Condalab

Reference: 6025 **Technical Data Sheet**

Product: **LEGIONELLA GVPC SUPPLEMENT ISO**

Specification

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

Presentation

	Packaging Details	Shelf Life	Storage
10 Freeze dried vial Vial with: 9 ± 0.1 g	22±0.25 x 55±0.5 mm glass vials, tag labelled, White plastic cap - 10 vials per box.	49 months	2-25 ºC

Composition

Compositon (g/vial)

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

NOTE : Each vial is sufficient to supplement 500 ml Legionella CYE Agar Base (1311).

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 developped 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 positive bacteria and cicloheximide or natanamycin 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 vial with 10 ml of sterile diluent in aseptic conditions and add it to 500 ml of melted Legionella BCYE Agar Base cooled to 50ºC 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 spiral method.
Incubate the plates, when the inoculum has been completely absorbed, in aerobic atmosphere at 36ºC from 4 to10 days.
To ensure the atmosphere in the incubator is humid, place a tray of water in the bottom of the incubator.
Incubation times longer than those mentioned above or different incubation temperatures may be required depending on the sample or the specifications
Examine the plates with a plate microscope on at least three occasions at intervals of 2 days to 4 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.
After incubation, count all the colonies that have appeared onto the surface of the agar.
Colonies of Legionella are often white-grey-blue-purple in colour, but may be brown, pink, lime-green or deep-red.
Presumptive isolation must be confirmed by further microbiological and biochemical tests.

Quality control

Physical/Chemical control

Color : Light beige pH: at 25ºC

Microbiological control

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

Inoculate: Practical range 100 ± 20 CFU. min. 50 CFU (productivity)/ 10^4-10^6 (selectivity).

Aerobiosis. Incubation at 36 ± 2 ºC. Reading 3 - 5 days, up to 10 days.

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

Microorganism	Growth
Escherichia coli ATCC® 8739, WDCM 00012	Inhibited
Enterococcus faecalis ATCC® 19433	Inhibited
Legionella pneumophila ATCC® 33152, WDCM 00107 (by MF)	Good (≥ 70%) grey-blue colonies
Legionella anisa ATCC® 35292, WDCM 00106 (by MF)	Good (≥ 70%) grey-blue colonies
Legionella anisa ATCC® 35292	Good (≥ 70%) grey-blue colonies
Legionella pneumophila ATCC® 33152	Good (≥ 70%) grey-blue colonies

the reference medium is GVPC validated.

Sterility Control

100 ml TSB and 100 ml Thioglycollate.
Incubation 48 hours at 30-35 ºC and 48 hours at 20-25 ºC: NO GROWTH.
Check at 7 days after incubation in same conditions.

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