Febrile Antigen Multi Kit

IVD CE

Multiple kit for rapid slide screening and titration on microplate or tube of specific bacterial antibodies

Instructions for Use (IFU)

ORDERING INFORMATIONS

Code	Suspension	Composition
REF CSI087370	Febrile Antigen Multi Kit	n° 7 vials x 3 ml
REF CSI087471	M.K. *Brucella abortus*	n° 1 vial x 6 ml
REF CSI087486	M.K. *Brucella melitensis*	n° 1 vial x 6 ml
REF CSI087473	M.K. *Salmonella typhi* O	n° 1 vial x 6 ml
REF CSI087474	M.K. *Salmonella typhi* H	n° 1 vial x 6 ml
REF CSI087476	M.K. *Salmonella paratyphi* AO	n° 1 vial x 6 ml
REF CSI087477	M.K. *Salmonella paratyphi* AH	n° 1 vial x 6 ml
REF CSI087479	M.K. *Salmonella paratyphi* BO	n° 1 vial x 6 ml
REF CSI087480	M.K. *Salmonella paratyphi* BH	n° 1 vial x 6 ml
REF CSI087481	M.K. *Salmonella paratyphi* CO	n° 1 vial x 6 ml
REF CSI087482	M.K. *Salmonella paratyphi* CH	n° 1 vial x 6 ml
REF CSI087387	M.K. Polyvalent Positive Control	n° 1 vial x 2 ml
REF CSI087388	M.K. Negative Control	n° 1 vial x 2 ml
REF CSI087389	M.K. Positive Control Plus	n° 1 vial x 2 ml

INTENDED USE

In vitro diagnostic medical device for the rapid slide screening and for the titration on microplate or tube of antibodies specific for Salmonella e Brucella. Test results should always be interpreted in relation to the clinical context. For professional use only.

CLINICAL SIGNIFICANCE

The serological diagnosis of infectious diseases characterized by persistent fever is based on the agglutination reaction that occurs between the antigen and specific antibodies present in the patient serum. Grumbaum and Widal firstly introduced the immunology applications in laboratory practice. Their method became universally known as "Widal test" and quantitatively determines antibodies (agglutinins) in the sera of patients with typhoid fever. In addition, the use of serological tests has become common even for the use in the diagnosis of brucellosis both in humans and animals.

PRINCIPLE

When serum containing specific agglutinins reacts with homologous antigen under optimized conditions, it is able to cause a visible agglutination. This reaction may be performed in test tubes, on slides or in microplate. The agglutination level depends on the antigen and antibody concentration, saline composition of the fluid and temperature.

Storage and Stability

= **Storage Temperature 2-8 °C**

Maintained at 2-8°C, avoiding direct light, reactive are stable until the expiry date printed on the label. Do not freeze. Avoid microbial contamination

Stability tests repeated on three different batches confirmed a validity for almost 36 months if stored 2-8°C. Slight variations in composition from batch to batch do not affect test result.

KIT COMPONENTS

All concentrations refer to ready to use reagents.

Kits for single antigens

Suspensions: 1 vl x 6 mL of dyed and inactivated bacterial suspension (the name is reported on the vial label)
Sodium azide 0,9 g/l

Multiple Kit (REF CSI087370)

Suspension *Brucella abortus*
Suspension Salmonella typhi O
Suspension Salmonella typhi H
Suspension Salmonella paratyphi AO
Suspension Salmonella paratyphi AH
Suspension Salmonella paratyphi BO
Suspension Salmonella paratyphi BH

Each vial contains 1x3 mL of dyed and inctivated bacterial suspension

Positive Control

1x2 mL, polyvalent positive serum, ready to use.
Contains Sodium Azide 0,9 g/L as preservative.

Negative Control

1x2 mL, negative serum, ready to use.
Contains Sodium Azide 0,9 g/L as preservative.

Cards

n. 10 cards white background with 6 circles for the execution of the rapid method

Microplates

n.2 96-wells microplates (U bottom) for the execution of micromethod

Separately are also available:

Ref. CSI087387 M.K. Polyvalent Positive Control
1x2 mL, polyvalent positive serum (positive for *S. typhi* O and H, *S. paratyphi* AO and AH, *S. paratyphi* BO and BH, Brucella)

Ref. CSI087388 M.K. Negative Control 1x2 mL

Ref. CSI087389 M.K. Positive Control Plus
1x2 mL, polyvalent positive serum (positive for *S. paratyphi* CO and CH, Brucella)

Ref. C18087246 96-wells microplates 14 microplates U bottom

Ref. C18087300 Slides white background Package with 50 slides with 6 circles.

