



BD BBL™ BCYE Selective Agar w/CCVC
BD BBL™ BCYE Selective Agar w/PAC
BD BBL™ BCYE Selective Agar w/PAV
BD BBL™ BCYE Differential Agar
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R_x Only

QUALITY CONTROL PROCEDURES

I INTRODUCTION

BD BBL™ BCYE Selective Agar with CCVC, **BD BBL™ BCYE Selective Agar with PAC**, **BD BBL™ BCYE Selective Agar with PAV** and **BD BBL™ BCYE Differential Agar** are used in qualitative procedures for isolation of *Legionella* species from clinical specimens and nonclinical (environmental) samples.

II PERFORMANCE TEST PROCEDURE

A. BD BBL BCYE Selective Agar with CCVC

- Inoculate representative samples with the cultures listed below.
 - For *Legionella* add 100 µL (0.1 mL) of a culture containing 30–300 CFU/0.1 mL to each plate and spread-inoculate using a sterile glass spreader.
 - For *E. coli*, *P. aeruginosa*, and *S. aureus* streak inoculate 1 µL (0.001 mL) from a 4–5 h culture of **BD BBL™ Trypticase™** Soy Broth diluted to yield 10⁶–10⁷ CFU/mL.
 - For *A. brasiliensis* test samples by inoculating with a fresh stock culture (less than one month in age).
 - Include plates of a previously tested lot of **BD BBL BCYE** for *Legionella* and **BD BBL Trypticase** Soy Agar with 5% Sheep Blood as controls for remaining organisms.
- Examine plates after 3–7 days of incubation at 35 ± 2 °C for ATCC 16404, 25922, 10145, and 25923 and 35 ± 2 °C in an atmosphere containing 3–5% CO₂ for ATCC 33152, for growth, selectivity, colony color and fluorescence.
- Expected Results

Organisms	ATCC®	Recovery	Colony Color	Fluorescence
<i>Aspergillus brasiliensis</i>	16404	Inhibition (partial to complete)	N/A	N/A
* <i>Escherichia coli</i>	25922	Inhibition (partial to complete)	N/A	N/A
* <i>Legionella pneumophila</i>	33152	Moderate to heavy growth	White-gray, gray to blue-gray	Yellow-green (colony and medium)
* <i>Pseudomonas aeruginosa</i>	10145	Inhibition (partial to complete)	N/A	N/A
* <i>Staphylococcus aureus</i>	25923	Inhibition (partial to complete)	N/A	N/A

*Recommended organism strain for User Quality Control.

B. BD BBL BCYE Selective Agar with PAC

- Inoculate representative samples with the cultures listed below.
 - For *Fluoribacter dumoffii* and *Legionella pneumophyllia*, add 100 µL (0.1 mL) of a culture containing 30–300 CFU/0.1 mL to each plate and spread-inoculate.
 - For all other organisms streak inoculate 0.01 mL of a dilution containing 10³–10⁴ CFUs.
 - Include plates of a previously tested lot of **BD BBL BCYE** for *Fluoribacter*, *Tatlockia* and *Legionella* organisms and **BD BBL Trypticase** Soy Agar with 5% Sheep Blood as controls for remaining organisms.
- Examine plates after 3–7 days of incubation at 35 ± 2 °C for ATCC 10231, 25922, and 25923 and 35 ± 2 °C in an atmosphere containing 3–5% CO₂ for ATCC 33217, 33279, 33218, 33152, and 33204, for growth, selectivity, colony color and fluorescence.
- Expected Results

Organisms	ATCC	Recovery	Colony Color	Fluorescence
* <i>Candida albicans</i>	10231	Inhibition (partial to complete)	N/A	N/A
* <i>Escherichia coli</i>	25922	Inhibition (partial to complete)	N/A	N/A
<i>Fluoribacter bozemanii</i>	33217	Complete Inhibition	N/A	N/A
<i>Fluoribacter dumoffii</i>	33279	Moderate to heavy growth	White-gray, gray to blue-gray	Blue-white (colony only)
<i>Tatlockia micdadei</i>	33218	Complete inhibition	N/A	N/A
* <i>Legionella pneumophila</i>	33152	Moderate to heavy growth	White-gray, gray to blue-gray	Yellow-green (colony and medium)
* <i>Staphylococcus aureus</i>	25923	Inhibition (partial to complete)	N/A	N/A
<i>Tatlockia micdadei</i>	33204	Complete inhibition	N/A	N/A

*Recommended organism strain for User Quality Control.

