



## Buffered Charcoal Yeast Extract Agar Medium(BCYE Medium) M813I

### 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.

### Composition\*\*

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

### Directions

Suspend 35.0 grams in 1000 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  $(121 \pm 3)^\circ\text{C}$  for  $(15 \pm 1)$  minutes. Cool to  $45\text{-}51^\circ\text{C}$ . Aseptically add sterile rehydrated contents of 2 vials each of Legionella Supplement (FD041A, Part A and Part B). Mix well and pour with constant stirring to ensure that charcoal particles get evenly distributed. Simultaneously a medium w/o L-Cysteine may be prepared by aseptically adding contents of two vials of Legionella Supplement (FD041A, Part B only).

For additional selectivity,

For BCYE+AB Agar : Aseptically add the rehydrated contents of one vial of PCP Supplement (FD347)

For GVPC Agar : Aseptically add rehydrated contents of 2 vials of Legionella (GVPC ) Selective Supplement (FD143).

For Modified Wadowsky Yee (MWY) Agar : Aseptically add the rehydrated contents of one vial of Legionella Selective Supplement IV (MWY) -(FD040- per 100 ml).

Note : As per standard it is recommended to use 2.8 grams of Potassium hydroxide pellets

### 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 tryptone 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 Peseulle (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 (FD347) containing Polymyxin B, Sodium cefazolin and Pimaricin or Legionella (GVPC ) Selective Supplement (FD143) containing glycine, Polymyxin B sulphate, vancomycin and cycloheximide 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.

## Type of specimen

Clinical samples - Blood; Water samples

## Specimen Collection and Handling

For clinical samples follow appropriate techniques for handling specimens as per established guidelines (11,12).

For water samples, follow appropriate techniques for sample collection, processing as per guidelines and local standards (13). 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 guidelines should be followed while handling clinical specimens. Safety guidelines may be referred in individual safety data sheets

## Limitations :

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

Cultural characteristics observed in 90% humid atmosphere with added Legionella Supplement (FD041A and FD040 or FD143 or FD347), after an incubation at 34-38°C for 2-5 days.

Organism	Inoculum (CFU)	Growth	Recovery	Colour of colony
<i>Legionella pneumophila</i> ATCC 33152 (00107*)	50-100	luxuriant	≥50%	white-grey-blue purple colonies with an entire edge exhibiting a characteristic ground glass appearance
<i>Legionella anisa</i> 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)
<i>Enterococcus faecalis</i> ATCC 29212 (00087*)	≥10 <sup>3</sup>	inhibited	0%	
<i>Enterococcus faecalis</i> ATCC 19433 (00009*)	≥10 <sup>3</sup>	inhibited	0%	
<i>Escherichia coli</i> ATCC 25922 (00013*)	50-100	none-poor	≤10%	
<i>Escherichia coli</i> ATCC 8739 (00012*)	50-100	none-poor	≤10%	
<i>Legionella dumofii</i> ATCC 33343	50-100	luxuriant	≥50%	light blue - grey

Key : (\*) - Corresponding WDCM numbers

## 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. 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 clinical sample must be decontaminated and disposed of in accordance with current laboratory techniques (11,12).

## Reference

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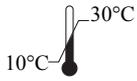
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In vitro diagnostic medical device



CE Marking



Storage temperature



Do not use if package is damaged



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