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TULIP's FEBRILE ANTIGEN SET™

(Febrile Antigen panel for serodiagnosis of antibodies to *S.typhi, S.paratyphi*, *Brucella* and *Proteus* antigens)

INTENDED USE

TULIP'S FEBRILE ANTIGEN SET™ can be used for the detection of antibodies produced in certain febrile diseases such as *Salmonellosis* (typhoid and paratyphoid), *Brucellosis* and *Rickettsial* diseases. Febrile antigen tests are serological applications of the classical Widal reaction for the diagnosis of typhoid and paratyphoid fever and the Weil-Felix test reactions where antigens prepared from *Proteus* organisms are used to detect related rickettsial antibodies.

SUMMARY

Serological diagnosis of patients suspected of having infectious diseases characterized by persistent fever is dependent upon demonstration of an agglutination reaction between the appropriate antigen and antibody.

The natural response to the invasion of pathogenic organisms is the production of antibodies. This immune response is highly individualized and in addition to the host's physiological status and genetic capabilities, a number of other factors are involved in the production of antibodies to the particular stimulus. These include the antigenicity of the organism, the total amount introduced to the host and the route of introduction, and whether the host has had previous exposure to the organism. These factors will determine the rate of antibody formation, the amount of antibodies produced and their persistence in the circulatory system.

The patient's serum is tested directly for specific antibodies by either slide or tube agglutination test. These tests are qualitative and semi-quantitative. The rapid slide test is used primarily as a screening procedure especially useful when large numbers of sera must be examined. The tube test should be used to confirm positive results obtained by the slide test.

REAGENT

TULIP'S FEBRILE ANTIGEN SET™ contains ready to use standardized, killed, stained, smooth antigen suspensions of the *Salmonella* bacilli; *S. typhi O, S. typhi H*, *S. paratyphi* AH, *S.paratyphi BH* antigens, *Brucella abortus* antigen and *Proteus OX19* antigen along with a polyspecific Febrile antigen positive control reactive with these antigens and Febrile antigen negative control nonreactive with these antigen.

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 reagent at 2-8^{0}C. DO NOT FREEZE. Keep the reagents away from direct sunlight.
2. The shelf life of reagent 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		6 x 5 ml
REF		105910065
Antigens		*S. typhi O, S. typhi H, S. paratyphi AH, S. paratyphi BH, Brucella abortus, Proteus OX19*
Control	+	1.0 ml
Control	-	1.0 ml
MIXING STICKS LADDER		4
DISPENSER PP TUBES		50
RUBBER TEAT		1
SLIDE		1
PACKAGE INSERT		1

PRINCIPLE

When the coloured, smooth attenuated TULIP'S FEBRILE ANTIGEN SET™ suspension is mixed / incubated with patient's serum, antibodies to the febrile antigen if present in the patient's serum reacts with the antigen suspension to produce an agglutination. Agglutination is a positive test result, indicating presence of antibodies to the febrile antigen in the patient's sample. No agglutination is a negative test result indicating absence of specific antibodies to the febrile antigen.

NOTE

(1). In vitro diagnostic reagent for laboratory and professional use only. Not for medicinal use. Keep the reagents away from direct sunlight.(2). The *S.typhi* 'O' reagent contains 0.5% Phenol, *S.typhi* 'H', *S.typhi* 'AH', *S.typhi* 'BH' reagents contain 0.3% Formaldehyde as preservative. *Proteus* OX19 antigen contains 0.1% sodium azide as preservative and *Brucella abortus* antigen contains 0.01% thimerosal as preservative. Polyspecific febrile antigen positive control contains 0.1% sodium azide as preservative. Negative control contains 0.1% sodium azide as preservative. Avoid contact with skin and mucosa. 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 positive and negative controls. (4). Shake the reagent vials well before use to disperse the antigen suspension uniformly and improve test performance. (5). Only clean and dry glass slides/tubes must be used. Clean the glass 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). The antigens of TULIP'S FEBRILE ANTIGEN SET™ are not from human sources hence contamination due to HBsAg and HIV is practically excluded. (8). 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 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^{0}C in case of delay in testing.

