Buffered Charcoal Yeast Extract HiCynth™ Agar Base MCD813

Intended Use

Recommended for selective isolation and cultivation of *Legionella* species from cooling towers and other specimens.

Composition**

Ingredients	Gms / Litre
HiCynth™ Peptone No.5*	10.000
Charcoal activated	2.000
ACES buffer	10.000
α-Ketoglutarate monopotassium salt	1.000
Agar	17.000
Final pH (at 25°C)	6.9±0.2

**Formula adjusted, standardized to suit performance parameters

* Chemically defined peptone

Directions

Suspend 20 grams in 500 ml purified / distilled water. Add 2.4 grams KOH pellets and mix to dissolve. 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 sterile rehydrated contents of 1 vial each of MWY Selective Supplement (FD040) and Legi Growth Supplement w/o SS (Twin Pack) (FD041A). Mix well and pour with constant stirring into sterile Petri plates, taking care that charcoal particles get evenly distributed. For additional selectivity,VCAT Selective Supplement (FD017), C3V Selective Supplement (FD037), PGV Selective Supplement (FD038) may be added to molten medium as per choice.

Principle And Interpretation

Feeley et al (1) originally formulated Charcoal Yeast Extract (CYE) Agar. This medium was a modification of the existing F-G Agar (2). F-G Agar had starch and casein enzymic hydrolysate as ingredients in the composition. Feely et al (1,2) replaced these two with charcoal and yeast extract respectively, and reported better recovery of *Legionella pneumophilla*. Later Paseulle (3) reported that supplementation of the Charcoal Yeast Agar with ACES buffer improved the performance of the medium. Edelstein (4) further modified the medium by adding alpha-ketoglutarate. This addition helped in improving the sensitivity of the medium. Buffered Charcoal Yeast Extract Agar Base is based on Edelsteins Modification. Buffered Charcoal Yeast Extract HiCynth™ Agar Base is the modification of the same prepared by replacing animal and vegetable peptones with chemically defined peptones to avoid BSE/TSE risks associated with animal peptones.

Legionella species are non-spore forming, narrow, gram-negative rods. *Legionella* causes pneumonia (Legionnaires disease) (5) or a milk, febrile disease (Pontiac fever). They do not oxidize or ferment carbohydrates in conventional media or grow on sheep blood agar. Growth is much better and more rapid on Buffered Charcoal Yeast Extract Agar (2,6). Amino acids are the major sources of energy for *Legionella*. The amino acid L-cystine holds an absolute requirement as it plays major role in growth metabolism of *Legionella* (7). This amino acid as well as ferric pyrophosphate helps for the growth of *Legionella*. The media contains charcoal, which acts as a detoxicant. HiCynth™ Peptone No.5 acts as a rich source of vitamins, nitrogen as well as carbon. ACES Buffer maintains optimal pH for growth while L-cystine hydrochloride; ferric pyrophosphate and α-ketoglutarate stimulate growth of *Legionella* species. For selective isolation, antibiotic supplements can be used to suppress contaminating microorganisms. C3V Selective Supplement (FD037) containing cephalothin, colistin, vancomycin and cycloheximide (8) or MWY Selective Supplement (FD040) containing glycine, polymyxin B, anisomycin, vancomycin, bromothymol blue and bromocresol purple (9) are often used. Wear gown, mask and gloves while handling *Legionella* cultures. Work in a safety hood.

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

Warning and Precautions

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. Further biochemical confirmation has to be carried out for complete identification.
2. Individual organisms differ in their growth requirement and may show variable growth patterns on the medium.

Performance and Evaluation

Performance of the medium is expected when used as per the direction on the label within theexpiry 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

Grey-black coloured opalescent gel forms in Petri plates.

Reaction

Reaction of 4.0% w/v aqueous solution at 25°C. pH : 6.9±0.2

pH

6.70-7.10

Cultural Response

Cultural characteristics observed in 90% humid atmosphere with added Supplement(FD041A and FD040), after an incubation at 35-37°C for 3-4 days.

Organism	Inoculum (CFU)	Growth	Recovery	Colour of colony
Escherichia coli ATCC 25922 (00013*)	50-100	none-poor	<=10%	
Legionella dumoffii ATCC 33343	50-100	luxuriant	>50%	light blue-grey
Legionella pneumophila ATCC 33153	50-100	luxuriant	>50%	white grey to blue grey
Staphylococcus epidermidis ATCC 12228 (00036*)	50-100	none-poor	<10%	

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 clinical sample must be decontaminated and disposed of in accordance with current laboratory techniques (11,12).

Reference

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