Febrile Antigen Kit

REF 2/034*, 2/040*

*Suffixes indicate change in kit presentation only.

BIOTEC

PRINCIPLE
Stained antigen suspensions are used for the identification and quantitative detection of specific antibodies in human sera for epidemiological and diagnostic purposes, primarily in the investigation of pyrexia and enteric infections with certain Salmonellae, Rickettsiae and Brucellae pathogens.

CLINICAL SIGNIFICANCE
Typhoid fever and Salmonellosis are endemic in many parts of the developing world. The Widal test is the most widely used test to demonstrate Salmonella O and H antigens in the patient serum. Its ease of use and the unavailability of microbiological facilities to perform blood culture, along with the time taken to confirm results has led to the preference of the Widal test. This however has led to physicians placing too much reliance on a single Widal test. It is essential follow up tests are performed to demonstrate a 2-4 fold increase in titre indicative of a current infection.

WARNINGS AND PRECAUTIONS
For *in vitro* diagnostic use only. For professional use only.
Health and Safety warnings:
All patient samples and reagents should be treated as potentially infectious and the user must wear protective gloves, eye protection and laboratory coats when performing the test.
Non disposable apparatus must be sterilised after use by an appropriate method.
Disposable apparatus must be treated as biohazardous waste and autoclaved or incinerated.
Spillages of potentially infectious material should be absorbed and disposed of as above. The site of spillage must be sterilised with disinfectant or 70% alcohol.
Do not pipette by mouth.
The serum used has been tested and found to be negative for HIV, HCV and HbsAg. Nonetheless the reagent must be treated as potentially infectious and appropriate precautions should be taken when handling and on disposal. The product also contains aqueous buffer salts including sodium azide and Thiomersal as preservative - see material safety data sheet
Analytical precautions:
Do not modify the test procedure.
All reagents are ready to use **do not** dilute or modify the reagents in any way.
Allow all reagents and samples to reach room temperature (18-30ºC) before use.
Do not interchange reagents from different kit batches.

COMPOSITION
Each febrile antigen is supplied in a vial with a dropper. Combinations of the below antigens and controls are available in various kit formats.

SALMONELLA ANTIGENS			
Typhi H	2/028	Typhi O	2/018
H paratyphi A	2/022	O paratyphi A	2/012
H paratyphi B	2/024	O paratyphi B	2/014
H paratyphi C	2/026	O paratyphi C	2/016
BRUCELLA ANTIGENS			
Abortus	2/002	Melitensis	2/004
PROTEUS ANTIGENS			
OX2	2/008	OX19	2/010
OXK	2/006		
CONTROLS			
Positive Control	2/030	Negative Control	2/032

SPECIMEN AND SAMPLE PREPARATION
Use fresh serum only obtained by centrifugation of clotted blood. The sample may be stored at 2-8°C for 48 hours before performing the test. For longer periods of time the serum must be frozen. Haematic, lipaemic or contaminated serum must be discarded.

MATERIALS AND EQUIPMENT REQUIRED BUT NOT PROVIDED
Small test tubes, Serological pipettes (10-100µl and 100-1000µl), 0.85% Saline (w/v NaCl), Water bath 37-50°C, Timer, Disposable stirring sticks, Reaction slides with white background

RECOMMENDED PROCEDURE

A Rapid Screening Test
1 Dispense 80µl of undiluted serum onto a row of 3 cm diameter circles of a reaction slide.
2 Gently shake the reagent bottle until fully resuspended and add one drop of the undiluted antigen suspension to each serum aliquot.
3 Mix well using a stirring stick and rotate the slide for one minute. Read the slide for result – see Interpretation of results

B Rapid Slide Agglutination
1 Dispense 80µl, 40µl, 20µl, 10µl and 5µl of undiluted serum onto a row of 3 cm diameter circles of a reaction slide.
2 Gently shake the reagent bottle until fully resuspended and add one drop of the undiluted antigen suspension to each serum aliquot.
3 Mix using a stirring stick and rotate the slide for one minute. Read the slide for result – see Interpretation of results.

