

LifescreeTMn - TYPHI - S

(STAINED WIDAL ANTIGENS)

CLINICAL SIGNIFICANCE:

When pathogenic microorganisms like *Salmonella typhi* & *Salmonella paratyphi A*, *S.paratyphi B*, *S.paratyphi C* infect the human body, the body responds by producing antibodies whose titre rises slowly in early stages to maxima and then tapers till it is undetectable. In enteric fever that follows *Salmonella* infection, once the patient is on medication it becomes difficult to isolate the organisms. Antibodies to *Salmonella* organisms may be detected in the patient serum from the second week after onset of infection.

The antigens of typhoid and paratyphoid consist of two distinct fractions, the stable somatic 'O' antigen and the labile flagellar 'H' antigen. The paratyphoid antigens are further classified into A&B species. In typhoid and paratyphoid, the 'H' antigen is type specific whereas the 'O' antigen is group specific.

The attenuated bacterial antigens provided for qualitative and semi-quantitative detection of *S.typhi* and *S.paratyphi* antibodies have different colours to facilitate the differentiation of antigens and clear reading of agglutination. It also avoids the possible error of mixing and/or misinterpretation. As undiluted serum is used in Slide Test, it is a simple, rapid and convenient screening test. The slide test antigens are standardized in such a way that they can be used for either slide or tube technique. In doubtful cases, it is recommended to perform the tube technique for obtaining conclusive results. 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. Diagnostically a rising antibody titre of at least four fold (two tube difference) is considered more significant than a single test. It is observed that individuals immunized with TAB vaccine may show a moderately high titre for all the antigens.

TEST PRINCIPLE:

LifescreeTMn-Typhi -S antigens are the ready to use, concentrated, smooth suspension of the bacilli, *S.typhi* 'O', *S.typhi* 'H', *S.paratyphi* 'AH' and *S.paratyphi* 'BH', along with a polyspecific positive control which reacts with these antigens. In serological tests the antibodies produced as a result of infection are detected by using these attenuated bacterial antigens. The antibodies from the patient's serum react with the corresponding antigens to cause clumping or agglutination.

KIT CONTENTS:

Reagent 1 *S.typhi* 'O' Antigen
 Reagent 2 *S.typhi* 'H' Antigen
 Reagent 3 *S.paratyphi* 'AH' antigen
 Reagent 4 *S.paratyphi* 'BH' antigen
 Reagent 5 Positive Control

Code No.
KTY1
(4x5ml)
 50T
 50T
 50T
 50T
 1 ml

ACCESSORIES:

White glass slide with 6- circles & glass droppers

SPECIMEN:

Fresh Serum. In case of a delay in testing, store at 2-8°C.

STABILITY & STORAGE :

All reagents are ready-to-use and are stable at 2-8°C till the expiry date mentioned on the labels.

PROCEDURE:

Rapid screening Slide Test:

- Place one drop of undiluted test serum in the first four circles (1-4) respectively.
- Add one drop of antigen 'O', 'H', 'AH' & 'BH' in circles 1, 2, 3 & 4 respectively.
- Mix the contents of each circle separately and spread it within the entire circle.
- Rock the slide gently for **one minute** and observe for agglutination.
- In circles 5 & 6, add a drop of positive control and saline respectively.
- Add one drop of any antigen separately in each of these circles.
- Mix as in the earlier circles and observe for agglutination / non agglutination, to validate the test results.

Semi-quantitative Slide Test:

- Using a pipette place 80µl, 40µl, 20µl, 10µl and 5µl of test serum on 5 different reaction circles (1-5), on the glass slide, respectively.
- The corresponding titres obtained will be 1:20, 1:40, 1:80, 1:160 & 1:320
- To each of the above circles, add one drop of the appropriate antigen, which gives agglutination in the screening slide test.
- Mix the contents of each circle separately and spread it in the entire circle.
- Rock the slide gently for **one minute** & observe for agglutination.

Tube technique using Slide Antigens (Quantitative Method) :

Perform the assay for all four antigens or for that which has given a positive result in the screening slide test.

Take a set of six test tubes (10x75mm) for each antigen. Dilute the serum sample and set up the test as indicated in the table.

Tube No.	1	2	3	4	5	6
Dilution	Saline Control	1:20	1:40	1:80	1:160	1:320
Normal Saline	1.0 ml	1.9 ml	1.0 ml	1.0 ml	1.0 ml	1.0 ml
Test Serum	----	0.1 ml				
Diluted Serum	----	-----	1.0 ml	1.0 ml	1.0 ml	1.0 ml
Appropriate Antigen	one drop	one drop	one drop	one drop	one drop	one drop

Mix well after each addition and incubate at 37°C for 16-20 hours. Observe for agglutination. The highest dilution of serum which shows clear-cut agglutination indicates the antibody titre.

INTERPRETATION OF RESULTS:

Screening Slide Test:

Granular agglutination in the case of 'O' antigen and flocculating agglutination in the case of 'H', 'AH' or 'BH' antigens indicates a positive reaction. This may be verified with the agglutination observed with the positive control provided and no agglutination with normal saline, which help to validate test results. No agglutination up to one minute is a negative test, and indicates the absence of corresponding antibodies.

Agglutination within **one minute** is a positive test, and indicates presence of corresponding antibodies. Then proceed for semi-quantitative slide or tube technique for determination of antibody titre.

Do not observe result after one minute

Semi-Quantitative Slide Test:

The lowest volume of serum, which shows clear agglutination, indicates the cut off level of the positive test and the corresponding antibody titre as per the tube technique is given below:

Serum Volume	Antibody Titre	Serum Volume	Antibody Titre
0.08 ml	1: 20	0.01 ml	1:160
0.04 ml	1: 40	0.005 ml	1:320
0.02 ml	1: 80		

Quantitative Method:

The titre of the patient serum using LifescreeTMn Typhi-S antigen is calculated as the highest dilution observed in the slide screening test that gives a visible agglutination.

NOTES:

- Bring all the reagents and samples to room temperature before use.
- Shake all the antigens thoroughly before use.
- Avoid using turbid, contaminated or inactivated serum.
- Sera from normal individuals may show agglutination up to 1:40 dilution.
- In a non inoculated person the titre as high as 1:80 between the 7th or 8th day of fever is of diagnostic value and the same titre increases gradually during subsequent period.
- In an inoculated person, the 'H' antigen titre should not be taken into account for the purpose of diagnosis unless there is a rising titre of the same during subsequent period.
- Lifescree Typhi- S is only a screening test. For confirmation of results, testing by the tube test and other microbiological investigations is recommended.
- Avoid performing the test directly under the fan.
- Care should be taken to empty the glass dropper after use in order to avoid the possibilities of false positive results.
- As with all diagnostic procedures, the physician should evaluate data obtained by use of this kit in light of other clinical information.
- For accuracy of results, the procedure has to be followed meticulously.

REFERENCES:

- Protell, R.I. *et al*, (1971), Lancet, 11:330
- Dey, N.C., (1970), Med. Bact., 259
- Cruickshank, R., (1982), Medical Microbiology, 12th Edition, 403.
- Felix, A., (1942), Brit. Med. J., 11: 597.



Kamineni Life Sciences Pvt. Ltd

Unit D 4-7, Industrial Estate, Moula-Ali,
 Hyderabad - 500 040.