TOKU-E Application Data Sheet

Modified Preston Campylobacter Selective Supplement

PRODUCT INFORMATION
P007-1MU - Polymyxin B Sulfate, Powder, 1MU
P007-10MU - Polymyxin B Sulfate, Powder, 10MU
P007-100MU - Polymyxin B Sulfate, Powder, 100MU
R003-1g - Rifampicin, Powder, 1g
R003-5g - Rifampicin, Powder, 5g
T011-5g - Trimethoprim, Powder, 5g
T011-25g - Trimethoprim, Powder, 25g
T011-100g - Trimethoprim, Powder, 100g
A007-100mg - Amphotericin B, Powder, 100mg
A007-250mg - Amphotericin B, Powder, 250mg
A007-1g - Amphotericin B, Powder, 1g
A007-5g - Amphotericin B, Powder, 5g

DESCRIPTION
Campylobacter Agar Base with Modified Preston Campylobacter Selective Supplement is a selective medium for the selective isolation of Campylobacter jejuni and C. coli from human, animal, avian and environmental specimens.

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.

Rifampicin is a bactericidal antibiotic drug of the rifamycin group. It is a semisynthetic compound derived from Amycolatopsis rifamycinica (formerly known as Amycolatopsis mediterranei and Streptomyces mediterranei).

Trimethoprim is a bacteriostatic antibiotic which belongs to the class of chemotherapeutic agents known as dihydrofolate reductase inhibitors.

Amphotericin B is a polyene antifungal drug. Two amphotericins, amphotericin A and amphotericin B are known, but only B is used clinically, because it is significantly more active in vivo.

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.

Rifampicin inhibits DNA-dependent RNA polymerase in bacterial cells by binding its beta-subunit, thus preventing transcription to RNA and subsequent translation to proteins. Its lipophilic nature makes it a good candidate to treat the meningitis form of tuberculosis, which requires distribution to the central nervous system and penetration through the blood-brain barrier.

Trimethoprim acts by interfering with the action of bacterial dihydrofolate reductase, inhibiting synthesis of tetrahydrofolic acid. Tetrahydrofolic acid is an essential precursor in the de novo synthesis of the intermediate Thymidine monophosphate (dTMP), precursor of DNA metabolite Thymidine triphosphate. Bacteria are unable to take up folic acid from the environment (i.e. the infection host) and are thus dependent on their own de novo synthesis. Inhibition of the enzyme starves the bacteria of nucleotides necessary for DNA replication causing, in certain circumstances, cell lethality due to thymineless death.

As with other polyene antifungals, amphotericin B associates with ergosterol, the main component of fungal cell membranes, forming a transmembrane channel that leads to monovalent ion (K+, Na+, H+ and Cl−) leakage, which is the primary effect leading to fungal cell death.

APPLICATION IN CAMPYLOBACTER AGAR BASE

The Preston campylobacter selective agar is based on the formulation described by Bolton and Robertson. This medium was specifically formulated to be suitable...
for isolation of *Campylobacter* species from all types of specimens (human, animal, avian and environmental).

In comparative studies of the selective media of Skirrow, Butzler, Blaser, Campy-BAP and Preston, the Preston medium was found to give the maximum isolation rate of *Campylobacter* species from all types of specimens tested and also to be the most selective.

Toku-e campylobacter agar base has been prepared from materials described by Bolton and Robertson. It is suitable as a basal medium for the selective supplements of Blaser-Wang, Skirrow and Butzler.

### Content concentrations

<table>
<thead>
<tr>
<th></th>
<th>mg/litre</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Campylobacter Agar Base</strong></td>
<td></td>
</tr>
<tr>
<td>'Lab-Lemco' powder</td>
<td>10</td>
</tr>
<tr>
<td>Peptone</td>
<td>10</td>
</tr>
<tr>
<td>Sodium chloride</td>
<td>5</td>
</tr>
<tr>
<td>Agar</td>
<td>12</td>
</tr>
<tr>
<td>Final pH 7.5 ± 0.2 @ 25°C</td>
<td></td>
</tr>
<tr>
<td><strong>Modified Preston Campylobacter Selective Supplement</strong></td>
<td></td>
</tr>
<tr>
<td>Polymyxin B</td>
<td>5,000 IU</td>
</tr>
<tr>
<td>Rifampicin</td>
<td>10</td>
</tr>
<tr>
<td>Trimethoprim</td>
<td>10</td>
</tr>
<tr>
<td>Amphotericin B</td>
<td>10</td>
</tr>
</tbody>
</table>

* Adjusted as required to meet performance standards

Table 1 typical formula for Campylobacter Agar Base and Modified Preston Campylobacter Selective Supplement

### METHOD

#### Preparation

Suspend appropriate amount of campylobacter agar base in distilled water and bring to the boil to dissolve completely. Sterilise by autoclaving at 121°C for 15 minutes. Cool to 50°C. Aseptically add 25ml of lysed horse blood, and supplements reconstituted as directed and appropriate amount of campylobacter growth supplement. Mix well and pour into sterile Petri dishes.

#### Protocol

Direct Selective Plating Method:

1. Prepare the campylobacter selective agar as directed.
2. Emulsify the specimen under test in 2 ml of 0.1% peptone water.
3. Inoculate onto the selective medium with cotton tipped swabs so that single isolated colonies are formed.
4. Incubate the plates in an atmosphere consisting of approximately 5-6% oxygen, 10% carbon dioxide and 84-85% nitrogen for 24-48 hours (When few Campylobacter colony forming units are present 48 hours incubation is necessary.) at 42°C.
5. Examine the plates and confirm the typical colonies as *Campylobacter jejuni* or *Campylobacter coli* by the standard methods.

Selective Enrichment Broth Technique:

1. Prepare the selective enrichment broth as directed.
2. Emulsify the specimen under test in the selective enrichment broth.
3. Incubate the broth aerobically at 42°C for 24 hours.
4. Subculture on to Preston Campylobacter Selective Agar or Campylobacter Blood-Free Selective Agar.

### Quality control

**Positive control:**

*Campylobacter jejuni* ATCC® 29428: Good growth; grey-brown coloured colonies

**Negative control:**

*Escherichia coli* ATCC® 25922: Inhibited

### REFERENCES