C Tube Agglutination Test
All positive rapid agglutination results should be confirmed using the following technique.
1 Label 8 small plastic tubes in a rack.
2 Dispense 1.9ml of 0.85% saline (w/v NaCl) into the first tube, and 1.0ml into the remaining seven.
3 Using a pipette, dispense 100µl of the patient's undiluted serum into the first tube and mix well.
4 Dispense 1.0ml from the first tube into the second tube and mix well.
5 Continue this method of doubling dilutions up to the seventh tube, discarding 1.0ml from the seventh tube. The eighth tube will contain only saline as a control and therefore should not contain any serum.
6 Gently Shake the reagent bottle until fully resuspended, and add 1 drop of the appropriate antigen suspension into each tube and mix well.
7 Incubate as indicated:

ANTIGEN	TEMPERATURE (°C)	TIME (Hours)
Salmonella O	**50**	**4**
Salmonella H	**50**	**2**
Brucella	**37**	**24**
Proteus	**50**	**4**

NOTE: It is vitally important that when the tubes are placed in a water bath, the level of water should come to approximately 2/3rd the way up the level of the tube content. This will maintain convection currents within the tube and thereby obviate false results.
8 Leave overnight in fridge, then allow to reach room temperature before reading. The titre to be taken is the last tube with visible agglutination.
The control tube containing no serum should show no sign of agglutination. The positive control provided is not suitable for the tube agglutination test.

INTERPRETATION OF RESULTS

A. Rapid Screening Test

Agglutination is an approximation to a serum titre of 1/20 in a tube test.

B. Rapid Slide Agglutination

If agglutinations are observed, the following approximate titres would be observed in a confirmatory tube test:

SERUM VOLUME (µL)	TITRE
80	1:20
40	1:40
20	1:80
10	1:160
5	1:320

In this way the rapid slide test provides an approximation to the expected results from a corresponding tube test.

NOTE: It is necessary to perform all dilutions in the slide test to obviate the 'prozone' effect where higher concentrations of the serum may give a positive result.

C. Tube Agglutination Test

A positive test will show an obvious floccular agglutination throughout the tube. A negative result and the saline control should show no change in appearance and should show a characteristic "swirl" when flicked. Tubes must not be shaken. In all forms of test the last sample showing signs of agglutination should be taken as the titre for that specimen. For negative results, all tests should remain clear of any agglutination. The control tube containing no serum should show no sign of agglutination. The positive control provided is not suitable for the tube agglutination test.

STORAGE AND SHELF LIFE

Store all reagents upright at 2-8ºC.
DO NOT FREEZE THE REAGENT.
Do not use reagents after the stated expiry date.
Discard reagents if they become contaminated.
ALL REAGENTS ARE SUPPLIED READY TO USE

LIMITATIONS OF THE METHOD

It has been found that many serotypes of salmonella possess somatic antigens of the same kind. Therefore, agglutination of any of the Salmonella antigens with human serum should not be taken as proof of infection by one particular organism, but rather as infection by an organism of like antigenic structure.
Tests should be read after the recommended incubation time to eliminate the possibility of false results.
Many populations or communities can show high levels of residual antibodies often in excess of 1/80 – 1/160. Patients can also show high levels of residual antibodies from previous infections or immunisation. For a test to be of clinical significance a rise in titre must be demonstrated not just a high titre for a one off test.
Chronic liver disease has also been shown to cause a rise in salmonella antibody titre.

PERFORMANCE CHARACTERISTICS

The generally accepted performance capabilities of the Widal test using stained febrile antigens are 70% specificity and sensitivity. This is because serological tests in the diagnosis of Salmonellae infections have important limitations, cultures of appropriate specimens are usually preferred if possible.

DISCLAIMER

The user is responsible for the performance of the reagent by any method other than those mentioned in the Recommended Techniques. Any deviations from the **Recommended Procedures** should be validated prior to use.

QUALITY CONTROL

Controls are provided within the kit and should be run at regular intervals to confirm that the test is working satisfactorily. Test results should be considered invalid if incorrect results are obtained with the control sera. The positive control provided is not suitable for use with the tube agglutination test.

BIBLIOGRAPHY

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TABLE OF SYMBOLS

SYMBOL	DEFINITION
LOT	Batch Number
IVD	*In-vitro* Diagnostics
REF	Catalogue reference
	Store at
	Expiry date
	Manufactured by
	Date of Manufacture
	Read the instructions for use
	Sufficient for

Lab21 Healthcare Ltd.
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