Multi Kit Proteus

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

Instructions for use (IFU)

IVD CE

ORDERING INFORMATIONS

Code	Suspension	Composition
REF CSI087257	Multi Kit Proteus	n° 3 vial x 3 ml

INTENDED USE

Multiple kit for rapid slide screening and titration on microplate or tube of specific bacterial Antibodies. Test results should always be interpreted in relation to the clinical context. For professional use only.

CLINICAL SIGNIFICANCE

Weil- Felix test was developed at the beginning of '900 and is based on the detection of antibodies against some species of Proteus containing antigens that cross-react with Rickettsia genus antigens. *Proteus vulgaris* OX19 reacts with sera of patients affected by Rickettsiae (typhus group e RMSF Rocky Mountains Spotted Fever), Proteus *vulgaris* OX2 reacts with sera of patients affected by Rickettsiae (Spotted Fever Group) and *Proteus mirabilis* OXK reacts with sera of patients affected by scrub typhus caused by Orientia tsutsugamushi.

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.

Multiple Kit (REF CSI087257)

Suspension *Proteus OX19*

Suspension *Proteus OX2*

Suspension *Proteus OXK*

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

Positive Control

1x2 mL, polyvalent positive 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 micro method

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.

Separately are also available:

Ref. C18087246 96-wells microplates 14 microplates U bottom

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

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. 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. Do not use the reagent if it is visibly degraded (e.g., presence of corpuscles).
5. All human samples shall be handled and disposed of as potentially infectious material.
6. The kit should only be used by qualified and properly trained technical personnel.
7. Diagnoses shall be carried out exclusively by authorized and qualified personnel.
8. Comply with national directives on occupational safety and quality assurance.
9. Use equipment that complies with current regulations.

Reporting of serious incidents

In the event of a serious incident in relation to the use of the device, please inform the manufacturer (via your distributor) and the competent authority of the European Union member state where the incident occurred. For other jurisdictions, reporting must be made in accordance with regulatory requirements. Reporting serious incidents helps provide more information about the safety of the diagnostic medical 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

Reactive and control sera, brought to room temperature, are ready to use. Mix gently suspension to obtain a 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).

Rapid Method

Screening test

Vortex vigorously the suspension for at least 15 seconds.

Distribute on the circle of the slide 50µl of serum and 50 µl of bacterial suspension, mix well and rotate the slide for 1 minute.

Positive Reaction: Presence of visible agglutination

Negative Reaction: Absence of visible agglutination

Microplate Method

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

1) **Vortex vigorously the suspension for at least 15 seconds.**
2) Distribute 90 µl of physiological saline in the first well (1A) of the microplate and 50 µl from the second (2A) to the tenth.
3) Distribute 50 µl of undiluted positive and negative controls in the wells 11 and12.
4) Add, in the first well (1A), 10 µl of serum and mix well by pipetting.
5) Transfer 50 µl from the first well to the second (up to the 10th) and mix well.
6) Repeat point 4 for each well and discard the last 50 µl.
7) Repeat points 1 to 5 for each sample to be analyzed.
8) **Dilute bacterial suspension 1:10 in physiological saline** (ex. 100 µL of suspension + 900 µL. physiological saline) and distribute 50µL of the diluted suspension to each well utilized.
9) Incubate *overnight* (at least 16 hours) at 37°C in a moist chamber in a thermostat without vibrations.
10) To improve reading characteristics is suggested, at the end of incubation, to maintain the plates for 30 minutes at RT on a flat surface, vibration-free.

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

For a correct diagnosis is discriminating detect a significant increase in titer between

Sclavo Diagnostics International
Loc. Pian dei Mori, via Po n° 26-28 • 53018 (SI) (Italy)
Phone +39 0577 390 41 • Fax +39 0577 390 444
www.sclavodiagnostics.com

Multi Kit Proteus

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

Instructions for use (IFU)

IVD CE

two samples taken between the 5th and 12th day from the beginning of the febrile state. For the interpretation of the result refer to the table reported in the Rapid Method paragraph.

Disease	Causative agent	Weil Felix Reagent		
		OX19	*OX2*	*OXK*
Thyphus	*R. prowazekii*	++	+	-
Murine thyphus	*R. moserii*	++	+	-
Rocky Mountain Spotted Fever	*R. ricketsii*	+/++	++	-/+
Spotted fever	*R. conori*	+/++	++	-
Japan River fever	*R. orientalis*	-	-	++
Q Fever	*R. burneti*	-	-	-

Tube Method

PREDILUTE POSITIVE AND NEGATIVE CONTROLS 1:10 in physiological saline
Vortex vigorously the suspension for at least 15 seconds.

1) Dilute the serum 1:20 (100 µl of serum in 1,90 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 4 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 serum + 9 ml of physiological saline), in two additional tubes.
7) Add **60µL** 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.
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.
Titer: the titer corresponds to the reciprocal of the highest dilution with a clearly visible agglutination
Results reading: For a correct diagnosis is discriminating detect a significant increase in titer between two samples taken between the 5th and 12th day from the beginning of the febrile state (for result interpretation refer to table above, in the Rapid Method paragraph).

Validation test
Specificity
Tests carried out with three different lots of bacterial suspensions of *Proteus OX19, OX2 e OXK* 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 *Proteus OX19, OX2 e OXK* 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 months at 2-8°C.

LIMITS OF THE TEST

Both sensitivity and specificity of the Weil-Felix test are low, but its predictive value can be increased by testing both acute and convalescent-phase samples and observing a rise in antibody titer. False-positive results are obtained in presence other diseases such as leptospirosis, and relapsing fever (diseases which require differentiating from Rickettsial infections), in Proteus infections, brucellosis, and acute febrile illness.

Symbols used in IFU and Packaging	
IVD In vitro diagnostic medical device	Manufacturer
REF Catalogue Number	Instruction for use
LOT Lot Number	Temperature limitation
Expiration date	

References

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.
3. **Amano K, Hatakeyama H, Okuta M, Suto T, Mahara F.** Serological studies of antigenic similarity between Japanese spotted fever rickettsiae and Weil-Felix test antigens. J Clin Microbiol. 1992 Sep;30(9):2441-6
4. **Kularatne SA, Gawarammana IB**. Validity of the Weil-Felix test in the diagnosis of acute rickettsial infections in Sri Lanka. Trans R Soc Trop Med Hyg. 2009 Apr;103(4):423-4.

REVISION	DATE	CHANGES
E	12/2022	New Issue for IVDR Regulation (UE) 2017/746 compliance

Sclavo Diagnostics International
Loc. Pian dei Mori, via Po n° 26-28 • 53018 (SI) (Italy)
Phone +39 0577 390 41 • Fax +39 0577 390 444
www.sclavodiagnostics.com