Febrile Antigens
(Serum)

REF IVD +2°C +8°C CE

PRINCIPLE
Stained antigen suspensions may be used to identify and quantitate specific antibodies in human sera following infection with certain Salmonellae, Rickettsiae and Brucellae pathogens. Proteus OX2, OX19 and OXK suspensions are used in the detection of Rickettsial antibodies as these species appear to share a polysaccharide with certain Rickettsia species and therefore produce agglutinins identical to them. Stained febrile antigens are suitable for both the rapid slide and tube agglutination tests against human sera for the detection of these agglutinins.
Stained antigen suspensions are killed bacteria, stained to enhance the reading of agglutination tests. The blue stained antigens are specific to the somatic '0' antigens whilst the red stained antigens are specific to the flagellar 'H' antigens.

PRECAUTIONS
For *in vitro* diagnostic and 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 ingest.
Control reagents contain rabbit serum. The product also contains aqueous buffer salts including sodium azide and Thiomersal as preservatives- see material safety data sheet
Do not inhale or ingest aerosols – wash splashes with copious amounts of water.
Analytical precautions:
Do not modify the test procedure.
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.

STORAGE
Store upright at 2°C - 8°C. Light Sensitive. Do not freeze under these conditions, kit performance characteristics will be maintained until the indicated expiry date on the vial label. Reagents should be discarded if they become contaminated or do not demonstrate correct activity with the controls.
The reagents in each kit have been standardized to produce the proper reaction and reagents should not be interchanged with those from other batches.

PRESENTATION OF STAINED FEBRILE ANTIGENS

F-BAA5	Brucella abortus	5 ml
F-BMA5	Brucella melitensis	5 ml
F-POX25	Proteus OX2	5 ml
F-POX195	Proteus OX19	5 ml
F-POXK5	Proteus OXK	5 ml
F-SHa5	Salmonella H paratyphi a	5 ml
F-SHb5	Salmonella H paratyphi b	5 ml
F-SHc5	Salmonella H paratyphi c	5 ml
F-SHd5	Salmonella typhi H	5 ml
F-SOA5	Salmonella O paratyphi A	5 ml
F-SOB5	Salmonella O paratyphi B	5 ml
F-SOC5	Salmonella O paratyphi C	5 ml
F-SOD5	Salmonella typhi O	5 ml
HF-BAA5	Brucella abortus	5 ml
HF-BMA5	Brucella melitensis	5 ml
HF-SHA5	Salmonella H paratyphi a	5 ml
HF-SHB5	Salmonella H paratyphi b	5 ml
HF-SHC5	Salmonella H paratyphi c	5 ml
HF-SHD5	Salmonella typhi H	5 ml
HF-SOA5	Salmonella O paratyphi A	5 ml
HF-SOB5	Salmonella O paratyphi B	5 ml
HF-SOC5	Salmonella O paratyphi C	5 ml
HF-SOD5	Salmonella typhi O	5 ml
F-FPC1	Positive control	1 ml
F-FNC1	Negative control	1 ml
F-KIT8x5	Febrile antigen kit	8x5 ml antigens
F-KIT8x5C	Febrile antigen kit	8x5 ml plus controls

HF-KIT8x5 Widal Kit Salmonella Ha,Hb,Hc,Hd,OA OB,OC,OD	8x5 ml antigens
HF-KIT8x5C Widal Kit Salmonella Ha,Hb Hc,Hd,OA,OB,OC,OD+tve	8x5 ml plus controls

Materials required but not provided
Small test tubes 75 x 12mm
Micropipettes
Reaction slides with white background

Specimen & sample preparation
Use fresh serum 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.

