

BUFFERED CYE SELECTIVE AGAR (BCYE w/ DGVP)

INTENDED USE

Remel Buffered CYE Selective Agar (BCYE w/ DGVP) is a solid medium recommended for use in qualitative procedures for selective and differential primary isolation of *Legionella* from clinical and environmental samples.

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 medium by substituting yeast extract and charcoal for casein hydrolysate and beef extract, creating Charcoal Yeast Extract (CYE) Agar.³ *Legionella* spp. grown on this medium were found to produce a fluorescent substance that could be detected by long wave (366 nm) UV light. Pasculle et al. added ACES buffer (N-2-acetamido-2-aminoethane-sulfonic acid) to CYE Agar to stabilize the pH of the medium and enhance the growth of *Legionella*.⁴ This medium became known as Buffered CYE (BCYE) Agar. In 1981, Edelstein added α -ketoglutarate to BCYE Agar which improved the recovery of *Legionella pneumophila* from contaminated clinical and environmental specimens.⁵ Vickers et al. added dyes to BCYE Agar to allow for differentiation of *Legionella* spp. based on colony color and morphology.⁶ In a further modification, glycine was added to BCYE Agar as a selective agent to inhibit gram-negative bacilli other than *Legionella* spp.⁷ BCYE w/ DGVP (dyes, glycine, vancomycin, and polymyxin B) is recommended for selective isolation of *Legionella* spp. from contaminated clinical specimens.⁸

PRINCIPLE

BCYE w/ DGVP 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. Polymyxin B and vancomycin are antibiotics added to inhibit the growth of contaminating bacteria. Glycine is a selective agent inhibitory to many bacteria found in environmental samples. Bromocresol purple and bromthymol blue are dyes that provide for differentiation of *Legionella* spp. based on colony color and morphology. Agar is a solidifying agent.

REAGENTS (CLASSICAL FORMULA)*

ACES Buffer.....	10.0 g	Ferric Pyrophosphate	0.25 g
Yeast Extract.....	10.0 g	Bromocresol Purple.....	0.01 g
Glycine	3.0 g	Bromthymol Blue	0.01 g
Charcoal.....	1.5 g	Vancomycin	1.0 mg
α -ketoglutarate	1.0 g	Polymyxin B	80,000 U
L-Cysteine Hydrochloride.....	0.4 g	Agar.....	15.0 g
		Demineralized Water	1000.0 ml

pH 6.9 \pm 0.2 @ 25°C

*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 plated media with a portion of 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 w/ DGVP Agar 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

**Tatlockia micdadei* ATCC® 33204
Legionella pneumophila ATCC® 33152
Escherichia coli ATCC® 25922
Staphylococcus aureus ATCC® 25923

* Also referred to as *Legionella micdadei*⁸

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

RESULTS

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

LIMITATIONS

1. Gram-negative bacilli other than *Legionella* may grow on BCYE w/ DGVP Agar. 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 w/ DGVP may inhibit the growth of some *Legionella* spp. For optimum recovery of *Legionella*, use a nonselective BCYE Agar in parallel with BCYE w/ DGVP.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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remel

12076 Santa Fe Drive, Lenexa, KS 66215, USA

General Information: (800) 255-6730 Website: www.remel.com Email: remel@remel.com

Local/International Phone: (913) 888-0939 International Fax: (913) 895-4128