Technical Data

BCYE Agar Plate

MP813I

Intended use

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 (E).

Composition**

BCYE Agar As per ISO 11731:2017(E)	
Ingredients	**g / L**
Yeast extract (Bacteriological grade)	10.000
Activated Charcoal	2.000
α-ketoglutarate, monopotassium salt	1.000
ACES Buffer	10.000
Agar	12.000
KOH pellets	2.800
L-cystine hydrochloride monohydrate	0.400
Iron (III) pyrophosphate	0.250
Final pH (at 25°C)	6.8±0.2

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Legi Growth Supplement w/o SS (Twin Pack)	**FD041A**
Part A	"
L-Cysteine hydrochloride	200mg
Part B	"
Ferric pyrophosphate,soluble	125mg
Distilled water	5ml

Directions

Either streak, inoculate or surface spread the test inoculum (50-100 CFU) aseptically on the plate. Membrane filters through which water sample has been filtered can be placed directly on the agar plate.

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 tryptone 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. The formulation of Buffered Charcoal Yeast Extract Agar Base is as per specification laid in ISO 11731-2 (5).

Legionella species are non-spore forming, narrow, gram-negative rods. *Legionella* causes pneumonia (Legionnaires disease) (6) 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 (2,7). 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 (8). This amino acid as well as ferric pyrophosphate helps for the growth of *Legionella.*

The media contains charcoal, which acts as 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. Wear gown, mask and gloves while handling *Legionella* cultures. Work in a safety hood (8).

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

Warning and Precautions :

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 further confirmation.
2. Individual organisms differ in their growth requirement and may show variable growth patterns on the medium
3. Each lot of the medium has been tested for the organisms specified on the COA. It is recommended to users to validate the medium for any specific microorganism other than mentioned in the COA based on the user's unique requirement.

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

Sterile Buffered Charcoal Yeast Extract Agar in 90mm disposable plate.

Colour

Grey-black coloured opalescent medium

Quantity of medium

25 ml of medium in 90mm plate

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.

Organism	Inoculum (CFU)	Growth	Recovery	Colour of colony
Legionella pneumophila ATCC 13152(00107*)	50-100	luxuriant	>=50%	white-grey-blue purple colonies with an entire edge exhibiting a characteristic ground glass appearance
Legionella anisa ATCC 35292 (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 sample must be decontaminated and disposed of in accordance with current laboratory techniques (9,10).

Reference

1. Feeley J. C., Gibson R. J., Gorman G. W. et al, 1979, J. Clin. Microbiol., 10:437.
2. Feeley J. C., Gorman G. W., Weaver R. E. et al, 1978, J. Clin. Microbiol., 8 : 320-325.
3. Paseulle, Feely et al, 1980, J. Infect. Dis., 191:727.

4.Edelstein P. H., 1981, J. Clin. Microbiol., 14:298.
5.Water quality-Detection and enumeration of Legionella-Part 2 Direct membrane filtration method for waters with low bacterial counts International Organization for Standardization (ISO), 2017, Draft ISO/DIS, 11731-2
6.Broome C. V., Fraser D. W., 1979, Epidemiol. Rev 1:1-16.
7.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.
8. George J. R. et al, 1980, J. Clin. Microbiol., 11:286
9. Isenberg, H.D. Clinical Microbiology Procedures Handbook. 2nd Edition.
10. 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.

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