Legionella Agar Base

M809

Intended Use:

With addition of supplements it is used for 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 purified/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 (3) by replacing starch by charcoal and casein hydrolysate by yeast extract which resulted in better recovery of *Legionella pneumophila* (4). Pasculle et al (7) reported that the addition of ACES (N-2-acetamido-2-amino ethane sulphonic acid) buffer improved the nutritive value of the medium. Edelstein (2) 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.

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 conditions.

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 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

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 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 (5,6).

Reference

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2. Edelstein P. H., 1981, J. Clin. Microbiol., 14:298.
3. Feeley J. C., Gorman G. W., Weaver R. E. Mackel D. G., Smith H. W.,1978, J. Clin. Microbiol., 8(3):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(4):437.
5. Isenberg, H.D. Clinical Microbiology Procedures Handbook 2nd Edition.
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7. 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.

Revision : 03/2021