BCYE Agars
BCYE Agar Base • BCYE Agar • BCYE Differential Agar BCYE Selective Agars (CCVC, PAC, PAV) • Legionella Agar Base • Legionella Agar Enrichment

Intended Use

These media are used in qualitative procedures for isolation of *Legionella* species from clinical specimens and nonclinical (environmental) samples.

Summary and Explanation

BCYE Agar is based on Edelstein's modification of previously described media. In 1979, Feely et al. described Charcoal Yeast Extract (CYE) Agar as a modification of an existing medium, F-G Agar.[1,2] They replaced the starch in the F-G Agar with activated charcoal and substituted yeast extract for casein hydrolysate, resulting in better recovery of *L. pneumophila*. In 1980, Pasculle reported that CYE Agar could be improved by buffering the medium with ACES Buffer.[3] A year later, Edelstein further increased the sensitivity of the medium by adding alpha-ketoglutarate (BCYE Agar).[4]

Legionella Agar is a modification of the BCYE Agar formula of Edelstein. In the formula, the concentration of ACES buffer was reduced from 10.0 g/L to 6.0 g/L.

BCYE Differential Agar is used for the presumptive identification and differentiation of *Legionella* spp. based on colony morphology and color.[5] This medium is based on the formulation of Vickers et al.,[6] and consists of the dyes bromcresol purple and bromthymol blue added to BCYE Agar.

BCYE Selective Agar w/CCVC is a highly selective medium consisting of BYCE Agar supplemented with cephalothin, colistin, vancomycin and cycloheximide. This medium is based on the formulation of Bopp et al.[7] They obtained improved recovery of *L. pneumophila* by using the selective medium in conjunction with an acid wash treatment to reduce the contaminating microbial flora present in environmental water samples.

BCYE Selective Agar with PAC was developed by Edelstein for isolation of *Legionella* spp. from specimens containing mixed flora.[4] He found that BYCE Agar supplemented with polymyxin B, cefamandole and anisomycin enhanced the recovery of *L. pneumophila* from contaminated clinical specimens. In conjunction with an acid wash treatment to reduce microbial flora, it also facilitated the recovery of the bacterium from potable water.

User Quality Control

NOTE: Differences in the Identity Specifications and Cultural Response testing for media offered as both **Difco**™ and **BBL**™ brands may reflect differences in the development and testing of media for industrial and clinical applications, per the referenced publications.

Identity Specifications

Difco™ Legionella Agar Base

Dehydrated Appearance:	Dark gray, free flowing, homogeneous.
Solution:	3.7% solution, insoluble in purified water. Suspension is black, opalescent.
Prepared Appearance:	Black, opaque with precipitate.
Reaction of 3.7% Solution at 25°C:	pH 6.85-7.0 (adjusted)

Difco™ Legionella Agar Enrichment

Lyophilized Appearance:	White to off-white powder, with or without traces of brown or green powder.
Solution:	After rehydration, white to off-white, opaque, milky suspension. Some evidence of browning may be present due to ferric pyrophosphate.

Cultural Response

Difco™ Legionella Agar Base

Prepare the medium per label directions. Inoculate and incubate at 35 ± 2°C under a humidified atmosphere containing approximately 2.5% CO_2 for 46-72 hours.

ORGANISM	ATCC™	INOCULUM CFU	RECOVERY	COLONY COLOR
Legionella dumoffii	33279	30-300	Good	Gray to off-white
Legionella pneumophila	33153	30-300	Good	Gray to off-white
Legionella pneumophila	33154	30-300	Good	Gray to off-white (may have a blue cast)

Identity Specifications

BBL™ BCYE Agar Base

Dehydrated Appearance:	Fine to coarse, homogeneous, free of extraneous material.
Solution:	3.83% solution, soluble in purified water upon boiling. Solution is dark, black-green to gray-black, moderately hazy to opaque.
Prepared Appearance:	Dark, black-green to gray-black, moderately hazy to opaque.
Reaction of 3.83% Solution at 25°C:	pH 6.85 ± 0.1 (adjusted)

