Legionella Agar Base w/o Charcoal

M1845

Intended Use

Recommended for isolation of *Legionella* species with addition of charcoal supplement.

Composition**

Ingredients	g / L
Yeast extract	10.000
Agar	15.000
Final pH (at 25°C)	6.9±0.2

**Formula adjusted, standardized to suit performance parameters

Directions

Suspend 12.5 grams in 430 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. Aseptically add the rehydrated contents of 1 vial of Sterile Charcoal Supplement for Legionella agar (FD280) and 1 vial containing 50ml of BCYE Growth Supplement (FD142). Aseptically add 10ml of sterile distilled water to bring the volume to 500 ml, when no selective supplement is added. The final pH of the medium should be 6.9 ± 0.2. Mix well to prevent the settling of charcoal particles and pour into sterile Petri plates.

If desired, the medium can be made selective by aseptically adding rehydrated contents of 1 vial of either BMPA Selective Supplement(FD144) or GVPC Selective Supplement(FD143), or GVPN Selective Supplement (FD242) along with 1 vial of BCYE Growth Supplement (FD142) and Sterile Charcoal Supplement(FD280) to 430 ml sterile molten, cooled Legionella Agar Base (M1845). Simultaneously, a medium without L-Cysteine may be prepared by aseptically adding contents of 1 vial of Legi Growth Supplement w/o L-Cysteine(FD206).

Principle And Interpretation

Legionella Agar initially called 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). Pasculle et al (3) reported that the addition of ACES (N-2-acetamido-2-amino ethane sulphonic acid) buffer improved the nutritive value of medium. Edelstein (4) suggested addition of a-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 (4,5). *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

Clinical samples - sputum , urine; Water samples

Specimen Collection and Handling

For clinical samples follow appropriate techniques for handling specimens as per established guidelines (6,7).

For water samples, follow appropriate techniques for sample collection, processing as per guidelines and local standards(8).

After use, contaminated materials must be sterilized by autoclaving before discarding.

Warning and Precautions

In Vitro diagnostic Use. For professional use only. 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 clinical specimens. Safety guidelines may be referred in individual safety data sheets.

Limitations

1.Individual organisms differ in their growth requirement and may show variable growth patterns on the medium.
2.Each lot of the medium has been tested for the organisms specified on the COA. It is recommended to users to validate the medium for any specific microorganism other than mentioned in the COA based on the user's unique requirement.

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

Cream to yellow homogeneous free flowing powder

Gelling

Firm,comparable with 1.5% Agar gel

Colour and Clarity of prepared medium

After addition of supplement (FD280) : Black coloured opaque gel forms in Petri plates.

Reaction

Reaction of 2.5% w/v aqueous solution on addition of BCYE Growth Supplement (FD142) and Sterile Charcoal Supplement (FD280) solution at 25°C. pH : 6.9±0.2

pH

6.70-7.10

Cultural Response

Cultural characteristics observed with added Sterile BCYE Growth Supplement (FD142) and GVPC Selective Supplement (FD143) or Legi Growth Supplement w/o L-Cystein (FD206) , after an incubation at 35-37°C for 48-72 hours.

Organism	Inoculum (CFU)	*Growth	**Growth	Recovery
Escherichia coli ATCC 25922 (00013#)	50-100	inhibited~	good	
Legionella dumoffii ATCC 33343	50-100	good-luxuriant	inhibited	>50%
Legionella pneumophila ATCC 33153	50-100	good-luxuriant	inhibited	>50%

Key: * = Growth on Legionella Agar Base with FD142 ~ = in presence of FD143
** = Growth on Legionella Agar Base with FD206 # 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 clinical sample must be decontaminated and disposed of in accordance with current laboratory techniques (6,7).

Reference

1. Feely J. C., et al, 1978, J. Clin. Microbiol., 8(3):320.
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3. Psculle, Feely, Gibson et al, 1980, J. Infect. Dis., 141:727.8
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.
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.

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Revision : 07/2024

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IVD ***In vitro*** **diagnostic medical device**

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10°C **Storage temperature**

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