COD 33001 Multiscreening 6 x 100 t	COD 33080 Salmonella 8 x 100 t	COD 33081 Salmonella 6 x 100 t
STORE AT 2-8ºC		
Reagents for determination of antibodies to febrile antigens Only for *in vitro* use in the clinical laboratory		

FEBRILE SERODIAGNOSTICS

CE

BioSystems
REAGENTS & INSTRUMENTS

FEBRILE SERODIAGNOSTICS

Agglutination

SLIDE AND TUBE TESTS

PRINCIPLE OF THE METHOD

Febrile antigens are standardized suspensions of stained bacteria used to identify and quantitate specific serum antibodies developed during some febrile infections such as brucellosis, salmonellosis and certain rickettsiosis. The antigen suspension agglutinates in the presence of the corresponding homologous antibody in the samples tested[1-3].

CONTENTS

Code	Component	Individual	33001	33080	33081
33309	*Brucella abortus*	1 x 5 mL	-	-	-
33315	*Brucella abortus*/Rose Bengal	1 x 5 mL	1 x 5 mL	-	-
33307	*Salmonella* typhi H (H: d)	1 x 5 mL	1 x 5 mL	1 x 5 mL	1 x 5 mL
33308	*Salmonella* typhi O (O: 9,12)	1 x 5 mL	1 x 5 mL	1 x 5 mL	1 x 5 mL
33301	*Salmonella* Paratyphi AH (H:a)	1 x 5 mL	1 x 5 mL	1 x 5 mL	1 x 5 mL
33302	*Salmonella* Paratyphi AO (O:1,2,12)	1 x 5 mL	-	1 x 5 mL	1 x 5 mL
33303	*Salmonella* Paratyphi BH (H:b)	1 x 5 mL	1 x 5 mL	1 x 5 mL	1 x 5 mL
33304	*Salmonella* Paratyphi BO (O:1,4,5,12)	1 x 5 mL	-	1 x 5 mL	1 x 5 mL
33305	*Salmonella* Paratyphi CH (H:c)	1 x 5 mL	-	1 x 5 mL	-
33306	*Salmonella* Paratyphi CO (O:6,7)	1 x 5 mL	-	1 x 5 mL	-
33311	*Proteus* OX19	1 x 5 mL	1 x 5 mL	-	-
33510	C+S: Positive Control *Salmonella*	1 x 1 mL	1 x 1 mL	-	-
33509	C+B: Positive Control *Brucella*	1 x 1 mL	1 x 1 mL	-	-
33502	C+P: Positive Control *Proteus*	1 x 1 mL	1 x 1 mL	-	-
33503	C-: Serology Negative Control	1 x 1 mL	1 x 1 mL	-	-

COMPOSITION

Febrile antigens: Suspension of nonviable bacterial cells stained (somatic blue, flagellar red) and containing sodium azide 0.95 g/L.

C- Negative Control: Negative human serum, sodium azide 0.95 g/L.

C+ Positive Controls: Serum containing the corresponding febrile antibodies, sodium azide 0.95 g/L.

Human sera used in the preparation of the negative control have been tested and found to be negative for the presence of antibodies anti-HIV and anti-HCV, as well as for HBs antigen. However, the controls should be handled cautiously as potentially infectious.

STORAGE

Store at 2-8ºC. Febrile antigens and Controls are stable until the expiry date shown on the label when stored tightly closed and if contaminations are prevented during their use.

Indications of deterioration (Note 1):

- Febrile antigens: Visible agglutination in the flask.
- Controls: Presence of particulate material.

REAGENT PREPARATION

Febrile antigens and controls are provided ready to use (Note 2).

ADDITIONAL EQUIPMENT

- Mechanical rotator adjustable to 100 r.p.m.

SAMPLES

Serum collected by standard procedures. Stable for 7 days at 2-8ºC. Hemolyzed or lipemic samples are not suitable for testing.

PROCEDURE

A. SLIDE TEST

1. Bring test reagents and samples to room temperature (Note 3).
2. Place 1 drop (50 µL) of the sample (Notes 4, 5) and 1 drop of each Control into separate circles on the glass slide.
3. Shake the antigen vial gently before using. Add 1 drop (50 µL) of antigen suspension to each circle next to the sample drop.
4. Mix with a disposable stirrer and spread over the entire area enclosed by the ring. Use a new stirrer for each sample.
5. Rotate the slide by hand or on a mechanical rotator at 100 r.p.m. for 2 minutes (B. Rose Bengal for 4 minutes).

