CE

TYDAL®

WIDAL ANTIGEN SET / ANTIGENS FOR SLIDE AND TUBE TESTS

INTENDED USE

TYDAL® is a Widal slide and tube agglutination test that detects the presence of the serum agglutinins (O, H) in the patient's serum, with typhoid and paratyphoid fever.

SUMMARY

Enteric fever occurs when pathogenic microorganisms like *S. typhi, S. paratyphi A, S. paratyphi B, S. paratyphi C* 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 TYDAL® 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.

REAGENT

TYDAL® contains ready to use concentrated, smooth antigen suspensions of the bacilli; *S. typhi 'O', S. typhi 'H', S. paratyphi 'AO', S. paratyphi 'BO', S. paratyphi 'AH', S. paratyphi 'BH', S. paratyphi 'CH', S. paratyphi 'CO'* and / or polyspecific positive control reactive with these antigens.

Each batch of reagents undergoes rigorous quality control at various stages of manufacture for its specificity and performance.

REAGENT STORAGE AND STABILITY

1. *Store the reagents at 2-8°C. DO* NOT FREEZE .Keep the reagents away from direct sunlight.
2. *The shelf life of* reagents is as per the expiry date mentioned on the reagent vial labels. *Do not use* beyond expiry date.
3. Once opened the shelf life of the reagent vial is as described on the reagent vial label provided it is not contaminated.

PRESENTATION

Σ		4x5 ml	8x5 ml*	2x5 ml	2x5 ml	2x5 ml	2x2x 5 ml	2x2x 5 ml	5 ml	5 ml	5 ml	5 ml	5 ml	5 ml	5 ml	5 ml
REF		105200045	105200085	105210025	105200025	105220025	105210225	105200225	105220005	105230005	105240005	105250005	105280005	105260005	105270005	105290005
Antigens		O, H, AH, BH	O, H, AH, BH, CH, AO BO, CO	O, H	O, H	O, H	O, H	O, H	O	H	AO	BO	CO	AH	BH	CH
Control	+	0.4 ml	2.0 ml	0.4ml	0.4ml	0.4ml	0.4ml	0.4ml								
Control	-		2.0 ml		0.4ml	0.4ml		0.4ml								
MIXING STICKS LADDER		4	6		4		4	4								
DISPENSER PP TUBES		50	50		50		50	50								
RUBBER TEAT		1	1		1		1	1								
SLIDE		1	1		1		1	1								
PACKAGE INSERT		1	1	1	1	1	1	1	1	1	1	1	1	1	1	1

** 8 x 5 ml pack is marketed as TYDAL® PLUS.*

ADDITIONAL MATERIAL REQUIRED

Slide test method: Stop watch, Variable Micropipettes.
Quantitative method: Timer, Kahn tubes / test tubes, Pipettes (0.1ml, 1ml), Physiological saline, Incubator (37°C), Test tube rack.

PRINCIPLE

When the coloured, smooth, attenuated TYDAL® antigen suspensions are mixed / incubated with patient serum, anti-*Salmonella* antibodies present in the patient serum react with the antigen suspensions to give agglutination. Agglutination is a positive test result, indicating presence of anti-*Salmonella* antibodies in the patient serum. No agglutination is a negative test result indicating absence of anti-*Salmonella* antibodies.

