



Ready Prepared Media

Technical Information

BCYE Selective Agar Plate

Product Code: PM 6383

Application: Recommended for selective isolation and cultivation of *Legionella*

Composition**

Ingredients	Gms / Litre
Yeast extract	10.000
Charcoal activated	2.000
ACES buffer	10.000
α -Ketoglutarate monopotassium salt	1.000
L-cysteine hydrochloride	0.400
Ferric pyrophosphate	0.250
Potassium hydroxide	2.800
Anisomycin	0.08
Colistin	0.01
Vancomycin	0.0005
Agar	17.000
Final pH (at 25°C)	6.9 \pm 0.2

**Formula adjusted, standardized to suit performance parameters

Principle & Interpretation

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 grown sheep blood agar. 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* (5). This amino acid as well as ferric pyrophosphate helps for the growth of *Legionella*. Growth is much better and more rapid on Buffered Charcoal Yeast Extract Agar (3,8). Feely et al (4) had originally formulated Charcoal Yeast Extract (CYE) Agar. This medium was a modification of the existing F-G Agar containing starch and casein enzymic hydrolysate (3). Feely et al (3, 4) replaced these two with charcoal and yeast extract respectively, and reported better recovery of *Legionella pneumophilla*. Later Paeulle (9) reported that supplementation of the Charcoal Yeast Agar with ACES buffer improved the performance of the medium. Edelstein (2) further modified the medium by adding alpha-ketoglutarate. This addition helped in improving the sensitivity of the medium. BCYE Agar (Buffered Charcoal Yeast Extract Agar) is based on Edelsteins Modification.

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. Antibiotic anisomycin, vancomycin and colistin in the medium suppress contaminating microorganisms. Anisomycin inhibits growth of fungi. Gram negative organisms are inhibited by colistin. Gram positive organisms are inhibited by Vancomycin.



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Type of specimen

Clinical specimen: Oropharyngeal flora

Non clinical specimen : Environmental samples, water sources such as cooling water etc.

Specimen Collection and Handling

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

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

Warning and Precautions

In Vitro diagnostic use. 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. Few strains of *Legionella* would grow well in CO₂ conditions in spite of medium supplemented with L-cysteine.
2. Suggested to isolate *Legionella* on non selective and selective BCYE plates in parallel.
3. Biochemical tests are necessary for confirmation of *Legionella*.

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. The plate should be directly inoculated with the clinical specimen or the transport medium that has been inoculated with the clinical specimen.

Quality Control

Appearance

Sterile BCYE Selective Agar in 90 mm disposable plates with smooth surface and absence of black particles/cracks/bubbles.

Colour

Grey-black coloured medium

Quantity of medium

25 ml of medium in 90 mm petri plate

pH

6.70-7.10

Sterility Check

Passes release criteria

Cultural Response

Cultural characteristics observed in 90% humid atmosphere after an incubation at 35-37°C for 3-4 days.



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Organism	Inoculum (CFU)	Growth	Recovery	Colour of Colony
<i>Candida albicans</i> ATCC 10231 (00054*)	50-100	inhibited	0%	
<i>Escherichia coli</i> ATCC25922 (00013*)	50-100	non-poor	<=10%	
<i>Legionella dumoffii</i> ATCC 33343	50-100	good-luxuriant	>50%	light-blue grey
<i>Legionella pneumophila</i> ATCC 33153	50-100	good-luxuriant	>50%	white grey to blue grey
<i>Staphylococcus epidermidis</i> ATCC 12228 (00036*)	50-100	non-poor	<10%	

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 sample must be decontaminated and disposed of in accordance with current laboratory techniques (6,7).

Further Reading

1. Broome C. V., Fraser D. W., 1979, Epidemiol. Rev 1:1-16.
2. Edelstein P. H., 1981, J. Clin. Microbiol., 14:298.
3. Feeley J. C., Gorman G. W., Weaver R. E. et al, 1978, J. Clin. Microbiol., 8 : 320-325.
4. Feeley J. C., Gibson R. J., Gorman G. W. et al, 1979, J. Clin. Microbiol., 10:437.
5. George J. R. et al, 1980, J. Clin. Microbiol., 11:286
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.
8. 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.
9. Paeulle, Feely et al, 1980, J. Infect. Dis., 191:727.



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Disclaimer

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