MATERIAL PROVIDED WITH THE KIT

Reagent pack

(1). *S.typhi* 'O' antigen (2). *S.typhi* 'H' antigen (3).*S. paratyphi* 'AH' antigen (4). *S. paratyphi* 'BH' antigen (5). *Brucella abortus* antigen (6). *Proteus* OX19 antigen (7). Polyspecific febrile antigen positive control (8). Febrile antigen negative control

ADDITIONAL MATERIAL REQUIRED

Slide Test Method: Stopwatch, appropriate pipettes/micropipettes,and high intensity direct light source.

Quantitative Method: Timer, test tubes (12 x 75 mm), test tube rack, appropriate pipettes/micropipettes, Incubator (37^{0}C), physiological saline.

TEST PROCEDURE

1. Bring all reagents to room temperature before testing.
2. Shake and mix antigens well before dispensing.

Rapid Slide screening method

1. Place one drop of Tulip's Febrile antigen polyspecific positive control onto the reaction circle of the glass slide.
2. Place one drop of Tulip's Febrile antigen negative control onto the next reaction circle of the glass slide.
3. Place one drop of patient serum to be tested onto each of the required number of reaction circles.
4. Add one drop of the appropriate Tulip's Febrile Antigen suspension to each circle using the reagent dropper.
5. Mix contents of each circle uniformly over the entire circle with separate mixing sticks.
6. Rock the slide gently, back and forth, and observe for agglutination macroscopically **at one minute.**
7. Repeat steps 1 to 6 for each antigen.

Semi-quantitative method

1. Using a pipette place 80 µl, 40 µl, 20 µl, 10 µl and 5 µl of patient serum or polyspecific positive control to be tested on 5 different circles on the glass slide. The corresponding titres obtained will be 1:20, 1:40, 1:80, 1:160, and 1:320 respectively.
2. Follow steps 4-7 of slide screen method.

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

Tube test method

1. Take 8 Test tubes and label them 1 to 8.
2. Pipette 1.9 ml of isotonic saline to tube No.1.
3. To each of the remaining tubes (2-7) add 1.0 ml of isotonic saline.
4. To the tube No. 1 add 0.1 ml of serum sample to be tested. Mix well.
5. Transfer 1.0 ml of the diluted serum from tube No.1 to tube No.2 and mix well.
6. Transfer 1.0 ml of the diluted serum from tube No.2 to tube No. 3 and mix well. Continue this serial dilution till tube No.7.
7. Discard 1.0 ml of the diluted serum from tube No. 7.
8. Pipette 1.0 ml of isotonic saline in tube No. 8, which serves as a negative control.
9. To all the tubes add 1 drop of appropriate Tulip's Febrile Antigen suspension and mix well. Repeat steps 1-9 for testing other antigens if required.
10. Cover the tubes and incubate at 37^{0}C overnight (approximately 18 hours)
11. Dislodge the sedimented button gently and observe for agglutination macroscopically in each tube of the dilution series.

INTERPRETATION OF RESULTS

Rapid Slide screening method

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

Semi-quantitative method

The reactions obtained are roughly equivalent to those which would occur in a tube agglutination test with serum dilutions of 1:20, 1:40,1:80, 1:160, 1:320 respectively. If a positive reaction is observed it is advisable to confirm the result and establish the titre by a tube test. A tube test is indicated when results do not conform to clinical findings. False results may be obtained if reagents are not allowed to reach room temperature (22-30^{0}C) before use. False positive reactions are also likely if the test is read beyond one minute after mixing.

Quantitative method

The titre of the patient serum using Tulip's Febrile Antigen suspensions is the highest dilution of the serum sample that gives a visible agglutination.

