



Legionella Agar Base w/o Charcoal

M1845

Legionella Agar Base without charcoal with the addition of charcoal supplement is used for the cultivation of *Legionella* species.

Composition**

Ingredients	Gms / Litre
Yeast extract	10.000
Agar	15.000
Final pH (at 25°C)	6.9±0.2

**Formula adjusted, standardized to suit performance parameters

Directions

Suspend 12.5 grams in 430 ml distilled water. Heat to boiling to dissolve the medium completely. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. Cool to 50°C. Aseptically add the rehydrated contents of 1 vial of Sterile Charcoal Supplement for Legionella agar (FD280) and 1 vial containing 50ml of Legionella Growth Supplement (BCYE) (FD142). Aseptically add 10ml of sterile distilled water to bring the volume to 500 ml, when no selective supplement is added. The final pH of the medium should be 6.9 ± 0.2. Mix well to prevent the settling of charcoal particles and pour into sterile Petri plates.

If desired, the medium can be made selective by aseptically adding rehydrated contents of 1 vial of either Legionella BMPA Selective Supplement (FD144) or Legionella (GVPC) Selective Supplement(FD143), or Legionella Selective Supplement (GVPN) (FD242) along with 1 vial of Legionella Growth Supplement (BCYE) (FD142) and Sterile Charcoal powder (FD280) to 430 ml sterile molten, cooled Legionella Agar Base (M1845). Simultaneously, a medium without L-Cysteine may be prepared by aseptically adding contents of 1 vial of Legionella Growth Supplement w/o L-Cysteine (FD206).

Principle And Interpretation

Legionella Agar initially called as F-G agar was modified by Feely et al (1) by replacing Starch with charcoal and casein hydrolysate with yeast extract which resulted in better recovery of *Legionella pneumophila* (2).

Pasculle et al (3) reported that the addition of ACES (N-2-acetamido-2-amino ethane sulphonic acid) buffer improved the nutritive value of medium. Edelstein (4) suggested addition of α-Ketoglutarate to increase the sensitivity of this medium.

The medium contains yeast extract to provide the necessary nitrogenous nutrients for *Legionella* growth. Activated charcoal nullifies toxic compounds that either accumulate in the medium during growth or develop during sterilization of medium. Addition of ACES buffer helps in maintaining proper pH of the medium for the optimal growth of *Legionella*. Antibiotics in the supplement inhibits the growth of various contaminating bacteria and fungi (4, 5).

Legionella species have an absolute nutritional requirement for L-Cysteine. Presumptive *Legionella* species colonies can be subcultured onto both Legionella Agar Base with FD142 and with FD206 (Medium without L-Cysteine). All plates are incubated at 35°C. Colonies which grow on Legionella Agar Base with FD142, with L-Cysteine, but not on Legionella Agar Base with FD206 without L-Cysteine, can be regarded as presumptive *Legionella* species.

Quality Control

Appearance

Cream to yellow homogeneous free flowing powder

Gelling

Firm, comparable with 1.5% Agar gel

Colour and Clarity of prepared medium

After addition of supplement (FD280) Black coloured opaque gel forms in Petri plates.

Reaction

Reaction of 2.5% w/v aqueous solution on addition of Legionella Growth Supplement (FD142) and Sterile Charcoal Supplement (FD280) solution at 25°C. pH : 6.9±0.2

pH

6.70-7.10

Cultural Response

M1845: Cultural characteristics observed with added Sterile Legionella Growth Supplement (BCYE) (FD142) and Legionella (GVPC) Selective Supplement (FD143) or Legionella Growth Supplement w/o L-Cysteine (FD206) , after an incubation at 35-37°C for 48-72 hours.

Organism	Inoculum (CFU)	*Growth	**Growth	Recovery
<i>Escherichia coli</i> ATCC 25922	50-100	inhibited~	good	
<i>Legionella dumoffii</i> ATCC 33343	50-100	good-luxuriant	inhibited	>50%
<i>Legionella pneumophila</i> ATCC 33153	50-100	good-luxuriant	inhibited	>50%

Key: * = Growth on Legionella Agar Base with FD142

~ = in presence of FD143

** = Growth on Legionella Agar Base with FD206

Storage and Shelf Life

Store below 30°C in tightly closed container and prepared medium at 2-8°C. Use before expiry date on the label.

Reference

1. Feely J. C., et al, 1978, J. Clin. Microbiol., 8(3):320.
2. Feely, Gibson, Gorman, et al, 1979, J. Clin. Microbiol., 10(4):437.
3. Psculle, Feely, Gibson et al, 1980, J. Infect. Dis., 141:727.
4. Edelstein, 1981, J. Clin. Microbiol., 14:298.
5. Dennis et al, 1984, Proceeding of the 2nd International Symposium, Washington D.C. Am. Soc. Microbiol. PP 294-296.

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