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. In 1978, Feeley et al. developed a medium containing iron salts and L-cysteine hydrochloride for isolation of Legionella from clinical specimens. Feeley modified the medium by substituting yeast extract and charcoal for casein hydrolysate and beef extract, creating Charcoal Yeast Extract (CYE) Agar. 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. 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. 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
Yeast Extract .............................................................. 10.0 g
Charcoal ........................................................................... 1.5 g
α-ketoglutarate ............................................................... 1.0 g
L-Cysteine Hydrochloride .................................................. 0.4 g
Ferric Pyrophosphate ........................................................ 0.25 g
Agar ............................................................................... 15.0 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:**
Polymyxin B ............................................................... 80,000 U
Anisomycin ................................................................. 80.0 mg
Vancomycin ................................................................. 0.5 mg

**BCYE w/ PAC:**
Polymyxin B ............................................................... 80,000 U
Anisomycin ................................................................. 80.0 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. Selective 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:**

“Fluoribacter bozemanae ATCC® 33217”
Ambient, up to 72 h @ 33-37°C
Growth, with blue-white fluorescence

“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

BCYE Agar w/ PAC:
- **Legionella pneumophila** ATCC® 33152
- **Candida albicans** ATCC® 10231
- **Escherichia coli** ATCC® 25922
- **Tatlockia micdadei** ATCC® 33204
- **Staphylococcus epidermidis** ATCC® 12228

### INCUBATION
- Ambient, up to 72 h @ 33-37°C

### RESULTS
- Growth
- Inhibition (partial to complete)

#### 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.

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.

### BIBLIOGRAPHY

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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