BCYE Agar (Legionella Isolation Medium) (NCM0037)

Intended Use
BCYE Agar is used (with appropriate supplementation) for the isolation of *Legionella* spp. from water and environmental samples. BCYE Agar is not intended for use in the diagnosis of disease or other conditions in humans.

Description
In 1977, McDade *et al*. identified *Legionella pneumophila* as the causative agent of Legionnaires' disease, a multisystem disease manifested primarily by pneumonia. In 1978 a new medium, F-G Agar, resulted in improved growth of *L. pneumophila*, a very fastidious organism. Freely *et al*. modified F-C Agar by substituting yeast extract as a vitamin source and replacing starch with activated charcoal, producing Charcoal Yeast Extract (CYE) Agar. In 1980, Pasculle *et al.* reported that CYE Agar could be improved by the addition of ACES (N-2-acetamido-2-aminoethane sulfonic acid) buffer. One year later, Edelstein further increased the sensitivity of the medium by adding the potassium salt of alpha-ketoglutaric acid.

Typical Formulation

Yeast Extract	10.0 g/L
Charcoal	2.0 g/L
Ferric Pyrophosphate	0.25 g/L
ACES Buffer	10.0 g/L
Potassium Carbonate	2.3g/L
Agar	14.0 g/L

Final pH: 6.9 ± 0.1 at 25°C
Formula may be adjusted and/or supplemented as required to meet performance specifications.

Supplements
GVPC Selective Supplement #X195 – 1 vial per 500mL of Agar
BCYE Growth Supplement #X196 – 1 vial per 500mL of Agar
BCYE Growth Supplement (without L-Cysteine) #X197 – 1 vial per 500mL of Agar

Precaution
Refer to SDS

Preparation
1. Suspend 38.5 g of the medium in 1L of purified water.
2. Heat with frequent agitation and boil for one minute to completely dissolve the medium.
3. Autoclave at 110°C for 10 minutes. Cool to 45 - 50°C.
4. Aseptically add 2 vials of X196 BCYE growth supplement to make BCYE agar with Legionella growth supplements.
5. To make GVPC selective medium additionally add 2 vials of X195 GVPC selective supplement
6. Mix thoroughly and continuing mixing throughout dispensing.

NOTE: X196 supplement can be substituted with X197 supplement (without the addition of X195 selective supplement) to prepare presumptive identification plates.

Test Procedure
According to ISO 11731:2017, preparation of the water sample depends on the sample type. Methods range from direct inoculation to concentration via membrane filtration with or without pre-treatment. After ensuring inocula has been absorbed invert the plates and incubate at 36±1°C for 7 to 10 days. Create a humid atmosphere to prevent desiccation of the plates.

Effective Date: 2/15/2019
Revision: 0

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Quality Control Specifications

Dehydrated Appearance: Powder is homogeneous, free flowing and black.

Prepared Appearance: Prepared medium is opaque and black.

Expected Cultural Response:.

Cultural response on BCYE Agar Supplemented with X196 Growth Supplement at 36 ± 2°C and examined for growth.

MICROORGANISM	ATCC	APPROX. INOCULUM (CFU)	EXPECTED RESULTS
Legionella bozemanii	33217	50-200	>50% grey/white colonies
Legionella pneumophila	33152	50-200	>50% grey/white colonies
Legionella pneumophila	33156	50-200	>50% grey/white colonies
Pseudomonas aeruginosa	9027	10^3-10^4	Growth
Escherichia coli	8739	10^3-10^4	Growth
Escherichia coli	25922	10^3-10^4	Growth
Enterococcus faecalis	19433	10^3-10^4	Growth
Enterococcus faecalis	29212	10^3-10^4	Growth

Cultural response on BCYE Agar Supplemented with X196 Growth Supplement and X195 GVPC selective supplement at 36 ± 2°C and examined for growth

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Pseudomonas aeruginosa	9027	10^3-10^4	Complete to partial inhibition
Escherichia coli	8739	10^3-10^4	Complete to partial inhibition
Escherichia coli	25922	10^3-10^4	Complete to partial inhibition
Enterococcus faecalis	19433	10^3-10^4	Partial inhibition
Enterococcus faecalis	29212	10^3-10^4	Partial inhibition

The organisms listed are the minimum that should be used for quality control testing.

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Results
Legionella pneumophila produces small to large, smooth, colorless to pale, blue-grey, slightly mucoid colonies that fluoresce yellow-green under longwave UV light. A gram stain, biochemical tests, and serological procedures should be performed for confirmation of *L. pneumophila*.

Storage
Store sealed bottle containing the dehydrated medium at 2 - 30°C. Once opened and recapped, place container in a low humidity environment at the same storage temperature. Protect from moisture and light by keeping container tightly closed.

Expiration
Refer to expiration date stamped on the container. The dehydrated medium should be discarded if it is not free flowing, or if appearance has changed from the original color. Expiry applies to medium in its intact container when stored as directed.

Limitations of the Procedure
1. Due to nutritional variation, some strains may be encountered that grow poorly or fail to grow on this medium.
2. Biochemical tests and serological procedures must be performed to confirm presence of *L. pneumophila*.

References

1. McDade, Shepard, Fraser, Tsai, Redus, Dowdle and the Laboratory Investigation Team. 1977. N. Engl. J. Med. 297:1197.
2. Edelstein. 1985. *In* Lennette, Balows, Hausler and Shadomy (eds.). Manual of clinical microbiology, 4th ed. ASM. Washington, D.C.
3. Freely, Gorman, Weaver, Mackel and Smith. 1978. J. Clin. Microbiol. 8:320.
4. Freely, Gibson, Gorman, Lansford, Rasheed, Mackel and Baine. 1979. J. Clin. Microbiol. 10:437.
5. Pasculle, Freely, Gibson, Cordes, Myerowitz, Patton, Gorman, Carmack, Ezzell and Dowling. 1980. J. Infect. Dis. 141:727.
6. Edelstein. 1981. J. Clin. Microbiol. 14:298.
7. ISO 11731:2017 – Water Quality – Enumeration of Legionella.

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