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BD BBL™ Prepared Plated Media for Isolation of *Legionella*

BCYE Selective Agar w/CCVC

BCYE Selective Agar w/PAC

BCYE Selective Agar w/PAV

BCYE Differential Agar

Legionella Selective Agar

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INTENDED USE

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

SUMMARY AND EXPLANATION

Charcoal Yeast Extract (CYE) Agar was developed by Feely et al. in 1979 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.⁴ BCYE Agar is based on Edelstein's modified formulation.

BCYE Selective Agar w/ CCVC is a highly selective medium consisting of BCYE Agar supplemented with cephalothin, colistin, vancomycin and cycloheximide. This medium is based on the formulation of Bopp et al.⁵ 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* species from specimens containing mixed flora.⁴ He found that BCYE 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.

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.

BCYE Differential Agar is used for the presumptive identification and differentiation of *Legionella* species based on colony morphology and color.⁶ This medium is based on the formulation of Vickers, et al.⁷ and consists of the dyes bromcresol purple and bromthymol blue added to BCYE Agar.

Legionella Selective Agar was developed by BD Diagnostics for isolation of *Legionella* species from materials containing mixed flora. Consisting of BCYE Agar supplemented with the antimicrobics vancomycin, colistin and anisomycin, it provides improved inhibition of contaminating bacteria without significantly inhibiting *Legionella* species.⁸

PRINCIPLES OF THE PROCEDURE

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

The base medium contains yeast extract to supply the nutrients necessary to support bacterial growth. L-cysteine HCL, ferric pyrophosphate and alphas-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 formulations have different spectra of activity. Vancomycin inhibits gram-positive bacteria; colistin and polymyxin B inhibit gram-negative bacteria, except for *Proteus* species; 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. After sufficient incubation, *L. pneumophila* produces light blue colonies with a pale green tint, while *L. micdadei* produces blue-gray to dark blue colonies.

REAGENTS

BCYE Agar

Approximate Formula* Per Liter Purified Water

Yeast Extract	10.0 g
L-Cysteine HCl	0.4 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

BCYE Selective Agar w/ CCVC

Approximate Formula* Per Liter Purified Water

BCYE Agar	38.65 g
Cephalothin	4.0 mg
Colistin	16.0 mg
Vancomycin	0.5 mg
Cycloheximide	80.0 mg

BCYE Selective Agar w/PAC

Approximate Formula* Per Liter Purified Water

BCYE Agar	38.65 g
Polymyxin B	80,000 units
Anisomycin	80.0 mg
Cefamandole	4.0 mg

BCYE Selective Agar w/PAV

Approximate Formula* Per Liter Purified Water

BCYE Agar	38.65 g
Polymyxin B	40,000 units
Anisomycin	80.0 mg
Vancomycin	0.5 mg

BCYE Differential Agar

Approximate Formula* Per Liter Purified Water

BCYE Agar	38.65 g
Bromcresol Purple	10.0 mg
Bromthymol Blue	10.0 mg

Legionella Selective Agar

Approximate Formula* Per Liter Purified Water

BCYE Agar	38.65 g
Vancomycin	2.0 mg
Colistin	3.75 mg
Anisomycin	10.0 mg

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

Warnings and Precautions:

For *in vitro* Diagnostic Use.

If excessive moisture is observed, invert the bottom over an off-set lid and allow to air dry in order to prevent formation of a seal between the top and bottom of the plate during incubation.

Storage Instructions: On receipt, store plates in the dark at 2 to 8°C. Avoid freezing and overheating. Do not open until ready to use. Minimize exposure to light. Prepared plates stored in their original sleeve wrapping at 2 to 8°C until just prior to use may be inoculated up to the expiration date and incubated for recommended incubation times. Allow the medium to warm to room temperature before incubation.

Product Deterioration: Do not use plates if they show evidence of microbial contamination, discoloration, drying, cracking or other signs of deterioration.

