

Legionella GVPC Supplement ISO

Cat. 6025

Selective supplement for the isolation of Legionella spp.

Practical information

Applications	Categories
Selective enumeration	Legionella
Selective isolation	Legionella

Industry: Water

Regulations: ISO 11133 / ISO 11731

Principles and uses

The Legionella BCYE Agar Base and its supplements have been shown to be optimal for Legionella culture with shorter incubation periods from environmental and clinical samples.

Feeley et al. described a modification of F-G Agar in which acid hydrolysed casein was replaced by yeast extract as the source of protein, and starch was replaced by activated charcoal. This medium, which they named CYE Agar has been further supplemented with ACES Buffer and α -ketoglutarate and is described in the literature as BCYE-a Medium. BCYE-a Medium has been shown to yield optimal recovery of Legionellaceae in a shorter incubation period from environmental samples and clinical specimens.

Cycloheximide is an antibiotic which inhibits saprophytic fungi but allows for growth of the pathogenic fungi. Vancomycin is a glycopeptide antibiotic used for the inhibition of Gram-positive bacteria. Polymyxin B sulfate is an antibiotic for gram-negative bacteria.

ISO 11731 recommends the following procedure for the isolation of Legionella and its enumeration in water samples. The samples are concentrated by membrane filtration, diluted or inoculated directly on the plate depending on the origin and characteristics of the sample. Independent fractions of the diluted sample should be subjected to heat or acid treatments in case of a high concentration of Legionella and other bacteria. These samples are transferred to the plates with the selective culture medium chosen for Legionella.

Formula per vial

Cycloheximide (mg)	40	Polymyxin B Sulfate (IU)	40000
Vancomycin (mg)	0,5	Glycine (g)	1,5

Preparation

Aseptically reconstitute 1 vial to about 80% of the volume with distilled or de-ionized water. Mix gently until complete dissolution and aseptically add to 500 ml medium Legionella BCYE Agar ISO (Cat. 1311 + Cat. 6022) cooled to 50 °C. Mix well and distribute into sterile containers.

Instructions for use

For the cultivation of legionella according to ISO 11731:

If the sample contains a high concentration of Legionella and a low concentration of interfering microorganisms:

- Directly inoculate 0,1-0,5 ml of the sample by distributing it uniformly on a plate of Agar BCYE (Cat. 1311 + Cat. 6022) and on a BCYE+AB plate.

If the sample contains a low concentration of Legionella and a low concentration of interfering organisms:

- Filter the initial sample by membrane.
- Place the filter on the BCYE plate.
- Repeat the process for GVPC Agar (Cat. 1311 + Cat. 6022 + Cat. 6025) and / or MWY Agar (Cat. 1311 + Cat. 6022 + Cat. 6067).

If the sample contains a high concentration of interfering microorganisms:

- It will be inoculated directly, concentrated or diluted.
- Divide each type of sample into three portions. One of them will be used untreated, the second one will be subjected to a thermal treatment and the third will be subjected to an acid treatment.
- Inoculate 0,1-0,5 ml on GVPC Agar plates and MWY Agar.

If the sample contains an extremely high amount of interfering microorganisms:

- It will be inoculated directly and diluted.
- Each sample is subjected to a combined thermal and acid treatment.
- Inoculate 0,1-0,5 ml on GVPC Agar plates and MWY Agar.
- Let the plates rest until the inoculum has been absorbed. Incubate at 36 ± 2 °C for 7-10 days.
- Confirm presumptive colonies of Legionella on BCYE Agar and BCYE-cys Agar.

Quality control

Solubility	Appearance	Color of the dehydrated medium	Color of the prepared medium	Final pH (25°C)
		N/A	N/A	N/A

Microbiological test

Incubation condition: (37 °C / 3 days)

Microorganisms	Specification	Characteristic reaction
Pseudomonas aeruginosa ATCC 25668	Inhibition	
Escherichia coli ATCC 25922	Inhibition	
Legionella pneumophila ATCC 33152	Good growth	White-grey-blue-purple colonies with an entire edge and exhibiting a characteristic ground-glass appearance.

Storage

Temp. Min.:2 °C
Temp. Max.:8 °C

Bibliography

ISO 11731 water quality- Detection and enumeration of Legionella.