# Legionella Agar Base

**M809A**

## Intended Use:

With addition of supplements it is used for 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 parameters

## Directions

Suspend 12.5 grams in 440 ml purified / distilled water. Heat to boiling to dissolve the medium completely. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. Cool to 45-50°C and aseptically add contents of 1 vial of Legionella Growth Supplement (BCYE) (FD142). In case of non incorporation of Legionella (GVPC) Selective Supplement (FD143), 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 (FD144) or Legionella (GVPC) Selective Supplement, (FD143) along with 1 vial of Legionella Growth Supplement (BCYE)(FD142) 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 (FD206).

## Principle And Interpretation

Legionella Agar initially called as F-G agar was modified by Feely et al (4) by replacing Starch with charcoal and casein hydrolysate with yeast extract which resulted in better recovery of *Legionella pneumophila* (5).
Pasculle et al (8) reported that the addition of ACES (N-2-acetamido-2-amino ethane sulphonic acid) buffer improved the nutritive value of medium. Edelstein (3) 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 (2,3).
*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.

## Type of specimen

Water samples

## Specimen Collection and Handling

For water samples, follow appropriate techniques for sample collection, processing as per guidelines and local standards (1). After use, contaminated materials must be sterilized by autoclaving before discarding.

## Warning and Precautions

Read the label before opening the container. Wear protective gloves/protective clothing/eye protection/face protection. Follow good microbiological lab practices while handling specimens and culture. Standard precautions as per established guidelines should be followed while handling specimens. Safety guidelines may be referred in individual safety data sheets.

## Limitations

1. Some strains may show poor growth due to variable nutritional requirements.

## Performance and Evaluation

Performance of the medium is expected when used as per the direction on the label within the expiry period when stored at recommended temperature.

## Quality Control

**Appearance**
Grey to black coloured homogeneous free flowing powder

**Gelling**
Firm, comparable with 1.3% Agar gel.

**Colour and Clarity of prepared medium**
Black coloured opaque gel forms in petri plates.

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

**pH**
6.70-7.10

**Cultural Response**
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 | Growth (with FD142 & FD143) | Growth (With FD206) |
|---|---|---|
| *Escherichia coli* ATCC 25922 (00013*) | inhibited | good |
| *Legionella dumoffii* ATCC 33343 | good-luxuriant | inhibited |
| *Legionella pneumophila* ATCC 33153 | good-luxuriant | inhibited |

Key : (*) corresponding WDCM numbers

## Storage and Shelf Life

Store between 10-30°C in a tightly closed container and the prepared medium at 2-8°C. Use before expiry date on the label. On opening, product should be properly stored dry, after tightly capping the bottle in order to prevent lump formation due to the hygroscopic nature of the product. Improper storage of the product may lead to lump formation. Store in dry ventilated area protected from extremes of temperature and sources of ignition. Seal the container tightly after use. Product performance is best if used within stated expiry period.

## Disposal

User must ensure safe disposal by autoclaving and/or incineration of used or unusable preparations of this product. Follow established laboratory procedures in disposing of infectious materials and material that comes into contact with sample must be decontaminated and disposed of in accordance with current laboratory techniques (6,7).

## Reference

1. Baird R.B., Eaton A.D., and Rice E.W., (Eds.), 2015, Standard Methods for the Examination of Water and Wastewater, 23rd ed., APHA, Washington, D.C.
2. Dennis et al, 1984, Proceeding of the 2nd International Symposium, Washington D.C. Am. Soc. Microbiol. PP 294-296.
3. Edelstein, 1981, J. Clin. Microbiol., 14:298.
4. Feely J. C., et al, 1978, J. Clin. Microbiol., 8(3):320.
5. Feely, Gibson, Gorman, et al, 1979, J. Clin. Microbiol., 10(4):437.
6. Isenberg, H.D. Clinical Microbiology Procedures Handbook 2nd Edition.
7. Jorgensen, J.H., Pfaller, M.A., Carroll, K.C., Funke, G., Landry, M.L., Richter, S.S and Warnock., D.W. (2015) Manual of Clinical Microbiology, 11th Edition. Vol. 1.
8. Pasculle, Feely, Gibson et al, 1980, J. Infect. Dis., 141:727.

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