SPECIMEN COLLECTION AND TRANSPORT

Refer to appropriate texts for details of specimen collection and handling procedures.^{5,9-11}

Pathogenic microorganisms, including hepatitis viruses and Human Immunodeficiency Virus, may be present in clinical specimens. "Standard Precautions"¹²⁻¹⁵ and institutional guidelines should be followed in handling all items contaminated with blood and other body fluids. Prior to discarding, sterilize specimen containers and other contaminated materials by autoclaving.

PROCEDURE

Material Provided: BCYE Selective Agar with CCVC, BCYE Selective Agar with PAC, BCYE Selective Agar with PAV, BCYE Differential Agar or Legionella Selective Agar.

Materials Required But Not Provided: Ancillary culture media, reagents, quality control organisms and laboratory equipment as required for this procedure.

Test Procedure

Observe aseptic techniques. The agar surface should be smooth and moist, but without excessive moisture.

Inoculate the medium as soon as possible after the specimen arrives at the laboratory. To culture a specimen from a swab, inoculate the medium by rolling the swab over a third of the agar surface and streak the remainder of the plate to obtain isolated colonies. Material not being cultured from swabs may be streaked onto the medium with a sterilized inoculating loop. The streak plate technique is used primarily to obtain isolated colonies from specimens containing mixed flora.

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

User Quality Control:

1. Examine plates for signs of deterioration as described under "Product Deterioration."
2. Check performance by inoculating a representative sample of plates with pure cultures of stable control organisms that produce known, desired reactions. The following test strains are recommended:

MEDIUM	TEST STRAIN	EXPECTED RESULTS
BCYE Selective w/CCVC	<i>Legionella pneumophila</i> ATCC™ 33152	Visible growth within 3 days
	<i>Escherichia coli</i> ATCC 25922	Inhibition (partial)
	<i>Pseudomonas aeruginosa</i> ATCC 10145	Inhibition (partial)
	<i>Staphylococcus aureus</i> ATCC 25923	Inhibition (partial)
BCYE Selective w/PAC BCYE Selective w/PAV	<i>Legionella pneumophila</i> ATCC 33152	Visible growth within 3 days
	<i>Candida albicans</i> ATCC 10231	Inhibition (partial)
Legionella Selective Agar	<i>Escherichia coli</i> ATCC 25922	Inhibition (partial)
	<i>Staphylococcus aureus</i> ATCC 25923	Inhibition (partial)
	<i>Legionella pneumophila</i> ATCC 33152	Light blue colonies with a pale green tint.
BCYE Differential Agar	<i>Legionella pneumophila</i> ATCC 33152	Light blue colonies with a pale green tint.
	<i>Legionella micdadei</i> ATCC 33218	Blue-gray to dark blue colonies within 3 days.

Quality control requirements must be performed in accordance with applicable local, state and/or federal regulations or accreditation requirements and your laboratory's standard Quality Control procedures. It is recommended that the user refer to pertinent NCCLS guidance and CLIA regulations for appropriate Quality Control practices.

EXPECTED RESULTS

After sufficient incubation, the plates should show isolated colonies in streaked areas and confluent growth in areas of heavy inoculation. On BCYE Selective media and Legionella Selective Agar, *Legionella pneumophila* produces small to large, smooth, colorless to pale blue-gray, slightly mucoid colonies.

On the BCYE Differential medium, *L. pneumophila* produces light blue colonies with a pale green tint. After sufficient incubation (3 days), *L. micdadei* produces blue-gray to dark blue colonies.

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

PERFORMANCE CHARACTERISTICS

BCYE Selective Agar w/CCVC: All lots of BCYE Selective Agar w/CCVC are tested for specific product performance characteristics. Samples are tested with cell suspensions of *Legionella*, *Escherichia coli*, *Pseudomonas aeruginosa* and *Staphylococcus aureus*, and inoculated by spreading the cell suspension, diluted in normal saline to yield 1×10^3 to 1×10^4 CFU/plate (30-300 CFU/plate for *Legionella*), over the agar surface. Plates are incubated at 35-37°C for up to 7 days in an aerobic atmosphere. Visible growth within 3 days and moderate to heavy growth within 7 days with correct color and fluorescence under long-wave UV light, of *Legionella* colonies are observed. Partial to complete inhibition of *E. coli*, *P. aeruginosa* and *S. aureus* is observed.