Warning: products that contain Sodium azide can react with lead and copper to form explosive metal azides deposits. To eliminate dilute with large amounts of water.

Other materials required but not supplied:

- Physiological Saline
- Automatic Pipettes (variable volumes)
- Serologic tubes

PRECAUTIONS AND WARNINGS

1. Reagents and waste materials shall be disposed of in accordance with Community waste provisions or national or regional provisions.
2. In addition to any risk claims relating to active components, reagents may contain non-active components such as preservatives and detergents. The total concentration of these components is below the limits set out in Regulation 1272/2008 EC and subsequent amendments and additions.
3. It is recommended that the reagent be handled in accordance with the rules of good laboratory practice and that appropriate personal protective equipment be used.
4. All human samples must be handled and disposed as potentially infectious materials. Components from human origin have been tested and found to be negative for the presence of HbsAg, HCV, and antibodies to HIV (1/2). However, handle cautiously as potentially infectious (Biosafety Level 2)
5. The kit should only be used by qualified and properly trained technical personnel.
6. Diagnoses shall only be carried out by authorised and qualified personnel.
7. It is recommended to handle the reagent according to the rules of good laboratory practice and to use appropriate personal protective equipment.
8. Comply with national directives on occupational safety and quality assurance.
9. Use equipment that comply with current standards.
10. Laboratory standards for protection against infection shall be used.

Reporting of serious incidents

Please inform the manufacturer (through your distributor) and the competent authority of the member state of the European Union in which the user and/or patient is established, of cases of serious incident that has occurred in relation to the device. For other jurisdictions, reports of serious incidents must be made in accordance with the regulatory requirements of the home Member State. By reporting serious incidents, you help provide more information about the safety of your in vitro medical diagnostic device.

PROCEDURE

Quality control

Kit suspensions must be analyzed with positive and negative control sera. Absence of reactions, respectively positive and negative indicates alteration of suspensions and/or controls.

REAGENT PREPARATION

Reactives and control sera, brought to room temperature, are ready to use. Mix gently suspension to obtain an homogeneous solution. After opening the reagents are stable if maintained as indicated in “Preservation and Stability”.

SPECIMEN COLLECTION AND PRESERVATION

Non inactivated serum must be used. The specimen must be clear, free of visible fat, excessive hemolysis, or bacterial contamination. If serum specimens are not to be tested within the day od collection, they should be stored at 2-8°C.

PROCEDURES

Kit suspensions can be used as rapid screening (slide) test or for titration both with macro (tubes) or micro methods (microplate U bottom).

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Instructions for Use (IFU)

Rapid Method

Screening test

Distribute on the circle of the slide 50µl of serum and 50 µl of bacterial suspension, mix well and rotate the slide for 2 minutes.

Positive Reaction: Presence of visible agglutination

Negative Reaction: Absence of visible agglutination

Microplate Method

1A	2A	3A	4A	5A	6A	7A	8A	9A	1A	11A	12A
1B	2B	3B	4B	5B	6B	7B	8B	9B	10B	11B	12B
1C	2C	3C	4C	Etc.							
1D	Etc.										
1E											
1F											
1G											
1H											

PREDILUTE POSITIVE AND NEGATIVE CONTROLS 1:10 in physiological saline

1) Distribute 90 µl of physiological saline in the first well (1A) of the microplate and 50 µl from the second (2A) to the tenth.

2) Distribute 50 µl of diluted positive and negative controls in the wells 11 and 12.

3) Add, in the first well, 10 µl of serum and mix well by pipetting.

4) Transfer 50 µl from the first well to the second (up to the 10th) and mix well.

5) Repeat point 4 for each well and discard the last 50 µl.

6) Repeat points 1 to 5 for each sample to be analyzed.

7) Dilute bacterial suspension 1:15 in physiological saline (ex. 10 µL of suspension + 140 µL. physiological saline) and distribute 50µL of the diluted suspension to each well utilized.

8) Incubate *overnight* (at least 16 hours) at 37°C in a moist chamber in a thermostat without vibrations.

