

## Specification

Sterile selective supplement for the isolation of *Legionella* species from environmental water samples.

## Presentation

10 Freeze dried vials  
Vial  
with:  $9 \pm 0.1$  g

### Packaging Details

23x60 mm glass vials, tag labelled, White plastic cap - 10 vials per box.

### Shelf Life

49 months

### Storage

2-25 °C

## Composition

Compositon (g/vial)

Glycine (ammonia free)..... 1.5000  
Vancomicin..... 0.0005  
Polymyxin B sulphate.....40000 IU  
Cycloheximide.....0.0400

Reconstitute the original freeze-dried vial

by adding :

Sterile Distilled Water..... 10 ml

## Description /Technique

### Description:

The discovery of the causative organism of Legionnaires' disease has permitted big progress in the studies around it. New media for the culture and the enumeration *Legionella spp* have been developed in the last years.

Legionella GVPC selective supplement, when added to the agar Base, gives the antibiotic support in order to obtain a selective final medium.

The selectivity is raised by the addition on vancomicin that acts against Gram positive bacteria, polymyxin B that inhibits Gram negative bacteria and cicloheximide or natamycin that are antifungal agents and inhibits the yeast growth.

### Technique:

Collect, dilute and prepare samples and volumes as required according to specifications, directives, official standard regulations and/or expected results.

Reconstitute the 1 vial of Selective Supplement of GVPC with 10 ml to the steril distilled water, in aseptic conditions, and add it to 500 ml of melted Legionella BCYE Agar Base cooled to 47-50°C supplemented before with Legionella BCYE growth Supplement. Do not overheat once supplemented.

Pour the complete medium into Petri dishes and, once solidified on a flat surface. Spread the plates by streaking methodology or by MF method.

Allow the inoculated plates to stand until the inocula has been absorbed. Invert the plates and incubate at  $36 \pm 2^\circ\text{C}$  for up to 2, 3, 5-10 days. To ensure the atmosphere in the incubator is humid, place a tray of water in the bottom of the incubator. Top up this tray with fresh water (if necessary) each time the plates are examined. Incubation in an atmosphere of air with 2,5% (volume fraction)  $\text{CO}_2$  may be beneficial for the growth of some *Legionella*, but it is not essential.

Examine the plates with a plate microscope on at least three occasions at intervals of 2,3 to 5 days during the 10-day incubation period, as *Legionella* grow slowly an can be masked by the growth of other organisms. Record the number of each type of colony present.

Colonies of *Legionella* are often white-grey-blue-purple in colour, but may be brown, pink, lime-green or deep-red. They are smooth with a smooth edges and exhibit a characteristic ground-glass appearance. Under ultraviolet light colonies of several species autofluoresce brilliant white, but others are red and *L. pneumophila* appear dull green often tinged with yellow. All presumptive colonies must be confirmed by cultural, biochemical, serological or genetic methods.

## Quality control

### Physical/Chemical control

Color : Light beige      pH: at 25°C

### Microbiological control

Reconstitute 1 vial as indicated in COMPOSITION; shake and dissolve completely

Add 1 vial to 500 ml of medium base. DO NOT HEAT once supplemented.

Analytical methodology according to ISO 11133:2014/A1:2018; A2:2020.

Aerobiosis. Incubation at  $36 \pm 2$  °C. Reading 2-5 days for *L. pneumophila*, 5-10 days for *L. anisa*, and 3 days for selectivity.

Microbiological control accor. to ISO 11133:2014/A1:2018 standard

### Microorganism

*Legionella anisa* ATCC® 35292, WDCM 00106 (by MF)

*Legionella anisa* ATCC® 35292

*Legionella pneumophila* ATCC® 33152, WDCM 00107 (by MF)

*Legionella pneumophila* ATCC® 33152

*Escherichia coli* ATCC® 25922, WDCM 00013

*Enterococcus faecalis* ATCC® 19433, WDCM 00009

the reference medium is GVPC validated.

### Growth

Good ( $\geq 70\%$ ) grey-blue colonies

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Inhibition (Partial to complet)

Inhibited

### Sterility Control

Add 5mL of the sample to 100 mL of TSB and to 100 mL Thioglycollate.

Incubation 48 h at 30-35 °C and 48 h at 20-25 °C: NO GROWTH.

Check at 7 days after incubation in same conditions.

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