

Also known as

CYE

Specification

Solid medium base used for the detection, isolation and enumeration of *Legionella* from water, according to ISO standard 11731:2017.

Formula * in g/L

Activated charcoal	2.00
Yeast extract	10.00
Agar	15.00

Final pH 6.8 ±0.2 at 25 °C

* Adjusted and /or supplemented as required to meet performance criteria

Directions

Suspend 13,5 g of powder in 500 mL of distilled water and bring to the boil dissolving completely. Sterilize by autoclaving at 121°C for 15 minutes. Allow to cool to 47-50°C and add aseptically a reconstituted vial (Ref. No. DSHB3069) of Legionella BCYE Growth Supplement. Mix gently and pour into Petri dishes. The final pH at 25 °C should be adjusted to 6.8 ± 0.2.

If a selective medium is desired it may be obtained by additionally adding a vial of GVPC Selective Supplement for Legionella (Ref.DSHB3070).

Description

The actual formulation of this medium is according to the ISO Standards 11731: 2017, but BCYE Agar is based in a modification of a previously described media. In 1979 Feeley and collaborators described Charcoal Yeast Extract (CYE) Agar as a modification of the F-G Agar. They replaced the starch in the F-G Agar with activated charcoal and substituted yeast extract for casein hydrolysate, resulting in a better recovery of *Legionella pneumophila*. Pasculle, in 1980, reported that CYE Agar could be improved by buffering the medium with ACES buffer and a year later Edelstein increased the sensitivity of the medium by adding a-ketoglutarate which is the present formulation (BCYE Agar).

The medium consist of a Medium base supplemented with growth factors (BCYE Agar) and the Selective Medium supplemented with inhibitors of undesirable accompanying flora. The yeast Extract supplies the basic nutrients as the medium contains no fermentable carbohydrates. L-Cysteine, Ferric pyrophosphate and a-ketoglutarate are incorporate to satisfy the specific nutritional requirements of *Legionella* species.

The activated charcoal decomposes hydrogen peroxide, a toxic metabolic product, and may also collect CO₂ and modify surface tension. The addition of the buffer helps maintain the proper pH for optimal growth. The selectivity is raised by the addition on Vancomycin / Sodium cefazolin they acts against gram-positive bacteria, Polymyxin B that inhibits gram-negative bacteria, anisomycin that has a broad spectrum of activity and Cycloheximide or Natamycin that as antifungal agents inhibits the yeast growth.

Necessary supplements to complete to the medium Base:

 -*Legionella* BCYE Growth Supplement (Art. No. DSHB3069)

Vial Contents:

Necessary amount for 500 mL of complete medium.

ACES Buffer.....	5,000 g
Potassium hydroxide.....	1,400 g
Ferric pyrophosphate.....	0,125 g
L-Cysteine HCl.....	0,200 g
Potassium a-ketoglutarate.....	0,500 g
Sterile Solvent	

 -*Legionella* GVPC Selective Supplement (Art. No.DSHB3070)

Vial Contents:

Necessary amount for 500 mL of complete medium.

Vancomycin.....	0,50 mg
Polymyxin B sulfate.....	40000,00 IU
Cycloheximide.....	40,00 mg
Glycine (ammonia free).....	1,50 g
Distilled water (Solvent)	

Technique

Refers to ISO 11731:2017 or other standard procedures to obtain isolated colonies from specimens and samples. Allow the inoculated plates to stand until the inocula has been absorbed. Invert the plates and incubate à 36 ±2 °C for up to 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) CO₂ may be beneficial for the growth of some Legionella, but it is not essential.

Examine the plates with a plate microscope on à least three occasions à intervals of 2 to 4-5 days during the 10 days incubation period, as Legionella grows slowly and 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 smooth edges and exhibit a characteristic ground-glass appearance. Under ultraviolet light colonies of several species are autofluorescent 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

Incubation temperature: 36 °C ± 2

Incubation time: 2 - 5 - 10 days

Inoculum: Practical range 100 ± 20 CFU. Min. 50 CFU (Productivity) / 10⁴-10⁶ CFU (Selectivity) according to ISO 11133:2014/Amd 1:2018. MF methods & Spiral Plate methods.

Microorganism	Growth	Remarks
<i>Legionella pneumophila</i> ATCC® 33152	Productivity > 0.50	Grey - white colonies (2-5 d)
<i>Leg. pneumophila</i> ATCC® 33152 (MF method)	Productivity > 0.50	Grey - white colonies (2-5 d)
<i>Legionella anisa</i> ATCC® 35292	Productivity > 0.50	Grey - white colonies (5-10 d)
<i>Legionella anisa</i> ATCC® 35292 (MF method)	Productivity > 0.50	Grey - white colonies (5-10 d)
<i>Escherichia coli</i> ATCC® 8739	Partial Inhibition	w. supplement GVPC (3 d)
<i>Pseudomonas aeruginosa</i> ATCC® 9027	Partial Inhibition	w. supplement GVPC (3 d)
<i>Enterococcus faecalis</i> ATCC® 19433	Inhibited	w. supplement GVPC (3 d)

References

- ATLAS, R.M. & L.C. PARKS (1993) Handbook of Microbiological Media. CRC Press. BocaRaton. Fla. USA.
- CLESCERI, L.S., A.E. GREENBERG & A.D. EATON (1998) Standard methods for the examination of water and wastewater. 9-106. 20th edition. APHA-AWWA-WEF. Washington DF, USA.
- EDELSTEIN, P.H., (1981) Improved semiselective medium for the isolation of Legionella pneumoniae from contaminated clinical and environmental specimens. J. Clin Microbiol. 14(3):298.
- FEELEY, J.C., R.J. GIBSON, G.W. GORMAN, N.C. LANGFORD, J.K. RASHEED, C.D. MACKEL, & W.B. BAINE (1979) Charcoal-Yeast Extract Agar: Primary isolation medium for Legionella pneumophila. J. Clin. Microbiol. 10 (4) 437.
- ISO 11731 Standard (2017) Water Quality - Enumeration of Legionella.
- ISO 11133:2014/ Adm 1:2018. Microbiology of food, animal feed and water. Preparation, production, storage and performance testing of culture media.
- MacFADDIN, J.F. (1985) Media for Isolation-Cultivation-Identification-Maintenance of Medical Bacteria.
- PASCULLE, A.W., J.C. FEELEY, R.J. GIBSON, L.G. CORDES, R.L. MYEROWITZ, C.M. PATTON, G.W. GORMAN, C.L. CARMACK, J.W. EZZELL & J.N. DOWLING (1980) Pittsburgh pneumonia agent: Direct isolation from human lung tissue. J. Infect. Dis., 141:727.
- WARD, K.W. (1995) Processing and interpretation of specimens for Legionella spp. In "Clinical Microbiology Procedures Handbook" Chap. 12.1 edited b H.D. Isenberg. ASM Press. Washington DC, USA.

Storage

For laboratory use only. Keep tightly closed, away from bright light, in a cool dry place (+4 °C to 30 °C).