B. TUBE TEST

1. Dilute serum samples 1/20 and Controls 1/10 with 9 g/L NaCl and make serial two-fold dilutions in 9 g/L NaCl.
2. Prepare for each antigen to be tested a row of tubes containing 1 mL of each of the serum and Controls (Positive and Negative) dilutions.
3. Shake the antigen vial gently and add 1 drop (50 µL) of the appropriate suspension to each tube. Mix thoroughly.
4. Incubate the tubes at 37°C for 24 h (Note 6).

READING

A. SLIDE TEST

Examine the presence of agglutination within a minute (Note 7).

Positive results: Presence of a visible agglutination. Positive sera should be quantified by the tube test. The presence of agglutination in the Brucella Rose Bengal test indicates a content of antibody ≥ 25 IU/mL.

Negative results: Absence of a visible agglutination.

B. TUBE TEST

Examine the presence and the pattern of visible agglutination.

Positive results: Partial or complete agglutination with variable degree of clearing of the supernatant fluid. A somatic reaction is characterized by coarse, compact agglutination which tends to be difficult to disperse, while the flagellar has a characteristic loose, flocculant agglutination. The serum titer is defined as the highest dilution showing a positive result.

Negative results: Absence of a visible agglutination.

QUALITY CONTROL

Positive (C+) and Negative (C-) Controls should be tested toghether with the patients samples, in order to verify the assay performance.

Positive Control (C+) should cause agglutination of the corresponding febrile antigens.

Negative Control (C-) should not cause any agglutination.

Each laboratory should establish its own internal Quality Control scheme and procedures for corrective action if controls do not perform within the acceptable tolerances.

ASSAY CHARACTERISTICS

- Detectability: 25 UI/mL for the Brucella abortus/ Rose Bengal suspension using the WHO 2nd International Standard.
- High dose (zone) effect: False negative results may be obtained with sera containing a high titer of antibodies. A dilution of these sera is positive.
- False results: Results obtained with these antigens did not show significative differences when compared with reference antigens. Details of the comparison are available on request.
- Interferences: Rheumatoid factors (300 IU/mL) do not interfere.

DIAGNOSTIC CHARACTERISTICS

Positive results are an aid in the diagnosis of some bacterial infections which are accompanied by a fever in the patient. However, agglutination cannot be taken as proof of infection by a particular organism because many types of microorganisms have antigens in common. Febrile serodiagnostics tests should be used in parallel with appropriate cultural techniques for the isolation and identification of the causative organism.

Titers greater than 1/80 for Salmonella or Brucella antigens are usually indicative of recent infection. Lower titres are often found in healthy individuals, specially in areas with a high prevalence of febrile infections. Sera of healthy individuals often contains antibodies to Proteus antigens. A titer of less than 1/160 should not be considered significant.

A single positive result has less clinical significance than the demostration of a rising or decreasing titre between successive serum specimens taken days apart.

A negative result does not preclude an active infection since the the specimen may have been taken before the patient produced antibodies to the etiological agent. False negative results can also be obtained in cases of immuno deficiency, prozone (Brucelosis) and antibiotic treatment.

Clinical diagnosis should not be made on the findings of a single test result, but should integrate both clinical and laboratory data.

NOTES

1. Color intensity of the antigen may vary from lot to lot. Results are not affected by these variations.
2. Rose Bengal Antigen is exclusively for the slide test.
3. The sensitivity of the test may be reduced at low temperatures.
4. It is recommended to reduce the sample to 20 µL for Brucella in order to avoid prozone.
5. In some geographical areas with a high prevalence of febrile infections, it is recommended to dilute the sample 1/4 in NaCl 9 g/L before to perform the assay.
6. Alternatively, incubate at 48-50ºC for 2 h (flagellar antigens) or 4 h (somatic and Proteus).
7. Delay in reading may cause false positive results.

BIBLIOGRAPHY

1. Felix A. Technique and interpretation of the Weil-Felix test in typhus fever. *Trans Roy Soc Trop Med Hyg* 1944; 37: 321-325
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3. Alton GG et al. Techniques for the brucellosis laboratory. INRA Paris, 1988.
4. Gualtney J B et al. Microagglutination procedures for febrile agglutination tests. *Appli Micr* 1971; 22: 635-640