NOTE

1. In vitro diagnostic reagent for laboratory and professional use only. Not for medicinal use.
2. *The S. typhi 'O', S. paratyphi 'CO'* reagents contain 0.5% Phenol, *S. typhi 'H', S. paratyphi 'AH', S. paratyphi 'BH', S. paratyphi 'CH'* reagents contain 0.3% Formaldehyde and *S. paratyphi 'AO', S. paratyphi 'BO'* reagents contain 0.7% Ethanol along with 0.1% Sodium azide as preservatives. Avoid contact with skin and mucosa. Do not breathe vapour. In case of contact with eyes, rinse immediately with plenty of water and seek medical advice. Sodium azide may react with lead and copper in plumbing and form highly explosive metal oxides, on disposal flush with large quantities of water.
3. The reagent can be damaged due to microbial contamination or on exposure to extreme temperatures. It is recommended that the performance of the reagent be verified with the positive and negative controls. Positive control provided with the kit only for TYDAL® 4 x 5 ml set (REF.: 105200045), 2 x 5 ml set (REF.: 105210025 & REF 105200025), 2 x 2 x 5 ml set (REF.: 105210225) and TYDAL® PLUS 8 x 5 ml set (REF.: 105200085). Negative Control provided with the kit only for 8 x 5 ml (REF.: 105200085), 2 x 5 ml (REF 105200025) and 2 x 2 x 5 ml (REF 105200225).
4. Shake the reagent vials well before use to disperse the antigen suspension uniformly and improve test readability.
5. Only clean and dry slides / tubes must be used. Clean the slide / tube with distilled water and dry.
6. It is necessary to use the calibrated dropper provided in the reagent vial to dispense a reagent drop.
7. TYDAL® antigen suspensions are not from human sources hence contamination due to HBsAg and HIV is practically excluded.
8. Accessories provided with the kit only must be used for optimum results. (Applicable only for TYDAL® 2 x 2 x 5 ml set (REF.: 105210225, 105200225), 2 x 5 ml set (REF.: 105200025), 4 x 5 ml set REF.: 105200045) and TYDAL® PLUS 8 x 5 ml set (REF.: 105200085).
9. Do not use damaged or leaking reagents.

SAMPLE COLLECTION AND STORAGE

1. No special preparation of the patient is required prior to sample collection by approved techniques. Do not use haemolysed and turbid samples.
2. Clean and dry glassware free from detergents must be used for sample collection.
3. Do not heat inactivate the serum.
4. Though freshly collected serum is preferable, store samples at 2-8°C in case of delay in testing, for upto 72 hours.

TEST PROCEDURE

Bring reagents and samples to room temperature before testing.
Shake and mix antigens well before dispensing.

Slide Screen Method

1. Place one drop of positive control onto a reaction circle of the slide.
2. Place 50 µl of physiological saline onto the next reaction circle of the slide.
3. Place one drop of patient's serum to be tested onto each of the required number of reaction circles.
4. Add one drop of appropriate TYDAL® antigen suspension to the reaction circles containing Positive control & physiological saline.
5. Add one drop of appropriate TYDAL® antigen suspensions to the reaction circles containing the patient's serum.
6. Mix contents of each circle uniformly over the entire circle with separate mixing sticks.
7. Rock the slide gently back and forth, and observe for agglutination **macroscopically at one minute.**

Slide Semi-Quantitative Method

1. Using a pipette place 80 µl, 40 µl, 20 µl, 10 µl, and 5 µl of patient serum to be tested on 5 different reaction circles on the slide. The corresponding titres obtained will be 1:20, 1:40, 1:80, 1:160, & 1:320 respectively.
2. Follow step No. 5-7 of slide screen method.

Note: This method is recommended for obtaining quick approximate titres only.

Quantitative Method

Tube-test Procedure

1. Take appropriate number of sets (as required; one set for each antigen suspension) of 8 Kahn tubes / test tubes and label them 1 to 8.
2. Pipette into tube No. 1 of all sets 1.9 ml of physiological saline.
3. To each of the remaining tubes (2 to 8) add 1 ml of physiological saline.
4. To tube No. 1 of all sets add 0.1 ml of serum sample to be tested and mix well.
5. Transfer 1 ml of the diluted serum sample from tube No. 1 to tube No. 2 and mix well.
6. Transfer 1 ml of the diluted serum sample from tube No. 2 to tube No. 3 and mix well. Continue this serial dilution till tube No. 7 in each set.
7. Discard 1.0 ml of the diluted serum from tube No.7 of each set.
8. Now the dilutions of the serum sample achieved from tube No. 1 to 7 respectively in each set is as follows: 1:20, 1:40, 1:80, 1:160, 1: 320, 1:640, 1: 1280. Tube No. 8 in all the sets, serves as a saline control.
9. To all the tubes (1 to 8) of each set add one drop of the respective well-mixed TYDAL® antigen suspensions from the reagent vials and mix well.
10. Cover and incubate at 37°C overnight (approximately 18 hours).
11. Dislodge the sedimented button gently and observe for agglutination.

