Legionella Agar Base

M809

Legionella Enrichment Agar Base with addition of supplements is used for the enrichment and cultivation of *Legionella* species.

Composition**

Ingredients	Gms / Litre
Yeast extract	10.000
Charcoal activated	1.500
ACES buffer	6.000
Alpha-Ketoglutarate	1.000
Potassium hydroxide	1.500
Agar	17.000
Final pH (at 25°C)	6.9±0.2

**Formula adjusted, standardized to suit performance parameters

Directions

Suspend 18.5 grams in 500 ml distilled water. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. Do not heat prior to sterilization. Cool to 45-50°C and aseptically add rehydrated contents of 1 vial of Legionella Growth Supplement (FD016A) or Legionella Supplement (FD041A) and Legionella Selective Supplement (FD017) . Mix well and pour into sterile Petri plates. Stir the medium during dispensing to prevent settling of charcoal particles.

Principle And Interpretation

Legionella is a gram-negative bacterium and is the causative agent of Legionnaires disease. Natural sources of *Legionella* are fresh water ponds and creeks. Transmission to humans takes place via inhalation of aerosols from cooling towers, hot water systems or fountains containing the bacteria.

Legionella Agar initially called as F-G Agar was modified by Feely et al (1) by replacing starch by charcoal and casein hydrolysate by 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 the medium. Edelstein (4) suggested addition of alpha-ketoglutarate to increase the sensitivity of this medium.

For the isolation of *Legionella* species from clinical samples, homogenize the specimens in sterile distilled water, examine microscopically for *Legionella* by fluorescent antibody (FA) method. Inoculate FA positive cultures on Legionella Agar Base. Incubate the plates at 35°C in 90% relative humidity atmosphere. Growth usually appears in 2-3 days but continue to examine the plates daily for 14 days before discarding them.

Legionella Agar Base contains yeast extract to provide the necessary nitrogenous nutrients for Legionella growth. alpha-Ketoglutarate satisfies the specific nutritional requirements of Legionella species. 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 optimal growth of *Legionella* . Antibiotics in the supplement inhibit the growth of various contaminating bacteria and fungi.

Quality Control

Appearance

Grey to black homogeneous free flowing powder

Gelling

Firm, comparable with 1.7% agar gel.

Colour and Clarity of prepared medium

Black coloured opaque gel forms in Petri plates

Reaction

Reaction of 3.7% w/v aqueous solution at 25°C. pH : 6.9±0.2

pH
6.70-7.10

Cultural Response
M809: Cultural characteristics observed with added Legionella Growth Supplement (FD016A),or Legionella Selective Supplement (FD017) and Legionella supplement (FD041A) after an incubation at 35-37°C for 48-72 hours.

Organism	Growth	Colour of colony
Legionella dumoffii ATCC 33343	good-luxuriant	light blue to grey white
Legionella pneumophila ATCC 33153	good-luxuriant	light blue to grey white

Storage and Shelf Life
Store at 30°C in tightly closed container and prepared medium at 2-8°C. Use before expiry period on the label.

Reference
1.Feeley J. C., Gorman G. W., Weaver R. E. Mackel D. G., Smith H. W.,1978, J. Clin. Microbiol., 8(3):320.
2.Feeley J. C., Gibson R. J., Gorman G. W., Langdard N. C., Rasheed J. K., Mackel D.C. and Baine W. B., 1979, J. Clin. Microbiol., 10(4):437.
3.Pasculle A. W., Feeley J. C., Gibson R. J., Cordes L. J., Myerowitz R. L., Patton C. M., Gorman G. W., Cormack C. L., Ezzell J. W., DowlingJ. N., 1980, J. Infect. Dis., 141:727.
4.Edelstein P. H., 1981, J. Clin. Microbiol., 14:298.

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