Buffered Charcoal Yeast Extract Agar

Intended Use
A medium used for selective cultivation of *Legionella* species from clinical and other specimens.

Summary
Feeley *et al.,* developed a medium to provide consistent isolation of *Legionella* species. The medium demonstrated differential growth characteristics to aid in the identification of *Legionella* species. Feeley later modified the medium by substituting yeast extract for casein hydrolysate and beef extract and replacing starch with activated charcoal. Feeley called this modified formula Charcoal Yeast Extract (CYE) Agar. A further modification was made by Pasculle *et al.,* in 1980. This version employed the addition of ACES (N-2-acetamido-2-aminoethane sulfonic acid) buffer in order to maintain the proper pH for optimal growth. The new medium, designated BCYE for Buffered Charcoal Yeast Extract, could also be incubated aerobically. In 1981, Edelstein et al. increased the sensitivity of the medium by adding the potassium salt of alpha-ketoglutaric acid.

Principle
The formulation employs the use of L-Cysteine, soluble ferric pyrophosphate, and alpha-ketoglutarate to enhance the growth of *Legionella* species. Activated charcoal removes toxic metabolic products. Protein and other growth nutrients are supplied by yeast extract.

Formula*

Ingredients	**g/L**
Yeast Extract	10.0
Charcol Activated	2.0
ACES Buffer	10.0
Alpha-Ketoglutarate Monopotassium Salt	1.0
Agar	17.0
Final pH (at 25°C)	6.9 ± 0.2

*Adjusted to suit performance parameters

Storage and Stability
Store dehydrated medium below 30°C in tightly closed container and the prepared medium at 2ºC-8°C. Avoid freezing and overheating. Use before expiry date on the label. Once opened keep powdered medium closed to avoid hydration.

Type of specimen
Clinical samples
Water samples

Specimen Collection and Handling
Ensure that all samples are properly labelled.
Follow appropriate techniques for handling samples as per established guidelines.
Some samples may require special handling, such as immediate refrigeration or protection from light, follow the standard procedure.
The samples must be stored and tested within the permissible time duration.
After use, contaminated materials must be sterilized by autoclaving before discarding

Directions
1. Suspend 20.00 g of the powder in 500 mL purified / distilled water. Add 2.4 g KOH pellets and mix to dissolve.
2. Heat to boiling to dissolve the powder completely.
3. Sterilize by autoclaving at 121°C (15 psi) for 15 minutes as per validated cycle.
4. Cool to 50°C. Aseptically add sterile rehydrate contents of 1 vial each of Legionella Supplement.
5. Mix well and pour with constant stirring to ensure that charcoal particles get evenly distributed.
6. For additional selectivity Legionella Selective Supplement may be added to molten medium as per choice.

Quality Control

Dehydrated Appearance: Grey to black coloured homogenous, free flowing powder.
Prepared Appearance: Black coloured, clear to slightly opalescent gel forms in petridishes.
Cultural Response: Cultural characteristics observed in 90% humid atmosphere with Legionella Supplement after an incubation at 35°C-37°C for 3-4 days.

Organism (ATCC)	Growth	Colour of colony
Escherichia coli (25922)	Partial Inhibition	-
Legionella dumoffii (33343)	Good	Grey
Legionella pneumophila (33153)	Good	White grey
Staphylococcus epidermidis strain PCI 1200 (12228)	Partial Inhibition	-

Interpretation of Results

1. In general, colonies of *Legionella* spp. present a white to gray colouration. They may also have blue, pink, purple, maroon, greenish-yellow or dark red pigmentation that fades, becoming whiter and more filamentous with age.
2. The colony surface is generally smooth with precise edges, but some strains may give a ground glass or "fried egg" appearance when viewed microscopically.
3. Some species fluoresce under UV light.

Performance and Evaluation

Performance of the product is dependent on following parameters as per product label claim:

1. Directions
2. Storage
3. Expiry

Precautions/Limitations

1. It is recommended that biochemical, immunological, molecular, or mass spectrometry testing be performed on colonies from pure culture for complete identification.
2. It is also recommended that more than one type of medium be used for isolating *Legionella* spp. and that non-selective and selective BCYE Agar plates be inoculated in parallel.
3. This medium is to be used for the isolation and presumptive identification of *Legionella*.
4. Colonies of *Legionella* that develop on white membrane filters may have a different appearance to those that develop against a black or dark background filter.
5. When handling *Legionella* spp., it is important to avoid aerosol formation. Thoroughly clean and disinfect all work areas.

Warranty

This product is designed to perform as described on the label and package insert. The manufacturer disclaims any implied warranty of use and sale for any other purpose.

Reference

1. Feeley J. C., Gorman G. W., Weaver R. E. et al, 1978, J. Clin. Microbiol., 8: 320-325.
2. Feeley J. C., Gibson R. J., Gorman G. W. et al, 1979, J. Clin. Microbiol., 10:437.
3. Paseulle, Feely et al, 1980, J. Infect. Dis., 191:727. 7.
4. Edelstein P. H., 1981, J. Clin. Microbiol., 14:298.
5. Data on file: Microxpress®, A Division of Tulip Diagnostics (P) Ltd.

Product Presentation:

Cat No.	Product description	Pack Size
201020360100	Dehydrated Culture Media	100 g
201020360500	Dehydrated Culture Media	500 g