HIMEDIA

Technical Data

Buffered Charcoal Yeast Extract Agar Medium(BCYE Medium) M813I

Intended use

Recommended for selective isolation and cultivation of *Legionella* species from cooling towers, all kinds of water samples and other materials. The composition and performance criteria of this medium are as per the specifications laid down in ISO 11731-1: 2017 & 11731-2: 2017 .

Composition**

BCYE Agar As per ISO 11731	
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
Potassium hydroxide (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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Agar	12.000
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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

Selective culture medium

For Buffered charcoal yeast extract agar with selective supplement (BCYE+AB)	
Polymyxin B sulfate	80,000 IU
Sodium cefazolin	0.009
Pimaricin (syn Natamycin)	0.070

PCP Supplement	**FD347**
Polymyxin B sulfate	80,000 IU
Cefazolin sodium	0.009
Pimaricin (Natamycin)	0.070

For Buffered charcoal yeast extract agar with selective supplement (BCYE+GVPC)	
Ammonium-free Glycine	3.000
Vancomycin hydrochloride	0.001
Polymyxin B sulphate	80,000 IU
Cycloheximide	0.080

GVPC Selective Supplement	**FD143**
Glycine	1.500g
Vancomycin hydrochloride	0.500mg
Polymyxin B sulphate	40000IU
Cycloheximide	40mg

For Modified Widomski Yee (MWY)	
Polymyxin B sulphate	50,000 IU
Ammonium-free glycine	3.000g
Anisomycin	0.080
Vancomycin hydrochloride	0.001
Bromo thymol blue	0.010
Bromo cresol purple	0.010

MWY Selective Supplement	**FD040**
Polymyxin B sulphate	25000Unit
Glycine	1.500g
Anisomycin	40mg
Vancomycin	0.500mg
Bromo thymol blue	5mg
Bromo cresol purple	5mg

Directions

Suspend 35.0 grams in 1000 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 (121 ± 3) °C for (15 ± 1) minutes. Cool to 45-51°C. Aseptically add sterile rehydrated contents of 2 vials each of Legi Growth Supplement w/o SS (Twin Pack) (FD041A, Part A and Part B). Mix well and pour into sterile Petri plates with constant stirring to ensure that charcoal particles get evenly distributed.
Note: As per standard it is recommended to use 2.8 grams of Potassium hydroxide pellets.

Buffered charcoal yeast extract agar without L-cysteine (BCYE–cys)
Prepare same as BCYE Agar composition and aseptically add contents of two vials of Legi Growth Supplement w/o SS (Twin Pack) (FD041A, Part B only).
For Buffered charcoal yeast extract agar with selective supplement (BCYE+AB)
Aseptically add rehydrated contents of one vial of PCP Supplement (FD347).
For Buffered charcoal yeast extract agar with selective supplement
Aseptically add rehydrated contents of two vials of GVPC Selective Supplement (FD143).
For Modified Widomski Yee (MWY) Agar:
Aseptically add the rehydrated contents of one vial of MWY Selective Supplement (FD040- per 100 ml).

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 α−ketoglutarate stimulate growth of Legionella species. For selective isolation, antibiotic supplements can be used to suppress contaminating microorganisms. PCP Supplement (FD347) containing Polymyxin B, Sodium cefazolin and Pimaricin or Legionella (GVPC) Selective Supplement (FD143) containing glycine, Polymyxin B sulphate, vancomycin and cycloheximide 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 (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 specimens. Safety guidelines may be referred in individual safety data sheets.

Limitations :

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

Quality Control

Appearance

Grey to black homogeneous free flowing powder

Gelling

Firm, comparable with 1.2% Agar gel.

Colour and Clarity of prepared medium

Grey-black coloured opalescent gel forms in Petri plates.

Reaction

Reaction of 3.5% w/v aqueous solution at 25°C. pH : 6.8±0.2

pH

6.60-7.00

Cultural Response

Productivity : Cultural response was observed after an incubation (90% humid atmosphere) at 36 ± 2°C for 2-5 days, with added sterile Legi Growth Supplement w/o SS (Twin Pack) (FD041A, Part A and Part B). Recovery rate is considered as >=50% on BCYE

Selectivity : Cultural response was observed after an incubation (90% humid atmosphere) at 36 ± 2°C for 2-5 days, with added sterile Legi Growth Supplement w/o SS (Twin Pack) (FD041A, Part A and Part B).

Organism	Inoculum (CFU)	Growth	Recovery	Colour of colony
Productivity				
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 pneumophila ATCC 33156 (00180*)	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)
Selectivity				
Enterococcus faecalis ATCC 29212 (00087*)	>=10^4	inhibited	0%	
Enterococcus faecalis ATCC 19433 (00009*)	>=10^4	inhibited	0%	
Escherichia coli ATCC 25922 (00013*)	50-100	none-poor	<=10%	
Escherichia coli ATCC 8739 (00012*)	50-100	none-poor	<=10%	
^*Pseudomonas paraeruginosa* ATCC 9027 (00026*)	50 -100	none-poor	<=10%	
Pseudomonas aeruginosa ATCC 27853 (00025*)	50 -100	none-poor	<=10%	

Key : (*) - Corresponding WDCM numbers

^ Formerly known as *Pseudomonas aeruginosa*

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 sample must be decontaminated and disposed of in accordance with current laboratory techniques (10,11).

Reference

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