Technical Information

Differential Buffered Charcoal Yeast Extract Agar Base

Product Code: DM 1814

Application: - Differential Buffered Charcoal Yeast Extract Agar is recommended for selective isolation and differentiation of *Legionella* species.

Composition**

Ingredients	Gms / Litre
Yeast extract	10.000
Charcoal activated	1.500
L-Cysteine hydrochloride	0.400
Ferric pyrophosphate, soluble	0.250
ACES buffer	10.000
Alpha - Ketoglutarate	0.200
Bromocresol purple	0.010
Bromothymol blue	0.010
Agar	15.000
Final pH (at 25°C)	6.9±0.2

**Formula adjusted, standardized to suit performance parameters

Principle & Interpretation

Legionella pneumophila is a gram-negative rod responsible for Legionnaires disease. It infects the respiratory passage when irborne droplets of water are inhaled. In nature, the bacterium lives within the cytoplasm of the waterborne protozoan *Hartmanella* (1).

Common sources of *Legionella* include cooling towers used in industrial cooling water systems as well as in large central air conditioning systems, domestic hot water systems, fountains, and similar disseminators that draw upon a public water supply. Natural sources include freshwater ponds and creeks (2). Initially F-G Agar developed by Feelay et al (3) was used for the isolation of *L. pneumophila* . F-G Agar was further modification for replacing beef extract and casein hydrolysate by yeast extract. Starch was also replaced by activated charcoal (4) The modified F-G Agar was improved by the addition of ACES Buffer (N-2-acetamido-2-aminoethane sulphonic acid) (5). Sensitivity of the resulting Buffered Charcoal Yeast Extract Agar was increased by the addition of alpha-ketoglutarate (6). Differential Buffered Charcoal Yeast Extract Agar Base used for the selective isolation and differentiation of *Legionella* species is based on the formulation of Vickers (7) containing the two dyes, bromocresol purple and bromothymol blue.The medium contains yeast extract, which supply necessary nutrients for bacterial growth. Ferric pyrophosphate, L-cysteine hydrochloride and alpha- Ketoglutarate stimulates the growth of *Legionella* species (6). Toxic metabolic products produced in the medium get neutralized by activated charcoal which modifies the surface tension of the medium. Bromocresol purple and bromothymol blue help in the identification of *Legionella* species based on colour and colony morphology (8). Polymyxin B inhibits most of the gram-negative bacilli while vancomycin suppresses the growth of most of the gram-positive bacteria. ACES buffer helps to buffer the medium. The test sample should be cultured as soon as possible. Culture swabs can be directly streaked on the plate. Legionella growth is usually visible within 3-4 days but some species may take upto 2 weeks to appear.

Methodology

Suspend 37.37 grams of dehydrated media in 1000 ml distilled water. Mix thoroughly & heat to boil to dissolve the medium completely. Sterilize by autoclaving at 15 lbs (121°C) pressure for 15 minutes. Cool to 45-50°C. . If desired aseptically add 50 units/ml of Polymyxin B and 1 mg/ ml Vancomycin or aseptically add the rehydrated contents of one vial of MS2349 (V.P. Supplement). Mix well and pour into sterile Petri plates.

Quality Control

Appearance
Light grey to black homogeneous free flowing powder

Gelling
Firm,comparable with 1.5% Agar gel

Colour and Clarity
Grey-black coloured, opaque gel forms in Petri plates

Reaction
Reaction of 3.74% w/v aqueous solution at 25°C. pH : 6.9±0.2

pH Range
6.70-7.10

Cultural Response
DM1814: Cultural characteristics observed with added 50 units/ml Polymyxin B and 1mg/ml Vancomycin, after an incubation at 35-37°C for 72-96 hours.

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

Storage and Shelf Life

Dried Media: Store below 30°C in tightly closed container and the prepared medium at 2 - 8°C. Use before expiry date on the label.
Prepared Media: 2-8° in sealable plastic bags for 2-5 days.

Further Reading

1. Alcamo E. I., 2001, Fundamentals of Microbiology, 6th Ed., Jones and Bartlett Publishers.
2. Winn, W. C. Jr., 1996, Legionella In : Barons Medical Microbiology, Barron S. et al, Eds., 4th Edition, University of Texas Medical Branch
3. Feeley J. C., Gorman G. W., Weaver R. E., Mackel D. C., and Smith H. W.,1978, J. Clin. Microbiol., 8:320.
4. 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., 10:437.
5. Pasculle, Feeley, Gibson, et al, 1980, J. Infect. Dis., 141:727.
6. Edelstein H., 1981, J. Clin. Microbiol., 14:298.
7. Vickers R. M., Brown A. and Garrity G. M., 1981, J. Clin. Microbiol. , 13:380.
8. MacFaddin J. F., 1985, Media for Isolation-Cultivation-Identification-Maintenance of Medical Bacteria, vol. I, Williams and Wilkins, Baltimore.

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