BUFFERED CYE (BCYE) SELECTIVE and NONSELECTIVE

INTENDED USE
Remel Buffered CYE (BCYE) Selective and Nonselective are solid media recommended for use in qualitative procedures for the isolation of *Legionella* spp. from clinical and environmental 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 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. In 1980, 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] Selective agents such as vancomycin, cefamandole, polymyxin B, and anisomycin may be added to inhibit contaminating bacteria and yeasts.

PRINCIPLE
BCYE 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. Polymyxin B, cefamandole, anisomycin, and vancomycin are selective agents which may be added to inhibit the growth of contaminating bacteria and yeasts. Agar is a solidifying agent.

REAGENTS (CLASSICAL FORMULAE)*

ACES Buffer	10.0 g	L-Cysteine Hydrochloride	0.4 g
Yeast Extract	10.0 g	Ferric Pyrophosphate	0.25 g
Charcoal	1.5 g	Agar	15.0 g
α-ketoglutarate	1.0 g	Demineralized Water	1000.0 ml

pH 6.9 ± 0.2 @ 25°C

The following antibiotic combinations are available per liter of medium:

BCYE w/ PAV:		**BCYE w/ PAC:**	
Polymyxin B	80,000 U	Polymyxin B	80,000 U
Anisomycin	80.0 mg	Anisomycin	80.0 mg
Vancomycin	0.5 mg	Cefamandole	4.0 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 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. Colonies may be examined for fluorescence with a long wave ultraviolet light in a darkened room.

Pour Tube: Melt the agar in a boiling water bath and cool to 45-50°C. Add BCYE Supplement (R45005) and mix thoroughly. The following selective supplements may also be added to the cooled base: PAC (R45006) or PAV (R45007) Supplement. Dispense melted agar into a sterile Petri dish, allow it to harden, and proceed with the procedure above.

QUALITY CONTROL
All lot numbers of Buffered CYE (BCYE) Selective and Nonselective Agar have been tested using the following quality control organisms and have been found to be acceptable. This quality control testing meets or exceeds CLSI standards.[6] 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	INCUBATION	RESULTS
BCYE Agar:		
*[a]*Fluoribacter bozemanae* ATCC® 33217	Ambient, up to 72 h @ 33-37°C	Growth, with blue-white fluorescence
*[b]*Tatlockia micdadei* ATCC® 33204	Ambient, up to 72 h @ 33-37°C	Growth
**Legionella pneumophila* ATCC® 33152	Ambient, up to 72 h @ 33-37°C	Growth, with yellow-green fluorescence
BCYE Agar w/ PAV:		
Tatlockia micdadei ATCC® 33204	Ambient, up to 72 h @ 33-37°C	Growth
Legionella pneumophila ATCC® 33152	Ambient, up to 72 h @ 33-37°C	Growth
Candida albicans ATCC® 10231	Ambient, up to 72 h @ 33-37°C	Inhibition (partial to complete)
Escherichia coli ATCC® 25922	Ambient, up to 72 h @ 33-37°C	Inhibition (partial to complete)
Staphylococcus epidermidis ATCC® 12228	Ambient, up to 72 h @ 33-37°C	Inhibition (partial to complete)

(Continued on back)

CONTROL	INCUBATION	RESULTS
BCYE Agar w/ PAC:		
Legionella pneumophila ATCC® 33152	Ambient, up to 72 h @ 33-37°C	Growth
Candida albicans ATCC® 10231	Ambient, up to 72 h @ 33-37°C	Inhibition (partial to complete)
Escherichia coli ATCC® 25922	Ambient, up to 72 h @ 33-37°C	Inhibition (partial to complete)
Tatlockia micdadei ATCC® 33204	Ambient, up to 72 h @ 33-37°C	Inhibition (partial to complete)
Staphylococcus epidermidis ATCC® 12228	Ambient, up to 72 h @ 33-37°C	Inhibition (partial to complete)

* CLSI recommended organism
[a] Also referred to as *Legionella bozemanii*[7]
[b] Also referred to as *Legionella micdadei*[7]

LIMITATIONS

1. Gram-negative bacilli other than *Legionella* may grow on BCYE Selective or Nonselective. 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.[7]
2. The selective agents contained in BCYE w/ PAV or PAC may inhibit the growth of some *Legionella* spp. For optimum recovery of *Legionella*, use nonselective BCYE Agar in parallel with BCYE w/ PAV or PAC.[7]

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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.

IFU 1342, Revised April 18, 2013 Printed in U.S.A.

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