INTERPRETATION OF RESULTS

Slide Screen Method

Agglutination is a positive test result and indicates presence of the corresponding antibody in the patient's serum. No agglutination is a negative test result and indicates absence of the corresponding antibody in the patient serum.

Slide Semi-Quantitative Method

Agglutination is a positive test result. The titre of the patient serum corresponds to the visible agglutination in the test circle with the least amount of serum sample.

Quantitative Method

The titre of the patient serum using TYDAL® antigen suspensions is the highest dilution of the serum sample that gives a visible agglutination.

REMARKS

1. Positive results obtained in the slide test should be confirmed with the tube test to establish whether the titres are diagnostically significant or not.
2. TAB vaccinated patients may show a high titre of antibodies to each of the antigens. Similarly, an amnestic response to other vaccines and unrelated fevers in case of patients who have had prior infection or immunization may give a false result.
3. Agglutinins usually appear by the end of the first week of infection, blood sample taken earlier may give a negative result.
4. A rising titre is more significant than a single high titre. It is therefore necessary to evaluate two or more serum samples taken at 4- 6 days intervals after the onset of the disease.
5. 'O' being a somatic antigen brings about a coarse, compact, granular agglutination whereas 'H' being a flagellar antigen brings about larger, loose, flocculant agglutination.
6. While the 'O' antigen is species specific, the 'H' antigen is specific to the serotype.
7. Serological findings are not intended as a substitute for culture. An appropriate attempt should be made to recover and identify the etiologic organisms through various culture and biochemical tests.
8. Generally antibody titres of 1:80 or more are considered clinically and diagnostically significant. However the significant titre may vary from population to population and needs to be established for each area.

9. False positive results are likely if the test is read more than one minute after mixing on the slide test.
10. Any deviation in test procedure could result in variable results.
11. Since techniques and standardization vary from lab to lab one tube difference in tube titres can be expected.
12. Use a separate disposable tip for each sample to prevent cross contamination.
13. After usage the antigen suspension should be immediately recapped and replaced at 2-8°C.
14. It is recommended that results of the tests should be correlated with clinical findings to arrive at the final diagnosis.
15. The performance of the reagents should be validated occasionally using the positive control provided. Good physiological saline may be used as a negative control.

PERFORMANCE CHARACTERISTICS

1. The positive control antisera should produce 1+ or greater agglutination at 1: 80 in the slide and tube test when tested with the TYDAL® antigen suspensions.
2. The negative control should show no agglutination with any of the TYDAL® antigen suspensions.
3. Generally accepted performance characteristic of this type of test is 70% specificity and sensitivity.
4. Reproducibility of TYDAL® antigen suspensions is 100% (+/- one double dilution).

WARRANTY

This product is designed to perform as described on the label and package insert. The manufacturer disclaims any implied warranty of use and sale for any other purpose.

BIBILIOGRAPHY

(1). Cruickshank R., (1982), Medical Microbiology, 12th Edition, 403. (2).Felix A., (1942), Brit. Med. J., 11, 597-600. (3). Data on file: Tulip Diagnostics (P) Ltd.

SYMBOL KEYS

Temperature limitation	Manufacturer	Contains sufficient for <n> tests
Use By	Consult Instructions for use	This way up
Date of Manufacture	REF Catalogue Number	CONTROL + Positive control
LOT Batch Number/ Lot Number	IVD *In vitro* Diagnostic Medical Device	CONTROL - Negative control
Danger Formaldehyde H350-H317 P201;P281; P308+P313 P280;P333+P313;P363	May be fatal if swallowed or enters airways Harmful if inhaled, or if swallowed or if in contact with skin May cause irritation to skin and/or eyes May cause irritation to the airways and/or drowsiness or dizziness. May cause an allergic skin reaction	EC REP Authorised Representative in the European Community

Manufactured by
TULIP DIAGNOSTICS (P) LTD.

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620-80421/VER-05