BCYE Selective Agar w/PAC: All lots of BCYE Selective Agar w/PAC are tested for specific product performance characteristics. Samples are tested with cell suspensions of *Legionella pneumophila*, *Candida albicans*, *Escherichia coli* and *Staphylococcus aureus*, and inoculated by spreading the cell suspension, diluted in normal saline to yield 1×10^3 to 1×10^4 CFU/plate (30-300 CFU/plate for *Legionella*), over the agar surface. Plates are incubated at 35-37°C for three days in an aerobic atmosphere. Moderate to heavy growth and correct color of *L. pneumophila* colonies and fluorescence under long-wave UV light, and partial to complete inhibition of *C. albicans*, *E. coli*, and *S. aureus* are observed.

BCYE Selective Agar w/PAV: All lots of BCYE Selective Agar w/PAV are tested for specific product performance characteristics. Samples are tested with cell suspensions of *Legionella*, *Candida albicans*, *Escherichia coli* and *Staphylococcus aureus*, and inoculated by spreading the cell suspension, diluted in normal saline to yield 1×10^3 to 1×10^4 CFU/plate (30-300 CFU/plate for *Legionella*), over the agar surface. Plates are incubated at 35-37°C for three days in an aerobic atmosphere. Moderate to heavy growth and correct color of *Legionella* colonies and fluorescence under long-wave UV light and partial to complete inhibition of *C. albicans*, *E. coli*, and *S. aureus* are observed.

BCYE Differential Agar: All lots of BCYE Differential Agar are tested for specific product performance characteristics. Samples are tested with cell suspensions of *Legionella* spp. and inoculated by spreading the cell suspension, diluted in normal saline to yield 30-300 CFU/plate, over the agar surface. Plates are incubated at 35-37°C for three days in an aerobic atmosphere. Moderate to heavy growth and correct color of *Legionella* colonies are observed.

Legionella Selective Agar: All lots of Legionella Selective Agar are tested for specific product performance characteristics. Samples are tested with cell suspensions of *Legionella*, *Candida albicans*, *Escherichia coli* and *Staphylococcus aureus*, and inoculated by spreading the cell suspension, diluted in normal saline to yield 1×10^3 to 1×10^4 CFU/plate (30-300 CFU/plate for *Legionella*), over the agar surface. Plates are incubated at 35-37°C for three days in an aerobic atmosphere. Moderate to heavy growth and correct color of *Legionella* colonies and fluorescence under long-wave UV light and partial to complete inhibition of *C. albicans*, *E. coli*, and *S. aureus* are observed.

LIMITATIONS OF THE PROCEDURE

These prepared plated media are intended for primary isolation. Some diagnostic tests may be performed with the primary plate. However, a pure culture is recommended for biochemical tests and serological procedures. Consult appropriate texts for detailed information and recommended procedures.^{9,11,16}

A single medium is rarely adequate for detecting all organisms of potential significance in a specimen. The agents in selective media may inhibit some strains of the desired species or permit growth of a species that were designed to inhibit, especially if the species is present in large numbers in the specimen. Specimens cultured on selective media should, therefore, also be cultured on nonselective media to obtain additional information and help ensure recovery of potential pathogens.

AVAILABILITY

Cat. No.	Description
297878	BBL™ BCYE Selective Agar with CCVC, Pkg. of 10 plates
297879	BBL™ BCYE Selective Agar with PAC, Pkg. of 10 plates
297880	BBL™ BCYE Selective Agar with PAV, Pkg. of 10 plates
215103	BBL™ BCYE Selective Agar with PAV, Ctn. of 100 plates
297881	BBL™ BCYE Differential Agar, Pkg. of 10 plates
297920	BBL™ Legionella Selective Agar, Pkg. of 10 plates

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