Differential Buffered Charcoal Yeast Extract Agar Base

M814

Intended use

Used for selective isolation and differentiation of *Legionella* species.

Composition**

Ingredients	g / L
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

Directions

Suspend 37.37 grams in 1000 ml purified / distilled water. Heat to boiling 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 FD349 (V.P. Supplement). Mix well and pour into sterile Petri plates.

Principle And Interpretation

Legionella pneumophila is a gram-negative rod responsible for Legionnaires disease. It infects the respiratory passage when airborne 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 modified by replacing beef extract and casein hydrolysate by yeast extract. Also starch was replaced by activated charcoal (4). The modified F-G Agar was improved by the addition of ACES Buffer (N-2-acetamido-2-aminoethane sulfonic 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 provide 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.

Type of specimen

Clinical samples - respiratory swabs; Water samples

Specimen Collection and Handling:

For clinical samples follow appropriate techniques for handling specimens as per established guidelines (9,10).

For water samples, follow appropriate techniques for sample collection, processing as per guidelines and local standards (11). 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. The test sample should be cultured as soon as possible. Culture swabs can be directly streaked on the plate.
2. *Legionella* growth is usually visible within 3-4 days but some species may take upto 2 weeks to appear.
3. Further biochemical confirmation has to be carried out for further confirmation.

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

Light grey to black homogeneous free flowing powder

Gelling

Firm,comparable with 1.5% Agar gel

Colour and Clarity of prepared medium

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

6.70-7.10

Cultural Response

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

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 (9,10).

Reference

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