Proteus Color Kit

IVD CE

Microplate agglutination test for the antibodies titration according to Weil Felix

Instructions for use (IFU)

ORDERING INFORMATIONS

Format	REF	Suspension	Composition
Kit 3 x 100 test	**CSI087254**	Proteus Color Kit	3 vials x 5 ml

INTENDED USE

Microplate agglutination test for antibody titration according to Weil – Felix. 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 reveling of antibodies against some species of Proteus that contain antigens that cross-react with Rickettsia genus antigens.
Proteus vulgaris OX19 reacts with sera of people infected with Rickettsiae (typhus group e RMSF Rocky Mountains Spotted Fever), Proteus vulgaris OX2 reacts with sera of people infected with Rickettsiae (Spotted Fever Group) and Proteus mirabilis OXK reacts with sera of people with scrub typhus caused dalla Orientia tsutsugamushi.

PRINCIPLE OF THE METHOD

When a serum, containing specific agglutinins, reacts with homologous antigen, in optimized and controlled conditions, produce a visible agglutination. This reaction can be performed in tube, microplate or on a slide. In the test on microplate the serum is twofold diluted. After addiction of the antigen, the reaction is incubated for the stated time and then is read.
The microorganisms are dyed to facilitate the reading. Positive reactions appear as a smooth mat of cells on the bottom of wells, while negative reactions show a totally or partially compact button of colored cells.

Preservation and Stability

= **Storage Temperature 2-8 °C**

Store reagents and controls at 2-8° C avoiding direct light. Do not freeze. Under above conditions reagents are stable until expiration date indicated on the label. Slight variations in composition among batches will not affect test results.

COMPONENTS

All concentrations refer to ready to use reagent:

Suspension Proteus OX19 1x5 ml
Proteus OX2 1x5 ml
Proteus OXK 1x5 ml

Dyed bacterial suspension at optimal concentration for microplate test.
Contains Sodium azide 0,95 g/L.

Positive Control Serum (polyvalent, animal source) 1x1 ml
Contains Sodium azide 0,95 g/L.

*__Warning__: Products that contain sodium azide may react with lead and copper in plumbing to form explosive metal azides. On disposal flush with large volumes of water to prevent azide build up.

4, 96-well microplates U bottom wells to perform analysis.

Other material required, but not supplied

PBS, automatic pipettes to distribute 10 µL, 50 µL, 90 µL.

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

REAGENT PREPARATION

Reagents are liquid ready to use. Avoid any chemical or bacteriological contamination. Mix well reagents before use. Suspension must appear uniform and free of visible particles.

SPECIMEN COLLECTION AND PRESERVATION

Not-inactivated sera must be used. Samples can be stored at 2-8° C for 48 h before testing. For longer time of storage samples must be frozen (-20° C). Highly hemolyzed, lipemic or contaminated samples cannot be used. Samples with the presence of fibrin should be centrifuged before testing.

PROCEDURE

Quality Control

Kit suspensions should be tested with positive control serum and with PBS as negative control.
Failure to obtain a positive and negative result with the respective controls evidence a deterioration of suspension and/or controls.

Technique

1 -Bring reagents and materials to room temperature. Low temperature can reduce the method sensitivity.
2 -Swirl gently the reagent before using. Suspension must appear uniform and free from visible particles.
3- Pre-dilute testing sera 1:25 with buffered PBS pH 7,2 and put in first column's wells (Column 1) of the microplate.

	ROWS											
COLUMN	1A	2A	3A	4A	5A	6A	7A	8A	9A	10A	11A	12A
	1B	2B	3B	4B	5B	6B	7B	8B	9B	10B	11B	12B
	1C	2C	3C	4C	5C	6C	7C	8C	9C	10C	10C	12C
	Etc											
Dil	***25***	***100***	***200***	***400***	***800***	***1600***	***3200***	***6400***	***12800***	***25600***		

4 - Starting from the first well of the first row (1A), dilute 10 µL of sample with 240 µL of PBS buffered
5 - Distribute 50 µL of PBS in all the wells of the row, until the 10 A; in 11 A add 50 µl of the undiluted Positive control.
6 - Dilute the sample transferring 50 µl from the first to the second well.
7 - Mix by pipetting several times and transfer 50 µl to well 3A.
8 - Follow up to well 10 A discarding the last 50 µL.
9 - Repeat this operation for each sample.
10 -Distribute 50 µL of bacterial suspension in each well used (except the column 1 used for predilution).
11 - Shake for 30 seconds with a plate mixer or manually.
12 - Incubate at 37° C in a moist chamber in absence of vibrations for 16 ± 4 hours.
To improve the reading a further incubation of 30 minutes at room temperature on a surface without vibrations is suggested.

Results

Positive Reaction (agglutination): Homogeneous layer of cells without sedimented bacteria (clear supernatant).
Negative reaction (absence of agglutination): sedimented cells forming a button on the well bottom.

Titer

The titer is defined as reciprocal dilution of serum showing a visible bacterial agglutination.
Sometimes we can have a border-line effect; for example, in a well there is s a smaller bacterial button than in the negative control with, around, an agglutination layer of cells.
In these cases the well which shows the border-line effect, and not the previous well, fix the titre.

Results interpretation

Generally, titers equal or higher than 1:100 are probative for the diagnosis of the disease.
If we consider + as a medium titre and ++ as a higher titre, the diagnosis can be made according to the following table:

Disease	Agent	Weil – Felix Reaction		
		OX 19	OX 2	OX K
Epidemic typhus	*R. prowazekii*	++	+	-
Murine typhus	*R. mooseri*	++	+	-
Rocky Mountains spotted Fever	*R. ricketsii*	+/++	++	-/+
Boutonneuse Fever	*R. conori*	+/++	++	-
Tsutsugamushi Fever	*R. orientalis*	-	-	++
Q fever	*R. burneti*	-	-	-

To have a correct diagnosis it is discriminating to have a significant growth of the titre between two samples drawn in the 5th and 12th days from the beginning of the fever.

Validation Test

Specificity

Tests carried out with three different lots of bacterial suspension of Proteus OX19, OX2 and OX K, on samples of human negative serum have given repeatedly negative results.

Sensibility

Tests carried out with three different lots of bacterial suspension "O", "H"," O+H", Brucella on human positive sera, had confirmed the titer obtained with reference methods.

Precision

Test of repeatability (within run) and reproducibility (between run) carried out with three different lots of bacterial suspension of Proteus OX19, OX2 and OX K on normal human samples and animal samples at known titre, have given constantly the expected results.

Method features

Results obtained by Plate test showed a good correlation (±1 dilution) with titers obtained by classical tube tests.

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	

Bibliografia

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

REVISION	DATE	CHANGES
D	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