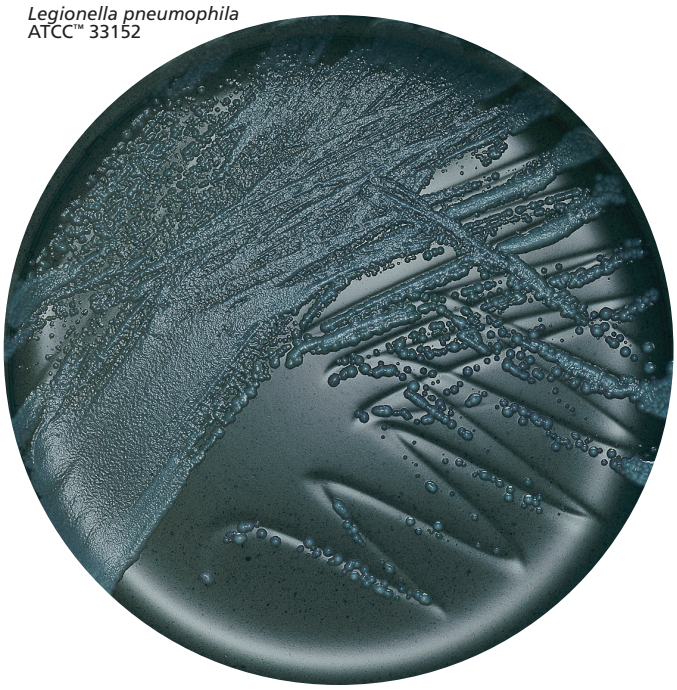
Cultural Response

BBL™ BCYE Agar Base

Prepare the medium per label directions. For *E. coli*, inoculate and incubate at 35 ± 2°C for 66-72 hours. For *Legionella* spp., inoculate test and control lots of medium with serial 10-fold dilutions of the test organisms and incubate at 35 ± 2°C for 66-72 hours. Growth on the test lot should be within 1 log of the control lot.

ORGANISM	ATCC™	INOCULUM CFU	RECOVERY	FLUORESCENCE
Escherichia coli	25922	10^3-10^4	Good	–
Legionella bozemanii	33217	N/A	Good (white-gray to blue-gray colonies)	Blue-white
Legionella pneumophila	33152	N/A	Good	Yellow green

Legionella pneumophila
ATCC™ 33152



BCYE Selective Agar with PAV is similar to the Edelstein formula, above, except that the concentration of polymyxin B is reduced by half, and vancomycin is substituted for cefamandole.

Principles of the Procedure

These media consist of a base medium (BCYE) supplemented with antibiotics or dyes. Antibiotics improve the recovery of *Legionella* spp. by inhibiting the growth of contaminating organisms. Dyes facilitate differentiation and identification of *Legionella* spp.

The base media (BCYE Agar Base and Legionella Agar Base) contain yeast extract to supply the nutrients necessary to support bacterial growth. L-cysteine HCl, ferric pyrophosphate and alpha-ketoglutarate are incorporated to satisfy the specific nutritional requirements of *Legionella* species. The activated charcoal decomposes hydrogen peroxide, a toxic metabolic product, and may also collect carbon dioxide and modify surface tension. The addition of the buffer helps maintain the proper pH for optimal growth of *Legionella* species.

Antibiotics incorporated in the various BCYE formulations have different spectra of activity. Vancomycin inhibits gram-positive bacteria; colistin and polymyxin B inhibit gram-negative bacteria, except for *Proteus* spp.; and cephalothin and cefamandole inhibit both gram-positive and gram-negative bacteria. Anisomycin and cycloheximide are antifungal agents.

BCYE Differential Agar contains the dyes bromcresol purple and bromthymol blue to aid in the differentiation and identification of *Legionella* species.

Formulae

Difco™ Legionella Agar Base

Approximate Formula* Per Liter

Yeast Extract	11.5	g
ACES Buffer	6.0	g
Charcoal, Activated	1.5	g
Alpha-Ketoglutarate, Monopotassium	1.0	g
Agar	17.0	g

Difco™ Legionella Agar Enrichment

Approximate Formula* Per Vial

L-Cysteine HCl	0.35	g
Ferric Pyrophosphate	0.14	g

BBL™ BCYE Agar Base

Approximate Formula* Per Liter

Yeast Extract	10.0	g
Ferric Pyrophosphate	0.25	g
ACES Buffer	10.0	g
Charcoal, Activated	2.0	g
Alpha-Ketoglutarate	1.0	g
Agar	15.0	g

**Adjusted and/or supplemented as required to meet performance criteria.*

Directions for Preparation from Dehydrated Product

Difco™ Legionella Agar Base

1. Dissolve 18.5 g of the powder in 500 mL of purified water.
2. Adjust to pH 7.1-7.2 with 1 N KOH. Do not heat prior to autoclaving.
3. Autoclave at 121°C for 15 minutes.
4. Cool to 45-50°C and aseptically add 5 mL rehydrated Difco™ Legionella Agar Enrichment. Mix thoroughly.
5. Check pH. If necessary, aseptically adjust to pH 6.85-7.0 with 1 N HCl or 1 N KOH.
6. Dispense into Petri dishes. Agitate while dispensing to keep charcoal in suspension.
7. Test samples of the finished product for performance using stable, typical control cultures.