C. BD BBL BCYE Selective Agar with PAV

- Inoculate representative samples with the cultures listed below.
 - For *Legionella* organisms, add 100 µL (0.1 mL) of a culture containing 30–300 CFU/0.1 mL to each plate and spread-inoculate using a sterile glass spreader.
 - For *E. coli*, *S. aureus*, *T. micdadei* and *C. albicans*, streak inoculate 1 µL (0.001 mL) from a 4–5 h culture of **BD BBL Trypticase** Soy Broth diluted to yield 10⁶–10⁷ CFU/mL.
 - Include plates of a previously tested lot of **BD BBL BCYE** for *Legionella* organisms and **BD BBL Trypticase** Soy Agar with 5% Sheep Blood as controls for remaining organisms.

- Examine plates after 3–7 days of incubation at 35 ± 2 °C for ATCC 10231, 25922, and 25923 and 35 ± 2 °C in an atmosphere containing 3–5% CO₂ for ATCC 33217, 33279, 33218, 33152, and 33204 for growth, selectivity, colony color and fluorescence.
- Expected Results

Organisms	ATCC	Recovery	Colony Color	Fluorescence
* <i>Candida albicans</i>	10231	Inhibition (partial to complete)	N/A	N/A
* <i>Escherichia coli</i>	25922	Inhibition (partial to complete)	N/A	N/A
<i>Legionella bozemanii</i>	33217	Moderate to heavy growth	White-gray, gray to blue-gray	Blue-white (colony only)
<i>Legionella dumoffii</i>	33279	Moderate to heavy growth	White-gray, gray to blue-gray	Blue-white (colony only)
<i>Legionella micdadei</i>	33218	Moderate to heavy growth	White-gray, gray to blue-gray	N/A
* <i>Legionella pneumophila</i>	33152	Moderate to heavy growth	White-gray, gray to blue-gray	Yellow-green (colony and medium)
* <i>Staphylococcus aureus</i>	25923	Inhibition (partial to complete)	N/A	N/A
<i>Tatlockia micdadei</i>	33204	Moderate to heavy growth	White-gray, gray to blue-gray	N/A

*Recommended organism strain for User Quality Control.

D. BD BBL BCYE Differential Agar

- Inoculate representative samples with the cultures listed below.
 - For all organisms, add 100 µL (0.1 mL) of a culture containing 30–300 CFU/0.1 mL to each plate and spread-inoculate.
 - Include plates of previously tested **BD BBL Trypticase** Soy Agar with 5% Sheep Blood as a control.
- Examine plates after 3 days of incubation at 35 ± 2 °C in an atmosphere containing 3–5% CO₂ for growth, selectivity, and colony color.
- Expected Results

Organisms	ATCC	Recovery	Colony Color
<i>Fluoribacter bozemanii</i>	33217	Moderate to heavy growth	Green
<i>Fluoribacter dumoffii</i>	33279	Moderate to heavy growth	Green
* <i>Tatlockia micdadei</i>	33218	Moderate to heavy growth	Blue-gray to dark blue
* <i>Legionella pneumophila</i>	33152	Moderate to heavy growth	Light blue with pale green tint
<i>Tatlockia micdadei</i>	33204	Moderate to heavy growth	Blue-gray to dark blue

*Recommended organism strain for User Quality Control.

III ADDITIONAL QUALITY CONTROL

- Examine plates as described under “Product Deterioration.”
- Visually examine representative plates to assure that any existing physical defects will not interfere with use.
- Determine the pH potentiometrically at room temperature for adherence to the specification of 6.9 ± 0.2 .
- Note the firmness of plates during the inoculation procedure.
- Incubate uninoculated representative plates at 33–37 °C for 72 h and examine for microbial contamination.

PRODUCT INFORMATION

IV INTENDED USE

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

V 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, Pascule 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 **BD BBL BCYE** Agar is based on Edelstein's modified formulation.

BD BBL BCYE Selective Agar w/CCVC is a highly selective medium consisting of **BD BBL BCYE** Agar supplement 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.

BD BBL BCYE Selective Agar with PAC was developed by Edelstein for isolation of *Legionella* species from specimens containing mixed flora.⁴ He found that **BD BBL 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.

