



# STAINED FEBRILE ANTIGENS

## (For Widal and Weil-Felix Test)

### INTENDED USE

**Plasmatec** stained antigen suspensions are for the identification and quantitative determination of specific antibodies in human sera following infection with certain Salmonellae, Rickettsiae and Brucellae pathogens.

### 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.

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 - 30°C) before use.

Do not interchange reagents from different kit batches.

### COMPOSITION

Presentation

#### Plasmatec Stained Febrile Antigens

FA/002 Salmonella typhi H 5 ml

FA/004 Salmonella H paratyphi A 5 ml

FA/006 Salmonella H paratyphi B 5 ml

FA/008 Salmonella H paratyphi C 5 ml

FA/010 Salmonella typhi O 5ml

FA/011 Salmonella typhi Vi 5ml

FA/012 Salmonella O paratyphi A 5 ml

FA/014 Salmonella O paratyphi B 5 ml

FA/016 Salmonella O paratyphi C 5 ml

FA/018 Brucella abortus 5 ml

FA/020 Brucella melitensis 5 ml

FA/022 Proteus OX2 5 ml

FA/024 Proteus OX19 5 ml

FA/026 Proteus OXK 5 ml

FA/030 Positive control 0.5 ml

FA/032 Negative control 0.5 ml

FA/040 Febrile antigen kit containing 8x5 ml antigens (FA/002-FA/016)

FA/042 Febrile antigen kit containing 8x5 ml antigens (FA/002-FA/016) with positive

(FA/030) and negative (FA/032) controls

Kit Insert

### STORAGE AND SHELF LIFE

Store upright at 2°C - 8°C. Light Sensitive. Do not freeze Under these conditions, kit performance characteristics will be maintained for 36 months from date of manufacture. See expiry date on kit 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 standardised to produce the proper reaction and reagents should not be interchanged with those from other batches.

### MATERIALS REQUIRED BUT NOT PROVIDED

Small test tubes

Pipettes

Reaction slides with white background

### SPECIMEN AND 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.

### PROCEDURE

#### Principle

**Plasmatec** febrile antigens are suitable for both the rapid slide and tube agglutination tests against human sera for the detection of these agglutinins.

**Plasmatec** stained antigen suspensions are killed bacteria, stained to enhance the reading of agglutination tests.

The blue stained antigens are specific to the somatic 'O' antigens whilst the red stained antigens are specific to the flagellar 'H' antigens.

### Rapid slide titration

- Using a pipettor, dispense 0.08 ml, 0.04 ml, 0.02 ml, 0.01 ml and 0.005 ml of undiluted serum onto a row of 3 cm diameter circles.
- Shake the reagent bottle well and add one drop of the undiluted antigen suspension to each serum aliquot.
- Mix well using a stirring stick and rotate the slide.

### Read after one minute

Agglutination seen in any circle is indicative of the following results should a tube test be carried out...

0.08 ml = 1:20, 0.04 ml = 1:40, 0.02 ml = 1:80,

0.01 ml = 1:160, 0.005 ml = 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.

### Tube agglutination test

All positive results obtained through a slide test should be confirmed using the following technique.

- Label up 8 small plastic tubes in a rack.
- Using a pipette, dispense 1.9ml of 0.85% saline into the first tube, and 1.0ml into the remaining seven.
- 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*)
- Using the pipette, dispense 1.0ml from the first tube into the second tube. Mix well.
- 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.
- Shake the reagent bottle well and add 1 drop of the appropriate antigen suspension into each tube and mix well.
- Incubate as follows:-

Salmonella 'O' Antigens and Proteus	=	50°C for 4 hours
Salmonella 'H' Antigens	=	50°C for 2 hours
Brucella antigens	=	37°C for 24 hours
Typhi i	=	37°C for 2 hours

(Leave overnight in fridge, then allow to reach room temperature before reading.)

It is vitally important that when the tubes are placed in a water bath, the level of water should come to approximately 2/3<sup>rd</sup> the way up the level of the tube content. This will maintain convection currents within the tube and thereby obviate false results.

- Examine the tubes 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.

Tests should be read after the recommended incubation time to eliminate the possibility of false results.

The last test showing signs of agglutination should be taken as the titre for that test. For negative results, all tests should remain clear of any agglutination.

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. 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 is 70% specificity and sensitivity. Because serological tests in the diagnosis of Salmonellae infections have important limitations, cultures of appropriate specimens is usually preferred.

### INTERNAL QUALITY CONTROL

Controls are provided in the kit and should be run at regular intervals to confirm that the test is working satisfactorily.

### REFERENCES:

- Huddleson. I.F and Bell (1928) J infect. Dis. 42 242
- Freter, R. (1980) Man of Cli. Imm. 2<sup>nd</sup> Ed. A.S.M. Washington DC 460-453
- Weil. E and felix. A. Wein Klin, 29 974 (1916)
- Cruikshank, R (1965) Med Mic 11<sup>th</sup> Ed 907