BBL™ BCYE Agar Base

1. To 500 mL of purified water, add 2.4 g KOH pellets and mix to dissolve.
2. Add 38.3 g of the powder and 500 mL of purified water. Mix thoroughly.
3. Heat with frequent agitation and boil for 1 minute to completely dissolve the powder.
4. Autoclave at 121°C for 15 minutes.
5. Cool to 45-50°C and add 4 mL of a 10% filter-sterilized solution of L-cysteine HCl.
6. Mix thoroughly. Check pH; if not 6.85 ± 0.1, adjust using 1 N HCl or KOH.
7. Dispense into Petri dishes. Agitate while dispensing to keep charcoal in suspension.
8. Test samples of the finished product for performance using stable, typical control cultures.

Procedure

Use standard procedures to obtain isolated colonies from specimens and samples.

Incubate the plates in an inverted position (agar side up) at 35 ± 2°C for a minimum of 3 days. Growth is usually visible within 3-4 days, but may take up to 2 weeks to appear.

Expected Results

On BCYE Agar, Legionella Agar and the selective media, *Legionella pneumophila* produces small to large, smooth, colorless to pale, blue-gray, slightly mucoid colonies that fluoresce yellow-green under long-wave UV light. Consult references for morphology and color of fluorescence of other species.[8,9]

On BCYE Differential Agar, *L. pneumophila* produces light blue colonies with a pale green tint. *L. micdadei* produces blue-gray to dark blue colonies.

A Gram stain, biochemical tests and serological procedures should be performed to confirm findings.

References

1. Feely, Gibson, Gorman, Langford, Rasheed, Mackel and Baine. 1979. J. Clin. Microbiol. *10*:437.
2. Feely, Gorman, Weaver, Mackel and Smith. 1978. J. Clin. Microbiol. *8*:320.
3. Pasculle, Feely, Gibson, Cordes, Myerowitz, Patton, Gorman, Carmack, Ezzell and Dowling. 1980. J. Infect. Dis. *191*:727.
4. Edelstein. 1981. J. Clin. Microbiol. *14*:298.
5. MacFaddin. 1985. Media for isolation-cultivation-identification-maintenance of medical bacteria, vol. 1. Williams & Wilkins, Baltimore, Md.
6. Vickers, Brown and Garrity. 1981. J. Clin. Microbiol. *13*:380.
7. Bopp, Sumner, Morris and Wells. 1981. J. Clin. Microbiol. *13*:714.
8. Edelstein. 2007. *In* Murray, Baron, Jorgensen, Landry and Pfaller (ed.), Manual of clinical microbiology, 9th ed. American Society for Microbiology, Washington, D.C.
9. Weaver. 1978. *In* Jones and Herbert (ed.), "Legionnaires": the disease, the bacterium and methodology. DHEW, Center for Disease Control, Atlanta, Ga.

Availability

Difco™ Legionella Agar Base

Cat. No. 218301 Dehydrated – 500 g

Difco™ Legionella Agar Enrichment

Cat. No. 233901 Vial, 5 mL – Pkg. of 6*

BBL™ BCYE Agar Base

SMWW

Cat. No. 212327 Dehydrated – 500 g

BBL™ BCYE Agar

BS12 CMPH2 MCM9

United States and Canada

Cat. No. 221808 Prepared Plates – Pkg. of 10*

Europe

Cat. No. 257321 Prepared Plates – Ctn. of 120*

Japan

Cat. No. 252164 Prepared Plates – Pkg. of 20*

BBL™ BCYE Differential Agar

Cat. No. 297881 Prepared Plates – Pkg. of 10*

BBL™ BCYE Selective Agar with PAC

BS12 CMPH2 MCM9

Cat. No. 297879 Prepared Plates – Pkg. of 10*

BBL™ BCYE Selective Agar with PAV

BS12 MCM9

Cat. No. 297880 Prepared Plates – Pkg. of 10*

BBL™ BCYE Selective Agar with CCVC

Cat. No. 297878 Prepared Plates – Pkg. of 10*

**Store at 2-8°C.*