Technical Information

Legionella Agar Base

Product Code: DM 1809A

Application :- Legionella Agar Base with the addition of supplement is used for the cultivation of *Legionella* species

Composition**

Ingredients	Gms / Litre
Charcoal activated	2.000
Yeast extract	10.000
Agar	13.000
Final pH (at 25°C)	6.9±0.2

**Formula adjusted, standardized to suit performance

Principle & Interpretation

Legionella Agar initially named 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] from clinical specimens.
Pasculle et al [3] showed that the addition of ACES (N-2-acetamido-2-amino ethane sulphonic acid) buffer improved the nutritive value of medium. Edelstein [4] was of the view that addition of a-Ketoglutarate increase the sensitivity of this medium.
The medium contains yeast extract to provide the necessary nitrogenous nutrients for *Legionella growth* . Activated charcoal neutralises 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] accompany theclinical specimens
Legionella species have an absolute nutritional requirement for L-Cysteine. Presumptive *Legionella* species colonies can be subcultured onto both Legionella Agar Base with MS2142 and with MS2206 (Medium without L-Cysteine). All plates are incubated at 3 5°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.

Methodology

Suspend 12.5 grams of powder media in 440 ml distilled water. Shake well & heat to dissolve the medium completely. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. Cool to 50°C and aseptically add contents of 1 vial of Legionella Growth Supplement (MS2142). In case of non incorporation of Legionella (GVPC) Selective Supplement (MS2143), add aseptically 10 ml sterile distilled water to bring the total volume to 500 ml of medium. The final pH of the medium will be 6.9 ± 0.2. Mix well and pour into sterile petri plates. Stir the medium while dispensing to prevent the settling of charcoal particles. If desired, the medium can be made selective by aseptically adding rehydrated contents of 1 vial of either Legionella BMPA Selective Supplement (MS2144) or Legionella (GVPC) Selective Supplement, (MS2143) along with 1 vial of Legionella Growth Supplement (MS2142) to 440 ml sterile molten, cooled Legionella Agar Base. Simultaneously, a medium without L-Cysteine may be prepared by adding aseptically contents of 1 vial of Legionella Growth Supplement w/o L-Cysteine (MS2206).

Quality Control

Physical Appearance

Grey to black coloured homogeneous free flowing powder solution

Gelling

Firm, comparable with 1.3% Agar gel.

Colour and Clarity of prepared medium

coloured opaque gel forms in petri plates.

Reaction

Reaction of 2.5% w/v aqueous solution on addition of Legionella Growth Supplement (MS2142) at 25°C. pH : 6.9±0.2

pH range

6.70-7.10

Cultural Response/Characteristics

DM 1809A: Cultural characteristics observed with added Sterile Legionella Growth Supplement (BCYE) (MS2142) and Legionella (GVPC) Selective Supplement (MS2143) or Legionella Growth Supplement w/o L-Cysteine (MS2206) , after an incubation at 35-37°C for 48-72 hours.

Organism	**Growth MS2142 & MS2143**	**(with Growth (With MS2206)**
Escherichia coli ATCC 25922	inhibited	good
Legionella dumofii ATCC 33343	good-luxuriant	inhibited
Legionella pneumophila ATCC 33153	good-luxuriant	inhibited

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: 2-8^0 in sealable plastic bags for 2-5 days.

Further Reading

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. Pasculle, 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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