Results

Negative reaction (absence of agglutination): Button shaped layer of sedimented cells in the well bottom.

Positive reaction (agglutination): homogeneous layer of cells without any visible button of sedimented bacteria on the bottom of the well.

Titer: the reciprocal of the highest dilution without button of sedimented cells.

Results interpretation

Generally, titers in the range 40 – 80 are considered suspect; only higher titers are probative for the diagnosis of the disease. Significant titers may be obtained from individuals immunized with typhoid vaccine.

Tube Method

PREDILUTE POSITIVE AND NEGATIVE CONTROLS 1:10 in physiological saline

1) Dilute the serum 1:20 (80 µl of serum in 1,92 ml of physiological saline).

2) Prepare a set of test tubes for each antigen. Each set contains 8 tubes.

3) Put 2 mL of diluted serum in the first tube and distribute in the other tubes 1 ml of physiological saline.

4) Mix well and transfer 1ml from the first to the second tube.

5) Repeat point 3 for all the tubes and discard the last ml.

6) Distribute 1 mL of positive control and 1 mL negative control, prediluted 1:10 physiological saline (ex. 1 ml of suspension + 9 ml of physiological saline), in two additional tubes 1.

7) Add **30µL** of **undiluted** bacterial suspension to each tube

8) Repeat points from 2 to 6 for each antigen to be analyzed.

It is advisable a waterbath incubation for 18 hours at 37°C. For a rapid execution of the test an incubation for 4 hours at 56°C is also possible.

9) **To improve the reading maintain the tubes for 30 min at room temperature, in a surface without vibrations.**

Negative reaction: absence of agglutination with cloudy supernatant or button presence on the bottom of the tube.

Positive reaction: presence of agglutination on the bottom of the tube with clear supernatant.

Warning: Only for anti-Salmonella antibodies (Wright reaction) a discrimination between O agglutination (somatic antigens) and H agglutination (ciliar antigen) can be made.

O antigen: Granular agglutination visible and stable after gently agitation.

H antigen: Flaky agglutination, easily destroyed after gently agitation.

Titer: the titer correspond to the reciprocal of the highest dilution with a clearly visible agglutination

Results reading: titers comprised between 40 and 80 must be considered as suspect, only higher titers are confirmatory for the diagnosis of the disease.

Validation test

Specificity

Tests carried out with three different lots of bacterial suspensions of *Salmonella typhi* and *paratyphi* A, B, C ant. "O", "H" and Brucella on samples of human negative serum have given repeatedly negative results.

Precision

Test of repeatability (within run) and reproducibility (between run) carried out with three different lots of bacterial suspension of *Salmonella typhi e paratyphi* A, B, C ant. "O", "H", "O+H" and Brucella on normal human samples and animal samples, at a known titer, have given the expected results.

Stability

Stability tests, in real time, was carried out on three different lots of each bacterial suspension have confirmed the functionality of the reagent for at least 36 mesi at 2-8°C.

Limits of the method

Prozone reactions, especially with Brucella reagent, are possible. If clumpings are visible at lower dilutions, care should be taken to observe agglutination at the higher dilutions (at least to 1:640 dilution). Apparently negative serum agglutination test may be due to an excess of antibodies, that, at low dilutions, prevents the agglutinate formation (prozone phenomenon). In case not significant positivity, the addition of anti - human Ig serum may reveal the presence of incomplete antibodies (Coombs test) present, above all, in case of chronic disease. As with all serological procedures, a single antibody determination should not be used for diagnosis. The significance of the presence of antibodies in a single serum can only provide the evidence of an exposure to etiologic agent. For a correct diagnosis it is necessary to observe a significant increasing of titer between samples collected in a 10-14 days interval.

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Bibliography

1. **Castaneda M.R**. Bull. WHO • 9: 399, 1953.
2. **Sonnerwirth A.C.** 1970, in: Gradwohl's Clinical Laboratory Methods and Diagnosis • 7th ed., p. 1482, The CV Mosby Co., St. Louis.

Symbols used for IFU and Packaging	
IVD In vitro diagnostics medical device	Manufacturer
REF Catalog number	Instruction for Use
LOT Lot Number	Storage Temperature
Expiration Date	Biological Risk

REVISION	DATE	CHANGES
G	12-2022	Change from Technical File to Instructions for Use

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