Rapid Screening Test.
1) Using a pipettor, dispense 0.08ml onto a 3cm diameter circle.
2) Shake the reagent bottle well and add one drop of the undiluted antigen suspension to the serum aliquot.
3) Mix well using a stirring stick and rotate the slide.
Read after 1 minute.
If agglutination is observed after one minute a significant titre should be obtained in confirmatory tube test. The reaction is roughly equivalent to a tube test dilution of 1:20.
At this dilution a prozone effect may be encountered, this can be obviated by either using a smaller volume of sample or performing the **Rapid Slide Titration**

Rapid Slide Titration:
1) Using a pipette, dispense 0.08ml, 0.04ml, 0.02ml, 0.01ml and 0.005ml of undiluted serum onto a row of 3cm diameter circles.
2) Shake the reagent bottle well and add one drop of the undiluted antigen suspension to each serum aliquot.
3) Mix well using a stirring stick and rotate the slide.
Read after 1 minute.
Agglutination seen in any circle is indicative of the following results should a tube test be carried out.
0.08ml = 1:20, 0.04ml = 1:40, 0.02ml = 1:80,
0.01ml = 1:160, 0.005ml = 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 negative result but further dilutions may give a positive result.

Febrile Antigens
(Serum)

Tube Agglutination Test:

1) Label up 8 small plastic tubes in a rack.
2) Using a pipette, dispense 1.9ml of 0.85% saline into the first tube, and 1.0ml into the remaining seven.
3) Using a pipette, dispense 0.1ml of the patients undiluted serum into the first tube. Mix well using the larger pipette volume and tip. (i.e. set to 1.0ml).
4) Using the pipette, dispense 1.0ml from the first tube into the second tube. Mix well.
5) Continue this method of doubling dilutions up to the seventh tube. Discard 1.0ml from the seventh tube. The eighth tube will contain only saline as a control and therefore should not contain any serum.
6) Shake the reagent bottle well and add 1 drop of the appropriate antigen suspension into each tube and mix well.
7) Incubate as follows: Brucella, Proteus and 'F-' code Salmonella. **37°C for 24 hours.**
However the HF Range: HF-SOA5, HF-SOB5,HF-SOC5, HF-SOD5, HF-SHa5, HF-SHb5, HF-SHc5, HF-SHd5.
Should be used with an alternative faster method.
'O' antigens = 50 deg C for 4 hours
'H' antigens = 50 deg C for 2 hours
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) Examine the tube after the appropriate incubation time and check for agglutination. The titre to be taken is the last tube to show agglutination.

INTERPRETATION OF RESULTS

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 a like antigenic structure.
Tubes should be read after the recommended incubation time to eliminate the possibility of false results. The last tube showing signs of agglutination should be taken as the titre for that test. For negative results, all tubes should remain clear of any agglutination. Titres in excess of 1:80 are usually significant, and may reflect recent infection, but low titres can be found in patients. Any deviation in test procedure could result in variable results. Since techniques and standardization vary from lab to lab one tube difference in tube titres can be expected. Use a separate disposable tip for each sample to prevent cross contamination. After usage the antigen suspension should be immediately recapped and replaced at 2-8°C. It is recommended that results of the tests should be correlated with clinical findings to arrive at the final diagnosis. 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 Widal antigen suspensions.
2. The negative control should show no agglutination with any of the Widal antigen suspensions.
3. Generally accepted performance characteristic of this type of test is 70% specificity and sensitivity.
4. Reproducibility of Widal antigen suspensions is 100% (+/- one double dilution).

LIMITATIONS

1. The use of samples other than serum has not been validated in this test
2. Cross reactions between Brucella antigens and other organisms have been reported
3. If prozone is suspected during the slide test dilute the serum 1/20 in saline and retest
4. This is a single use test
5. Both Brucella abortus and Brucella melitensis share a common Brucella antigen. A sample should be tested using the Brucella abortus and the Brucella melitensis suspensions by rapid slide test and confirmed by tube agglutination test to determine the type of Brucella antibodies. The higher titre determines the specific type of Brucella anitbodies present.

WARRANTY

This product is designed to perform as described on the label and package insert. We do not recommend any other purpose or procedure.

BIBILIOGRAPHY

1. Cruickshank R.(1982) Medical Microbiology, 12th Edition.403.
2. Felix A.,(1942),Brit.Med.J.,11,597.
3. Data held on file by Rapid Labs

Index of Symbols

	Consult instructions for use	IVD	For *in vitro* diagnostic use only		Use by
REF	Catalog Number	LOT	Lot Number		Tests per kit
	Store between 2-8°C		Do not re-use		
	Manufacturer		Date of manufacture		

 Manufactured by: Rapid Labs Ltd

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