

BUFFERED CYE (BCYE) DIFFERENTIAL AGAR w/ and w/o PAV

INTENDED USE

Remel Buffered CYE (BCYE) Differential Agar w/ and w/o PAV are solid CYE media recommended for use in qualitative procedures for differential isolation of *Legionella* species from clinical specimens.

SUMMARY AND EXPLANATION

McDade et al. isolated the Legionnaires' disease bacterium in 1977 using guinea pigs and embryonated chicken eggs.¹ In 1978, Feeley et al. developed a medium containing iron salts and L-cysteine hydrochloride for isolation of *Legionella* from clinical specimens.² Feeley modified the formula by adding yeast extract and charcoal in place of casein hydrolysate and beef extract, creating Charcoal Yeast Extract (CYE) Agar.³ In 1980, Pasculle et al. added ACES buffer (N-2-acetamido-2-aminoethane-sulfonic acid) to CYE Agar to stabilize the pH and enhance the growth of *Legionella*.⁴ This medium became known as Buffered CYE (BCYE) Agar. Edelstein further modified BCYE Agar by adding α -ketoglutarate which improved the recovery of *Legionella pneumophila* from contaminated clinical and environmental specimens.⁵ In 1981, Vickers et al. added dyes to BCYE Agar to provide for differentiation of *Legionella* spp. based on colony color and morphology.⁶ BCYE Differential Agar was used by Stout et al. to differentially isolate *Legionella* organisms in hospital potable water supplies.⁷

PRINCIPLE

BCYE Differential Agar contains charcoal and yeast extract to enhance the growth of *Legionella*. Charcoal also serves to absorb toxic metabolic products and modify the surface tension of the medium. Ferric pyrophosphate and L-cysteine hydrochloride are added to satisfy the specific nutritional requirements of *Legionella*. ACES Buffer serves to maintain proper pH and α -ketoglutarate is added to stimulate growth. Bromcresol purple and bromthymol blue are dyes which provide for differentiation of *Legionella* spp. Agar is a solidifying agent. Vancomycin, polymyxin B, and anisomycin are selective agents which may be added to inhibit contaminating bacteria and fungi.

REAGENTS (CLASSICAL FORMULAE)*

ACES Buffer.....	10.0 g	Ferric Pyrophosphate	0.25 g
Yeast Extract.....	10.0 g	Bromcresol Purple.....	0.01 g
Charcoal.....	1.5 g	Bromthymol Blue	0.01 g
α -ketoglutarate	1.0 g	Agar.....	15.0 g
L-Cysteine Hydrochloride.....	0.4 g	Deminerlized Water.....	1000.0 ml

pH 6.9 \pm 0.1 @ 25°C

The following optional ingredients are available per liter of medium:

PAV Additives:

Polymyxin B	80,000 U	Vancomycin.....	0.5 mg
Anisomycin.....	80.0 mg		

*Adjusted as required to meet performance standards.

PROCEDURE

1. Inoculate and streak the specimen as soon as possible after it is received in the laboratory. Selective and nonselective media should be inoculated to ensure recovery of microorganisms that may be inhibited on selective agar.
2. If a swab specimen is received, roll the swab over a small area of the agar surface and streak for isolation.
3. If a fluid specimen is received, inoculate plate with a portion of the specimen and streak for isolation.
4. Incubate plate(s) aerobically at 33-37°C for a minimum of 4 days. Growth is usually visible within 3 to 4 days but may take up to two weeks to appear.
5. Examine plate(s) for typical colony morphology and color. Colonies of *L. pneumophila* are white with a barely discernible green color; *Tatlockia micdadei* colonies are shiny and purple.

QUALITY CONTROL

All lot numbers of BCYE Differential Agar w/ and w/o PAV have been tested using the following quality control organisms and have been found to be acceptable. Testing of control organisms should be performed in accordance with established laboratory quality control procedures. If aberrant quality control results are noted, patient results should not be reported.

CONTROL

BCYE Differential Agar:

**Tatlockia micdadei* ATCC®33204
Legionella pneumophila ATCC®33152

BCYE Differential Agar w/ PAV:

**Tatlockia micdadei* ATCC®33204
Legionella pneumophila ATCC®33152
Candida albicans ATCC®10231
Escherichia coli ATCC®25922
Staphylococcus epidermidis ATCC®12228

INCUBATION

Aerobic, up to 72 h @ 33-37°C
Aerobic, up to 72 h @ 33-37°C

Aerobic, up to 72 h @ 33-37°C
Aerobic, up to 72 h @ 33-37°C
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Aerobic, up to 72 h @ 33-37°C
Aerobic, up to 72 h @ 33-37°C

RESULTS

Growth (purple colonies)
Growth (green colonies)

Growth (purple colonies)
Growth (green colonies)
Inhibition (partial to complete)
Inhibition (partial to complete)
Inhibition (partial to complete)

* Also referred to as *Legionella micdadei*⁸

LIMITATIONS

1. Gram-negative bacilli other than *Legionella* may grow on BCYE Differential Agar w/ and w/o PAV. Additional biochemical and/or serological tests are required for definitive identification of *Legionella* spp. Follow established laboratory procedures and consult appropriate references for further instructions.⁸
2. The selective agents contained in BCYE Differential Agar w/ PAV may inhibit the growth of some *Legionella* spp. For optimum recovery of *Legionella* spp., use a nonselective BCYE Agar in parallel with BCYE Differential Agar w/ and w/o PAV.⁸

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Refer to the front of Remel *Technical Manual of Microbiological Media* for **General Information** regarding precautions, product storage and deterioration, specimen collection, storage and transportation, materials required, quality control, and limitations.

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