable. Antibodies to Salmonella organisms 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 Pratham WIDAL antigen suspensions. Usually tube titres of 1:80 and above are taken as diagnostically significant, however for endemic areas higher cut-offs may need to be established.

TEST PRINCIPAL

PRATHAM® WIDAL Test contains ready to use concentrated, smooth antigen suspensions of the bacilli; S.typhi 'O', S. paratyphi 'AH' and S. paratyphi 'BH'. Agglutination reaction occurs when specimen containing corresponding antibodies is mixed with antigen suspension. Agglutination reaction indicates positive result. Usually specimen having titres of 1:80 and above are taken as diagnostically significant. No agglutination indicates negative result.

KIT COMPONENTS

1. S. typhi "O" Antigen
2. S. typhi "H" Antigen
3. S. Paratyphi "AH" Antigen
4. S. Paratyphi "BH" Antigen
5. Positive Control
6. Testing Slide
7. Product Insert

MATERIAL REQUIRED BUT NOT PROVIDED

Micro pipettes, Vortex mixer, Tissue paper, Normal saline, Test Tube, Centrifuge Marker, Timer, Specimen Collection Device.

STORAGE AND STABILITY

PRATHAM® WIDAL Test slide test components are stable up to expiry date indicated on the component label/box label. WIDAL kit needs to be stored at 2-8º C.

SPECIMEN COLLECTION AND STORAGE

1. Use fresh serum collected by centrifuging clotted blood.
2. If the test cannot be carried out on the same day, the serum may be stored between 2-8º C for no longer than 48 hours after collection. For longer periods the sample must be frozen.
3. As in all serological tests, hemolytic or contaminated serum must not be used.
4. Do not use plasma.

WARNING AND PRECAUTION

1. Bring all the reagents and samples to room temperature before use.
2. Shake all the antigens throughly before use.
3. Avoid using turbid, contaminated or inactivated serum.
4. Discard the reagent if they become contaminated or do or demonstrate correct activity with controls.
5. Do not interchange the reagents form other batches.

TEST PROCEDURE

A. QUALITATIVE TEST:

1. Allow the reagents and samples to reach room temperature. The sensitivity of the test may be reduced at low temperature.
2. Place 50µl of undiluted serum to be tested in each of first four circles (1-4).
3. Add one drop of antigen O, H, AH and BH in Circles 1,2,3,4 respectively.
4. Mix the contents of each circle with separate stick and spread to fill the entire circle area.
5. Rock the slide foe one minute and observe for agglutination.
6. If agglutination is visible within one minute, then proceed for quantitative estimation.

B. SEMI-QUANTITATIVE TEST:

1. Clean the glass slide supplied in the kit properly and wipe it free of moisture.
2. Place 0.005ml, 0.01ml, 0.02ml, 0.04ml, 0.08ml, of undiluted serum in 5 circles on the slide.
3. Add one drop of appropriate antigen suspension which showed agglutination in screening slide test to each of the above circles.
4. Mix the contents of each circle with separate stick and spread to fill the entire circle area.
5. Rotate the slide slowly for one minute and observe for agglutination.
6. The titre of the antibody is the highest dilution of serum upto which there is clear agglutination.
7. Repeat steps 1 to 6 with all the antigens, which showed agglutination is screening slide test.

Circle	Volume of serum	Volume of antigen	Titre
1	0.08 mL	1 drop	1:20
2	0.04 mL	1 drop	1:40
3	0.02 mL	1 drop	1:80
4	0.01 mL	1 drop	1:160
5	0.005 mL	1 drop	1:320

INTERPRETATION OF RESULTS

A. QUALITATIVE TEST:

Granular agglutination in case of 'O' and flocculating agglutination in case of H or A (H), or BH indicates positive reaction.

B. SEMI-QUANTITATIVE TEST:

A diagnostic titre of 1:80 suggest positive reaction.

INTERNAL QUALITY CONTROL

1. Positive and negative controls should be included in each test batch.
2. Acceptable performance is indicated when a uniform milky suspension with no agglutination in observed with the WIDAL Negative Control and agglutination with large aggregates is observed with the WIDAL Positive Control.

PERFORMANCE CHARACTERISTICS

1. Sensitivity: Titer greater than 1:80.
2. Specificity: Specificity was observed with mono specific as well as poly-specific antisera for all the four antigens. Cross reaction was not observed amongst the antigens.

LIMITATION OF THE TEST

- Bring all the reagents and samples to room temperature before use.
- Serum should not be inactivated.
- Use clean and dry glassware.
- Include positive and negative control sera (normal saline) for greater proficiency in interpretation of results.
- Shake antigen vial well before use.
- Test serum should be clear.
- Avoid performing the test directly under the fan.
- Before giving the final result, patient history should be taken into consideration.
- In non vaccinated persons the titre as high as 1;80 between 7th or 10th day of fever is of diagnostic value and the same titre increases gradually during subsequent period.

- In vaccinated persons 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' in subsequent period.
- Care should be taken to empty the dropper after use in order to avoid the possibilities of false positive results.

DISPOSAL

Consider all test run with human specimen as potentially infectious and discard using standard biosafety practices.

DISCLAIMER:

Whilst every precaution has been taken to ensure the diagnostic ability and accuracy of this product the product is used outside of the control of the Manufacturer and Distributor and the result may accordingly be affected by environmental factors and / or user error. A person who is the subject of the diagnosis should consult a doctor for further confirmation of the result.

The manufacturer and distributors of this product shall not be liable for any losses, liability, claims, coasts or damages whether direct or indirect or consequential out of or related to an incorrect diagnosis, whether positive or negative in the use of this product.

REFERENCES

1. Kenneth E.Sanderson, Shu-LinLiu1, LeTang and Randal N.Johnston. Kenneth E.Sanderson, Shu-LinLiu1, LeTang and Randal N.Johnston. Medical Microbiology (2 eds.). (2):1275-1306.
2. Parry CM, Hien TT, Dougan G, White NJ, Farrar JJ. Typhoid fever. NEngl J Med. 2002 (347); 22:1770-1782.
3. Song JH, Park M, Na DS, Moon HB, Pai CH. Detection of Salmonella typhi in the blood of patients with typhoid fever by PCR. J Clin Microbiol 1993;31:1439-1443.

SYMBOLS

	See instruction for use		Storage temeprature	IVD	For in-vitro diagnostic use
	Expiry date	REF	Catalogue number		Manufactured by
	Keep away from sunlight		Manufacturing date		No. of tests
	Do not reuse				

Manufactured By:
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