Note:

The chart below gives an approximate indication of the significance of serum titres

Infection	Febrile antigens	Serum agglutinins		
		Appear	Maximum	Titre and significance
Typhoid fever	*S.typhi 'O'* *S.typhi 'H'*	7 to 10 days later	3 to 5 weeks later	1:80* (in early stages) = suspicious 1:160* and rising=strongly suggestive 1:40*=suspicious 1:160*=strongly suggestive
Paratyphoid fever	*S.typhi 'AH'* *S.typhi 'BH'*	Those characterized by prolonged fever and typhoid like symptoms present. Antibodies of titres similar to above; lower titres may be more significant depending on the prevalence of a particular Salmonella species		
Typhus fever	*Proteus* OX19	7 to 10 days	By 14th day	1:40 to 1:80 (in early stages)= suspicious 1:160 = strongly suggestive
Rocky mountain fever	*Proteus* OX19	7 to 10 days	By 14th day	Peak titres usually not above 1:160 to 1:320
Brucellosis	*Brucella abortus*	2 to 3 weeks	3 to 5 weeks	1:80 to 1:160= strongly suggestive

* May be higher in vaccinated individuals

Analysis of results of Proteus OX19 antigen

Agglutination pattern for several rickettsial diseases are shown in the chart below:

Infection	Vector	Proteus OX19 antigen suspension
Epidemic typhus	Louse	+++
Murine typhus	Flea	+++
Endemic typhus	Flea	+++
Rocky mountain spotted fever	Tick	+++
Tsutsugamushi fever	Mite	-
Scrub typhus fever	Mite	-
Boutonneuse fever	Tick	+
South-African tick-bit fever	Tick	+
Brills, disease	Louse	Usually negative
Trench fever	Louse	-
Q fever	Tick	-

REMARKS

(1). Sera from normal patients may show positive agglutination with febrile antigens due to previous immunization, past infection or the presence of antibodies to related antigens. In general the titres found in these cases will be lower and remain at a constant level. (2). Titres detected as a result of active infection or recent immunization with an organism containing homologous antigens will be higher and tend to rise over a period of time. It is therefore, necessary to evaluate two or more serum samples at 3- to 5-day interval after the onset of disease. A progressive (four-fold) increase in antibody titer is indicative of recent infection or immunization. (3). Positive results obtained in the slide test should be confirmed with the tube test to establish whether the titres are diagnostically significant or not. (4). Cross reactions, previous vaccinations, amnestic responses, antibiotic therapy, other diseases known or unknown, prozones , and autoagglutinations, as well as other factors, may affect results. (5). In certain geographic regions and occupations, typhoid fever, *Salmonella* and *Brucella* are endemic and high level of natural agglutinins may be present. (6). 'O' being a somatic antigen brings about coarse, compact, granular agglutination, whereas 'H' being a flagellar antigen brings about larger, loose, flocculant agglutination. (7). 'H' antigen, being species specific, is more reliable in determining the type of infection. (8).Turbid and contaminated sera should not be used for testing. (9).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. (10). Weil Felix reactions may vary unduly from case to case of spotted fever and therefore may be of little help in either detecting the disease or differentiating it from murine typhus. (11). It is recommended that results of the tests should be correlated with clinical findings to arrive at the final diagnosis. (12). Since techniques and standardization vary from lab to lab one tube difference in tube titres can be expected. (13). False positive reactions may occur with *Brucella* antigen in sera of patient's infected with *Pasteurella tularensis* or vaccinated with *Vibrio cholerae.* (14). 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.

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.

BIBLIOGRAPHY

(1).Cruickshank R. (1982), Medical Microbiology, 12th Ed.; 403. (2). Felix A., (1942), Brit. Med. J., 11, 597-600. (3). J. G. Collee, J. P. Duguid, A. G. Fraser, Practical Medical Microbiology, 14th Ed.: 473-478 & 573-588. (4). G. Galton, L. M. Jones, R. D. Angus, J. M. Verger, Techniques for the brucellosis laboratory, INRA, Paris, 1988. (5). 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	PS Production Site
		EC REP Authorised Representative in the European Community

Manufactured by

TULIP DIAGNOSTICS (P) LTD.

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