Legionella BCYE Agar Base ISO

Cat. No. MM-M-311

Selective medium for the cultivation of Legionella.

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 hydrolised 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 a-ketoglutarate and is described in the literature as BCYE-a Medium. BCYE-a Medium has been shown to yield optimal recovery of Legionellacea in a shorter incubation period from environmental samples and clinical specimens.
Yeast extract provides vitamins, particularly of the B-group, and other growth co-factors. L-Cysteine provides the required nutritional source. Activated charcoal is a protective agent neutralizing and absorbing toxic metabolites produced by bacterial growth. It decomposes hydrogen peroxide, a toxic metabolic product, and can also collect CO_2 and modify surface tension.
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 Litre:

Activated charcoal	2.0g	Bacteriological Agar	13g
Yeast Extract	10g		

Preparation:

Suspend 12.5 grams of the medium in 450 ml of distilled water. Heat until boiling and until the medium is completely dissolved. Distribute into appropriate containers and sterilize in autoclave at 121ºC for 15 minutes. Cool to 48±3ºC and aseptically add 5 vials of Legionella BCYE Growth Supplement (Cat. MMS-M-X022), previously reconstituted in 10 ml of warm sterile distilled water each one. To obtain GVPC agar also add 1 vial of Legionella GVPC Supplement (Cat. MMS-M-X025), previously reconstituted with sterile distilled or deionized water to about 80% of the volume (see preparation of Cat. MMS-M-X025), or 1 vial of Legionella MWY Growth Supplement (Cat. MMS-M-X067), previously reconstituted in 80% of the total volume of distilled or deionized water, to obtain MWY agar. Mix well and distribute into appropriate 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. MM-M-311 + Cat. MMS-M-X022) 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. MM-M-311 + Cat. MMS-M-X025) and / or MWY agar (Cat. MM-M-311 + Cat. MMS-M-X067).

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 sown plates rest until the inoculum has been absorbed. Incubate at 36±2ºC 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 (25ºC)
w/o rests	Fine powder	Black	Black	6.9±0.2

Microbiological test:

According to ISO 11133:
Incubation condition: (36±2ºC / 2-5 days).
Inoculation conditions: Productivity quantitative (100±20. Min. 50 CFU). Reference medium: Batch of BCYE medium already validated.

Microorganisms	Specification	Characteristic reaction
Legionella pneumophila ATCC 33152	Good growth >70%	White-grey-blue-purple colonies with an entire edge and exhibiting a characteristic

Storage:

Temperature: 2ºC - 25ºC

Bibliography:

Feeley J.C., Groman G.W.Weaver R.E., Mackel D.C.. International standard ISO 11731 water quality- Detection and enumeration of Legionella.