Technical Information

Legionella Agar Base

Product Code: DM 1809

Application: - Legionella Enrichment Broth 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 (25°C)	6.9±0.2

**Formula adjusted, standardized to suit performance parameters

Principle & 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. This resulted in better recovery of Legionella pneumophila [2]. Pasculle et al [3] found that the addition of ACES (N-2-acetamido-2-amino ethane sulphonic acid) buffer improved the nutritive value of the medium. Edelstein [4] was of the view that addition of alpha-ketoglutarate 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. α-Ketoglutarate satisfies the specific nutritional requirements of Legionella species. Activated charcoal detoxifies 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.

Methodology

Suspend 18.5 grams of powder media in 500 ml distilled water. Shake well & sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. Do not heat prior to sterilization. Cool to 50°C and aseptically add rehydrated contents of 1 vial of Legionella Growth Supplement (MS2016) or Legionella Supplement (MS2041) and Legionella Selective Supplement (MS2017) .Mix well and pour into sterile Petri plates. Stir the medium during dispensing to prevent settling of charcoal particles.

Quality Control

Physical 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
Reactionof 3.7% w/v aqueous solution at 25°C.pH:- 6.9±0.2

pH range 6.70-7.10

Cultural Response/ characteristices
DM 1809: Cultural characteristics observed with added Legionella Growth Supplement (MS2016),or Legionella Selective Supplement (MS2017) and Legionella supplement (MS2041), after an incubation at 35-37°C for 48-72 hours.

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

Storage and Shelf Life

Dried Media: Store below 30°C in tightly closed container and use before expiry date as mentioned on the label.
Prepared Media: 2-8^{o} in sealable plastic bags for 2-5 days.

Further Reading

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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