Legionella BMPA-α Selective Supplement

PRODUCT INFORMATION

C079-100mg - Cefamandole, Powder, 100mg

P007-1MU - Polymyxin B Sulfate, Powder, 1MU

P007-10MU - Polymyxin B Sulfate, Powder, 10MU

P007-100MU - Polymyxin B Sulfate, Powder, 100MU

DESCRIPTION

Legionella Cye Agar Base supplemented with Legionella Bcye Growth Supplement a selective supplement and Legionella BMPA-α Selective Supplement can be used for the isolation of legionellae.

BACKGROUND

Cefamandole is a second-generation broad-spectrum cephalosporin antibiotic.

Polymyxin is an antibiotic primarily used for resistant gram-negative infections. It is derived from the bacterium Bacillus polymyxa. Polymyxin B is a mixture of two closely related compounds, polymyxin B1 and polymyxin B2. It has a bactericidal action against almost all gram-negative bacilli except the Proteus group.

Anisomycin is an antibiotic produced by Streptomyces griseolus which inhibits protein synthesis. Partial inhibition of DNA synthesis occurs at anisomycin concentrations that effect 95% inhibition of protein synthesis.

Mechanism of action

Polymyxins bind to the cell membrane and alter its structure, making it more permeable. The resulting water uptake leads to cell death.

APPLICATION IN LEGIONELLA CYE AGAR BASE

The discovery of the causative organism of Legionnaires' disease has been reviewed by Fallon. Since that review further progress has been made in culturing the organism from clinical specimens and also in the enumeration of Legionella species from environmental samples. Feeley et al. described a modification of F-G Agar in which acid hydrolysed casein was replaced by yeast extract as the source of protein and starch was replaced by activated charcoal (Norit A) at a final concentration of 0.2% (w/v). This medium, which they named CYE Agar has been further supplemented with ACES Buffer and a-ketoglutarate and is described in the literature as BCYE-a Medium. BCYE-a Medium has been shown to yield optimal recovery of *Legionellaceae* in a shorter incubation period from environmental samples and clinical specimens.

BCYE Medium is based on the formulation of Edelstein and is prepared from Legionella CYE Agar Base and Legionella BCYE Growth Supplement. The sterile lyophilised supplement contains ACES Buffer/potassium hydroxide, a-ketoglutarate, ferric pyrophosphate and L-cysteine HCl. When added to CYE Agar Base it stabilises the pH of the medium at 6.9 ± 0.2 and provides essential growth factors.

Legionellaceae have an absolute nutritional requirement for L-cysteine. Presumptive *Legionella* spp. colonies can be subcultured onto both BCYE Medium with L-cysteine, and BCYE Medium without L-cysteine.

All plates are incubated at 35°C. Colonies which have grown on BCYE Medium with L-cysteine, but not on BCYE Medium without L-cysteine, can be regarded as presumptive *Legionella* spp.

Content concentrations

Typical Formula*	mg/litre
Legionella Cye Agar Base	
Activated charcoal	2
Yeast extract	10
Agar	13
Final pH 6.9 ± 0.2 @ 25°C	
Legionella Bcye Growth Supplement	
Buffer/Potassium hydroxide	10000
Ferric pyrophosphate	250
L-cysteine HCl	400
a-Ketoglutarate	1000
Legionella BMPA-α Selective Supplement	
Cefamandole	4
Polymyxin	80,000 IU
Anisomycin	80
* Adjusted as required to meet performance standards	

Table 1 - Typical Formula for Legionella Cye Agar Base, Legionella Bcye Growth Supplement and Legionella BMPA-α Selective Supplementhfh

METHOD

Preparation

Suspend appreciate amount of Legionella CYE Agar Base in the distilled water and bring gently to the boil to dissolve completely. Sterilise by autoclaving at 121°C for 15 minutes. Reconstitute Legionella Bcye Growth Supplement and Legionella BMPA-α Selective Supplement as directed. Allow medium to cool to 50°C Mix gently and pour into sterile Petri dishes. The final pH of the medium should be 6.9 ± 0.2.

Protocol

1. Prepare Legionella BMPA-α Selective agar as described in the preparation.

2. Emulsify approximately 0.5 g of the sample in 5 ml of sterile 0.1% peptone water to form an approximate 1:10 dilution.

3. Inoculate onto the selective medium with cotton tipped swabs so that single isolated colonies are formed.

4. Incubate the plates for 48 hours at 35°C.

Quality control

Positive control:

Legionella pneumophila ATCC® 33152: Good growth; grey/white-blueish coloured colonies

Legionella pneumophila NCTC 12821: Good growth; grey/white-blueish coloured colonies

Negative control:

Staphylococcus epidermidis ATCC® 12228: Inhibited

REFERENCES

1. Fallon J. Oxoid Limited. Culture September 1979, P. 3-4.

2. Feeley J.C., Gibson R.J., Gorman G.W., Langford N.C., Rasheed J.W., Mackel D.C. and Baine W.B. (1979) J. Clin. Micro. 10. 437-441.

3. Feeley J.C. Gorman G.W., Weaver R.E., Mackel D.C. and Smith H.W. (1978) J. Clin. Micro. 8. 320-325.

4. Edelstein P.H. (1981) J. Clin. Micro. 14. 298-303.

5. PHLS Communicable Diseases Report (1983) CDR 83/49.