BD BBL 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.

BD BBL 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 bromocresol purple and bromthymol blue added to **BD BBL BCYE** Agar.

VI PRINCIPLES OF THE PROCEDURE

These media consist of a base medium (**BD BBL 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.

BD BBL 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.

VII REAGENTS

BD BBL 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

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

BD BBL 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

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

BD BBL 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

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

BD BBL 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	0.625 mg

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

BD BBL BCYE Differential Agar

Approximate Formula* Per Liter Purified Water

BCYE Agar	38.65 g
Bromcresol Purple	10.0 mg
Bromthymol Blue	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 2–8 °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 2–8 °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 inoculation.

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

VIII SPECIMEN COLLECTION AND HANDLING

Refer to appropriate texts for details of specimen collection and handling procedures.^{5,8-10}

Pathogenic microorganisms, including hepatitis viruses and Human Immunodeficiency Virus, may be present in clinical specimens.

"Standard Precautions"¹¹⁻¹⁴ 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.

IX PROCEDURE

Material Provided: **BD BBL BCYE Selective Agar with CCVC**, **BD BBL BCYE Selective Agar with PAC**, **BD BBL BCYE Selective Agar with PAV** or **BD BBL BCYE Differential Agar**.

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

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 either at 35 ± 2 °C or at 35 ± 2 °C in an atmosphere containing 3–5% CO₂ as instructed in Section II. Growth is usually visible within 3 to 4 days, but may take up to 2 weeks to appear.

User Quality Control: See “Quality Control Procedures.”

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 CLSI guidance and CLIA regulations for appropriate Quality Control practices.

X RESULTS

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

On the BYCE 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.

XI 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.^{8,10,15}

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.

XII PERFORMANCE CHARACTERISTICS

BD BBL BCYE Selective Agar w/CCVC: All lots of **BD BBL 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 streak plating (spread plating for *Legionella*) the cell suspension, diluted in normal saline to yield 1 x 10³ to 1 x 10⁴ CFU/plate (30–300 CFU/plate for *Legionella*), over the agar surface. For *A. brasiliensis* samples are tested by inoculating with a fresh stock culture (less than one month in age). Plates are incubated at 35 ± 2 °C for up to 7 days in an aerobic atmosphere or atmosphere containing 3–5% CO₂ for *Legionella* cultures. 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 *A. brasiliensis*, *E. coli*, *P. aeruginosa* and *S. aureus* is observed.

BD BBL BCYE Selective Agar w/PAC: All lots of **BD BBL 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 streak plating (spread plating for *Legionella*) the cell suspension, diluted in normal saline to yield 1 x 10³ to 1 x 10⁴ CFU/plate (30–300 CFU/plate for *Legionella*), over the agar surface. Plates are incubated at 35 ± 2 °C for three days in an aerobic atmosphere or atmosphere containing 3–5% CO₂ for *Legionella* cultures. 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.

BD BBL BCYE Selective Agar w/PAV: All lots of **BD BBL 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 streak plating (spread plating for *Legionella*) the cell suspension, diluted in normal saline to yield 1 x 10³ to 1 x 10⁴ CFU/plate (30–300 CFU/plate for *Legionella*), over the agar surface. Plates are incubated at 35 ± 2 °C for three days in an aerobic atmosphere or atmosphere containing 3–5% CO₂ for *Legionella* cultures. 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.

BD BBL BCYE Differential Agar: All lots of **BD BBL BCYE Differential Agar** are tested for specific product performance characteristics. Samples are tested with cell suspensions of *Legionella* ssp. 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 ± 2 °C for three days in an atmosphere containing 3–5% CO₂. Moderate to heavy growth and correct color of *Legionella* colonies are observed.

XIII AVAILABILITY

Cat. No.	Description
297878	BD BBL™ BCYE Selective Agar with CCVC , Pkg. of 10 plates
297879	BD BBL™ BCYE Selective Agar with PAC , Pkg. of 10 plates
297880	BD BBL™ BCYE Selective Agar with PAV , Pkg. of 10 plates
297881	BD BBL™ BCYE Differential Agar , Pkg. of 10 plates

XIV REFERENCES

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Technical Information: In the United States contact BD Technical Service and Support at 1.800.638.8663 or www.bd.com.

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