microgen
Ready Prepared Media

Technical Information

Buffered Charcoal Yeast Extract Agar Plate (BCYE Medium)

Product Code: PM 1813I

Application : Recommended for selective isolation and cultivation of *Legionella* species from cooling towers, water samples, clinical and other materials. The composition and performance criteria of this medium are as per the specifications laid down in ISO 11731-2017.

Composition**

Buffered Charcoal Yeast Extract Agar Base	**DM8131I**
Ingredients	**Gms / Litre**
Yeast extract	10.000
Activated Charcoal	2.000
alpha ketoglutarate, monopotassium salt	1.000
ACES Buffer	10.000
Agar	12.000
Final pH (at 25°C)	6.8±0.2

Formula adjusted, standardized to suit performance parameters

Legionella Supplement (Twin Pack)	**MS2041A**
Ingredients	**Concentration**
Part A	
L-Cysteine hydrochloride	200mg
Part B	
Ferric pyrophosphate,soluble	125mg
Distilled water	5ml

Principle & 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. The formulation of Buffered Charcoal Yeast Extract Agar Base is as per specification laid in ISO 11731-2(10).

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 a-ketoglutarate stimulate growth of Legionella species. For selective isolation, antibiotic supplements can be used to suppress contaminating microorganisms. PCP Supplement (MS2347) containing Polymyxin B, Sodium cefazolin and Pimaricin or Legionella (GVPC) Selective Supplement (MS2143) containing glycine, Polymyxin B sulphate, vancomycin and cycloheximide or Legionella Selective Supplement IV (MWY) (MS2040) 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.

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Type of specimen

Clinical samples - Blood : Food and dairy samples : Water samples

Specimen Collection and Handling

For clinical samples follow appropriate techniques for handling specimens as per established guidelines (11,12).
For food and dairy samples, follow appropriate techniques for sample collection and processing as per guidelines (13,14,16).
For water samples, follow appropriate techniques for sample collection, processing as per guidelines and local standards.(15)
After use, contaminated materials must be sterilized by autoclaving before discarding.

Warning and Precautions

In Vitro diagnostic 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 guidleines should be followed while handling clincal specimens. Saftey guidelines may be referred in individual safety data sheets

Limitations

- Further biochemical tests should be carried out for 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.

Methodology

Either streak, inoculate or surface spread the test inoculum (50-100 CFU) aseptically on the plate.

Quality Control

Appearance
Sterile Buffered Charcoal Yeast Extract Agar in 90mm disposable plate**.**
Colour of medium
Grey-black coloured opalescent medium
Quantity of medium
25 ml of medium in 90 mm plates.
Reaction
6.60-7.00
Sterility Test
Passes release criteria
Cultural Response
Cultural characteristics observed in 90% humid atmosphere after an incubation at 34-38°C for 2-5 days.
Cultural Response

Oragnism	Inoculum (CFU)	Growth	Recovery	Colour of Colony
Legionella pneumophila ATCC 33152 (00107)*	50-100	luxuriant	>=50%	white-grey-blue purple colonies with an entire edge exhibiting a characteristic ground glass appearance
Legionella anisa ATCC35292 (00106)*	50-100	luxuriant	>=50%	white-grey-blue purple colonies with an entire edge exhibiting a characteristic ground glass appearance (incubated for 5-10 days)
Legionella dumofii ATCC 33343	50-100	luxuriant	>=50%	light-blue grey

Key : * - Corresponding WDCM numbers

Storage and Shelf Life

On receipt store between 2-8°C. Use before expiry date on the label. 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 clinicalsample must be decontaminated and disposed of in accordance with current laboratory techniques (11,12).

Further Reading

(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.
(10) Water quality-Detection and enumeration of Legionella-Part 2 Direct membrane filtration method for waters with low bacterialcounts International Organization for Standardization (ISO), 2017, Draft ISO/DIS, 11731-2
(11) Isenberg, H.D. Clinical Microbiology Procedures Handb0ook. 2nd Edition.
(12) 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.
(13) American Public Health Association, Standard Methods for the Examination of Dairy Products, 1978, 14th Ed., Washington D.C.
(14) Yvonne Salfinger and Mary Lou Tortorello (Ed.), 2001, Compendium of Methods for the Microbiological Examination of Foods, 5th Ed., American Public Health Association, Washington, D.C.
(15) Greenberg A. E., Clesceri L. S. and Eaton A. D., (Eds.), 2005, Standard Methods for the Examination of Water andWastewater, 21st ed., APHA, Washington, D.C.
(16) Wehr H. M. and Frank J. H., 2004, Standard Methods for the Microbiological Examination of Dairy Products, 17th Ed., APHA Inc., Washington, D.C.

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Disclaimer :

- User must ensure suitability of the product(s) in their application prior to use.
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