

Febrile Antigens



CONTENTS

Reagents for the determination of antibodies against febrile antigens

For *in vitro* diagnostic use only

Febrile Antigen Kit

Stained bacterial suspensions

SLIDE AND TUBE TESTS

PRINCIPLE

The CROMATEST stained antigens are standardized suspensions of killed bacteria prepared for the detection and semi-quantitation by agglutination in either slide or tube tests of human serum agglutinins, a group of antibodies developed during some febrile infections such as brucellosis, salmonellosis and certain rickettsiosis^{1,2}.

The assay is performed by testing the stained antigens –somatic, blue; flagellar, red– against unknown samples. The presence or absence of a visible agglutination is usually related with the presence or absence of the corresponding homologous antibody in the sample tested.

REAGENT COMPOSITION

Ag	Febrile Antigen. Stabilized suspension of stained killed bacteria in a buffered solution.
CONTROL +	Brucella, Salmonella, Proteus: Animal serum containing the corresponding febrile antibodies.
CONTROL -	Agglutinin Control Negative. Non-reactive animal serum.

Warning: The reagents in this kit contain 0.95 g/L of sodium azide. Do not contact with skin or mucous membranes.

PACKAGING CONTENTS

REF	REAGENTS	2199435	2199300
2100005	<i>Brucella Abortus</i>	1 x 5 mL	–
2104005	<i>Brucella Melitensis</i>	–	–
2135005	<i>Salmonella typhi</i> H (d-H)	1 x 5 mL	1 x 5 mL
2139005	<i>Salmonella typhi</i> O (9, 12-O)	1 x 5 mL	1 x 5 mL
2113005	<i>Salmonella paratyphi</i> A-H (a-H)	1 x 5 mL	1 x 5 mL
2117005	<i>Salmonella paratyphi</i> A-O (1,2,12-O)	–	1 x 5 mL
2119005	<i>Salmonella paratyphi</i> B-H (b-H)	1 x 5 mL	1 x 5 mL
2123005	<i>Salmonella paratyphi</i> B-O (1,4,5,12-O)	–	1 x 5 mL
2125005	<i>Salmonella paratyphi</i> C-H (c-H)	–	1 x 5 mL
2127005	<i>Salmonella paratyphi</i> C-O (6, 7-O)	–	1 x 5 mL
2107005	<i>Proteus OX19</i>	1 x 5 mL	–
2109005	<i>Proteus OX2</i>	–	–
2111005	<i>Proteus OXK</i>	–	–
2921205	<i>Brucella Positive Control</i>	1 x 1 mL	–
2921305	<i>Proteus Positive Control</i>	1 x 1 mL	–
2921405	<i>Salmonella Positive Control</i>	1 x 1 mL	–
2929910	<i>Agglutinin Control Negative</i>	1 x 1 mL	–

STORAGE AND STABILITY

Store at 2-8°C. Do not freeze. Frozen reagents could change the functionality of the test. Antigens and Controls are stable until the expiry date stated on the label.

REAGENT PREPARATION

Antigens and Controls are ready to use.

SAMPLES

Fresh, clear serum.

After the clear serum has been separated it may be stored at 2-8°C up to one week or for longer periods at –20°C.

MATERIAL REQUIRED

- Glass or white test cards.
- Disposable stirrers.
- Test tubes (12 x 100 mm).
- Automatic pipettes.
- Saline solution (NaCl 0.9%).
- Mechanical rotator, adjustable.
- Thermostatic bath (30-50°C).

PROCEDURE

I. Qualitative Test

1. Bring the test reagents and samples to room temperature.
2. Resuspend the antigen vial gently. Aspirate dropper several times to obtain a thorough mixing.
3. Place 50 µL of the serum under test into a row of circles on the card. When testing for *Brucella* antibodies, 20 µL is sufficient (Note 1 and Note 2). Dispense 1 drop of Positive Control serum and 1 drop of Negative Control serum into two additional circles.
4. Add 1 drop of the appropriate well-shaken suspension to each circle next to the sample to be tested.
5. Mix the contents of each circle with a disposable stirrer and spread over the entire area enclosed by the ring. Use separate applicators for each mixture.
6. Rock the slide gently by hand or by means of a mechanical rotator (100 r.p.m.) for a period of **1 minutes**.
7. Observe immediately under a suitable light source for any degree of agglutination.

Reading

Nonreactive: Smooth suspension with no visible agglutination, as shown by negative control.

Reactive: Any degree of agglutination visible macroscopically.

