

# BUFFERED CYE (BCYE) AGAR BASE

## INTENDED USE

Remel Buffered CYE (BCYE) Agar Base is a solid medium 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.<sup>1</sup> In 1978, Feeley et al. developed a medium containing iron salts and L-cysteine hydrochloride for isolation of *Legionella* from clinical specimens.<sup>2</sup> In a later modification, Feeley replaced casein hydrolysate and beef extract with yeast extract and charcoal, creating Charcoal Yeast Extract (CYE) Agar.<sup>3</sup> *Legionella* spp. were found to produce a fluorescent substance detectable with long wave (366 nm) UV light when grown on this medium. In 1980, Pasculle et al. created Buffered CYE Agar (BCYE) by adding ACES buffer (N-2-acetamido-2-aminoethane-sulfonic acid).<sup>4</sup> Edelstein added  $\alpha$ -ketoglutarate to BCYE Agar which increased the recovery of *Legionella pneumophila* from contaminated clinical and environmental specimens.<sup>5</sup> BCYE Agar is recommended in *Standard Methods for the Examination of Water and Wastewater* for isolation of *Legionella* from environmental water samples.<sup>6</sup>

## PRINCIPLE

BCYE Agar Base contains charcoal and yeast extract to enhance the growth of *Legionella*. Charcoal also absorbs toxic metabolic products and modifies 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  $\alpha$ -ketoglutarate is added to stimulate growth. Agar is a solidifying agent.

## REAGENTS (CLASSICAL FORMULAE)\*

ACES Buffer.....	10.0 g	$\alpha$ -ketoglutarate.....	1.0 g
Yeast Extract.....	10.0 g	Ferric Pyrophosphate.....	0.25 g
Charcoal.....	1.5 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.

## PRECAUTIONS

This product is For Laboratory Use only. It is not intended for use in the diagnosis of disease or other conditions.

## PREPARATION OF DEHYDRATED CULTURE MEDIUM

1. Suspend 38 g of medium in 900 ml of demineralized water.
2. Adjust the pH to 6.9 using 1N KOH.
3. Add demineralized water to bring volume to 1000 ml.
4. Heat to boiling with agitation to dissolve.
5. Sterilize at 121°C for 15 minutes or following established laboratory procedures.
6. Cool to 45-50°C and add 10 ml of filter sterilized 4% solution of L-cysteine hydrochloride. If using Buffered CYE Supplement (R450041) rehydrate the vial with 10 ml of sterile demineralized water and add to 1000 ml of medium.
7. Mix thoroughly and dispense into appropriate containers.

## PROCEDURE

1. Consult current editions of appropriate references for the recommended procedure for sample preparation, inoculation, and testing.<sup>6</sup>

## QUALITY CONTROL

Each lot number of Buffered CYE Agar Base has been manufactured, packaged, and processed in accordance with current Good Manufacturing Practice regulations. All lot numbers have been tested using the following quality control organisms and 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, sample results should not be reported.

## CONTROL

\**Fluoribacter bozemanii* ATCC® 33217  
\*\**Tatlockia micdadei* ATCC® 33204  
*Legionella pneumophila* ATCC® 33152  
*Legionella pneumophila* ATCC® 33156  
*Escherichia coli* ATCC® 25922  
*Staphylococcus aureus* ATCC® 25923

## INCUBATION

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

## RESULTS

Growth, blue-white fluorescence  
Growth  
Growth, yellow-green fluorescence  
Growth, yellow-green fluorescence  
Growth  
Growth

\* Also referred to as *Legionella bozemanii*

\*\* Also referred to as *Legionella micdadei*

## BIBLIOGRAPHY

1. McDade, J.E., C.C. Shepard, D.W. Fraser, T.R. Tsai, M.A. Redus, and W.R. Dowdle. 1977. N. Engl. J. Med. 297:1197-1203.
2. Feeley, J.C., G.W. Gorman, R.E. Weaver, D.C. Mackel, and H.W. Smith. 1978. J. Clin. Microbiol. 8:320-325.
3. Feeley, J.C., R.J. Gibson, G.W. Gorman, N.C. Langford, J.K. Rasheed, D.C. Mackel, and W.B. Baine. 1979. J. Clin. Microbiol. 10:437-441.
4. Pasculle, A.W., J.C. Feeley, R.J. Gibson, L.G. Cordes, R.L. Myerowitz, C.M. Patton, G.W. Gorman, C.L. Carmack, J.W. Ezzell, and J.N. Dowling. 1980. J. Infect. Dis. 141:727-732.
5. Edelstein, P.H. 1981. J. Clin. Microbiol. 14:298-303.
6. Eaton, A.D., L.S. Clesceri, E.W. Rice, and A.E. Greenberg. 2005. Standard Methods for the Examination of Water and Wastewater. 21<sup>st</sup> ed. APHA, Washington, D.C.

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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