Legionella GVPC Selective Supplement

PRODUCT INFORMATION
V001-250mg - Vancomycin HCl, Powder, 250mg
V001-1g - Vancomycin HCl, Powder, 1g
V001-5g - Vancomycin HCl, Powder, 5g
P007-1MU - Polymyxin B Sulfate, Powder, 1MU
P007-10MU - Polymyxin B Sulfate, Powder, 10MU
P007-100MU - Polymyxin B Sulfate, Powder, 100MU
C001-1g - Cycloheximide, Powder, 1g
C001-5g - Cycloheximide, Powder, 5g

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

BACKGROUND
Polymyxin B 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.

Vancomycin is a glycopeptide antibiotic used in the prophylaxis and treatment of infections caused by Gram-positive bacteria.

Cycloheximide is widely used in biomedical research to inhibit protein synthesis in eukaryotic cells studied in vitro (i.e. outside of organisms). Its effects are rapidly reversed by simply removing it from the culture medium.

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.

Vancomycin acts by inhibiting proper cell wall synthesis in Gram-positive bacteria. Due to the different mechanism by which Gram-negative bacteria produce their cell walls and the various factors related to entering the outer membrane of Gram-negative organisms, vancomycin is not active against Gram-negative bacteria (except some non-gonococcal species of Neisseria).

Cycloheximide is an inhibitor of protein biosynthesis in eukaryotic organisms, produced by the bacterium Streptomyces griseus. Cycloheximide exerts its effect by interfering with the translocation step in protein synthesis (movement of two tRNA molecules and mRNA in relation to the ribosome) thus blocking translational elongation.

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

<table>
<thead>
<tr>
<th>Typical Formula*</th>
<th>mg/litre</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Legionella CYE Agar Base</strong></td>
<td></td>
</tr>
<tr>
<td>Activated charcoal</td>
<td>2</td>
</tr>
<tr>
<td>Yeast extract</td>
<td>10</td>
</tr>
<tr>
<td>Agar</td>
<td>13</td>
</tr>
<tr>
<td><strong>Final pH 6.9 ± 0.2 @ 25°C</strong></td>
<td></td>
</tr>
<tr>
<td><strong>Legionella Bcy Growth Supplement</strong></td>
<td></td>
</tr>
<tr>
<td>Buffer/Potassium hydroxide</td>
<td>10000</td>
</tr>
<tr>
<td>Ferric pyrophosphate</td>
<td>250</td>
</tr>
<tr>
<td>L-cysteine HCl</td>
<td>400</td>
</tr>
<tr>
<td>a-Ketoglutarate</td>
<td>1000</td>
</tr>
<tr>
<td><strong>Legionella GVPC Selective Supplement</strong></td>
<td></td>
</tr>
<tr>
<td>Glycine (Ammonia free)</td>
<td>1500</td>
</tr>
<tr>
<td><strong>Vancomycin hydrochloride</strong></td>
<td>0.5</td>
</tr>
<tr>
<td><strong>Polymyxin B sulphate</strong></td>
<td>40000 IU</td>
</tr>
<tr>
<td><strong>Cycloheximide</strong></td>
<td>40</td>
</tr>
</tbody>
</table>

* Adjusted as required to meet performance standards

| Table 1 - Typical Formula for Legionella CYE Agar Base, Legionella Bcy Growth Supplement and Legionella GVPC Selective Supplementth |

### 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 Bcy Growth Supplement and Legionella GVPC 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 GVPC 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