Buffered Charcoal Yeast Extract Agar Base

M813

Buffered Charcoal Yeast Extract Agar Base with added supplements is used for selective cultivation of *Legionella* species from clinical and other specimens.

Composition**

Ingredients	Gms / Litre
Yeast extract	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

Directions

Suspend 20 grams in 500 ml 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 50°C. Aseptically add sterile rehydrated contents of 1 vial each of Legionella Supplement (FD041A and FD040). Mix well and pour with constant stirring to ensure that charcoal particles get evenly distributed. For additional selectivity, Legionella Selective Supplements (FD017, FD037, FD038) may be added to molten medium as per choice.

Principle And Interpretation

Feeley et al (5) originally formulated Charcoal Yeast Extract (CYE) Agar. This medium was a modification of the existing F-G Agar (3). F-G Agar had starch and casein enzymic hydrolysate as ingredients in the composition. Feely et al (3, 5) replaced these two with charcoal and yeast extract respectively, and reported better recovery of *Legionella pneumophilla* . Later Paseulle (6) reported that supplementation of the Charcoal Yeast Agar with ACES buffer improved the performance of the medium. Edelstein (7) 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.

Legionella species are non-spore forming, narrow, gram-negative rods. *Legionella* causes pneumonia (Legionnaires disease) (1) 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 (3, 4). 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* (2). This amino acid as well as ferric pyrophosphate helps for the growth of *Legionella* .

The media contains charcoal, which acts as a detoxicant. Yeast extract 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. Legionella Selective Supplement II (CCVC) (FD037) containing cephalothin, colistin, vancomycin and cycloheximide (8) or Legionella Selective Supplement IV (MWY) (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.

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 Legionella Supplement(FD041 and FD040), after an incubation at 35-37°C for 3-4 days.

Cultural Response

Organism	Inoculum (CFU)	Growth	Recovery	Colour of colony
Cultural Response				
Escherichia coli ATCC 25922	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	50-100	none-poor	<10%	

Storage and Shelf Life
Store below 30°C in tightly closed container and the prepared medium at 2-8°C. Use before expiry date on the label.

Reference
1. Broome C. V., Fraser D. W., 1979, Epidemiol. Rev 1:1-16.
2. George J. R. et al, 1980, J. Clin. Microbiol., 11:286
3. Feeley J. C., Gorman G. W., Weaver R. E. et al, 1978, J. Clin. Microbiol., 8 : 320-325.
4. Jones G. T., Hebert G. A., (Eds.), 1979, US Department of Health, Education and Welfare Publication No. (CDC) 79-8375, Atlanta, Centers for Disease Control.
5. Feeley J. C., Gibson R. J., Gorman G. W. et al, 1979, J. Clin. Microbiol., 10:437.
6. Paseulle, Feely et al, 1980, J. Infect. Dis., 191:727.
7. Edelstein P. H., 1981, J. Clin. Microbiol., 14:298.
8. Bopp C. A., Sumner J. W., Morris G. K. and Wells J. G., 1981, J. Clin. Microbiol., 13:714.
9. Vicker R., Brown and Garrity, 1981, J. Clin. Microbiol., 13:380.

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