II. Semi-quantitative Test

1. For each specimen to be tested place 80, 40, 20, 10 and 5 µL of serum into each of the circles of a card.
2. Test each dilution as described in steps 4-7 for the Qualitative Test (Note 3).

Reading

Same as in Qualitative Test. The titer of the specimen is reported as the highest dilution that shows reactivity.



III. Tube Agglutination Test

- Using saline solution as a diluent, prepare for each antigen to be tested a row of doubling dilutions of the specimen as follows:

Tube	1	2	3	4	5	6	Control Susp.	Control +	Control -
Saline Sol. 0.95% (mL)	1.9	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
Serum (μL)	+	+	+	+	+	+	-	+	+
	100	1 mL serial dilutions					-	2 drops	2 drops
	Mix								

- Add 1 drop of the appropriate well-shaken suspension to each tube of a given row. Mix. Final serum dilutions will be: 1:20, 1:40, 1:80, 1:160, 1:320, 1:640.
- Incubate at 37°C for 24 hours. (Note 4).
- Examine macroscopically for agglutination.

Reading

Read the results of all control tubes first. After the examining the pattern of the sediment shake the tube gently.

Nonreactive: In a negative reaction and in the Suspension Control tube there is no clumping visible. Suspension shows a typical swirl when the tube is flicked.

Reactive: Partial or complete agglutination with variable degree of clearing of the supernatant fluid.

The titer is reported as the highest dilution that shows agglutination. The next higher dilution should be negative.

QUALITY CONTROL

Positive and negative serums as well as Suspension Control tubes should be run daily to check the operativity of the system.

EXPECTED VALUES

Salmonella and Brucella: Titers greater than 1/80 (somatic and brucella antigens) and 1/160 (flagellar antigens) indicates recent infection.

Proteus: Titer of less than 1/160 should not be considered significant.

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

CLINICAL SIGNIFICANCE

Febrile Antigen is a term which has been accepted generally as referring to bacterial suspensions representative of a number of pathogenic microorganisms to human, involved in some bacterial infections (brucellosis, salmonellosis and certain rickettsiosis) which are accompanied by a fever in the host. The best option to establish the etiology of an infectious disease is by isolation and identification of the causative agent. However, these culture techniques may be difficult to use and the febrile serodiagnostic tests become important to detect the antibodies produced in the patient serum during the infection (indirect method of diagnosis).

Testing Febrile Antigens has a high diagnostic value as their exclusion or detection can support or place doubt on a tentative diagnosis made on the basis of case history data and clinical findings.

ANALYTICAL PERFORMANCE

- There is not a Reference Material for the sensitivity standardization of these reagents. For this reason, Linear Chemicals adjust the sensitivity of their reagents against to specific antisera and commercial reagents of certified quality.
- Prozone effect: False negative results may be obtained with sera containing a high titer of antibodies. A dilution of these sera will give a positive result.
- Results obtained with this reagent did not show significant differences when compared with reference reagents. Details of the comparison experiments are available on request.
- Hemoglobin (<10 g/L), bilirubin (<20 mg/dL), lipemia (<10 g/L) and rheumatoid factors (<300 IU/mL) do not interfere.

LIMITATIONS OF PROCEDURE

- Biological false negative reactions can occur early in disease and in cases of immuno-unresponsiveness.
- False negative somatic (O) tests may be given by typhoid patients which have been treated with antibiotics.
- False positives (cross-reactions) may be found with the brucella antigen in cases of infection with some strains of *Vibrio* (*Campylobacter*), *Pasteurella*, *Proteus* OX19 and *Y. enterocolitica* (serotype 9), as well as in patients vaccinated with *V. cholerae*.

NOTES

- Never test suspected cases of chronic brucellosis by this test. Samples should be tested by the Rose Bengal and the Tube Agglutination tests.
- In some geographical areas with a high prevalence of febrile antibodies, it is recommended to dilute de sample 1:4 in saline solution (0.95%) before perform the assay.
- Final dilutions are approximately 1:20, 1:40, 1:80, 1:160, and 1:320, respectively.
- Alternatively incubate: Somatic (O) and Proteus antigens at 48-50°C for 4 hours.
Flagellar (H) antigens at 48-50°C for 2 hours.

SOURCES OF ERROR

- Antigens used beyond their expiry dates may give false negative reactions.
- Bacterial contamination of antigens, specimens or saline solution, as well as the freezing of the suspensions may lead to false positive results.

REFERENCES

- Felix, A. Trans. Roy. Soc. Trop. Med. Hyg. 37: 321 (1944).
- Joint FAO/WHO Expert Committee on Brucellosis. Wld. Hlth. Org. Tech. Rep. Ser. 148: 1 (1958).