Campylobacter Enrichment Supplement (Exeter)

SV59 For the isolation of *Campylobacter* spp. from food and water samples in conjunction with Campylobacter Growth Supplement (FBP) (SV61).

**Introduction**

*Campylobacter* spp. are now of world wide significance in human and animal disease, in particular *C.jejuni* which is recognised as one of the commonest causes of acute bacterial diarrhoea in man\(^1\). The importance of controlling *Campylobacter* contamination of foods has become more apparent as raw milk, meat, and especially poultry have been implicated as the main vehicles of infections. *Campylobacter* spp. are often associated with foods that have been stored or treated by chilling, freezing and heating. Such treatment has been shown to damage the outer membrane of *C.jejuni* sublethally injuring the cells to produce an increase in sensitivity to the antibiotics and selective agents used in many isolation media. Consequently isolation of *Campylobacter* spp. from food specimens is difficult when selective supplements designed for clinical samples are employed\(^2\). Recovery of injured cells can be enhanced by pre-enrichment in broth medium, allowing sublethally injured organisms to repair lesions\(^3\), and to tolerate certain selective antibiotics.

The antibiotics in Campylobacter Enrichment Supplement (Exeter) have been chosen and their content optimised for the ability to both inhibit competitive flora and maintain viability of campylobacters. In particular cefoperazone, which has a wide spectrum of activity against contaminating Gram negative organisms, is used in preference to cephalothin found in some other selective supplements as certain *Campylobacter* strains, especially *C.coli*, may be sensitive to the latter antibiotic\(^4\).

Rifampicin is included for its activity against Gram positive organisms, but when in the presence of the oxygen derivative, hydrogen peroxide, the antibiotic also has an inhibitory effect on *Campylobacter* spp. especially *C.jejuni*\(^5\). The use of quenching agents such as those incorporated in Campylobacter Growth Supplement (FBP) helps combat this effect preventing further cellular damage and increasing the possibility of *Campylobacter* isolation from a food or water sample. In Campylobacter Enrichment Supplement (Exeter) the concentration of the antibiotic has also been minimised in order to further enhance the recovery of injured *Campylobacter* strains\(^6\).

*Campylobacter* spp. especially *C.jejuni*, are also less able to tolerate elevated growth temperatures of 42ºC following exposure to heating or freezing. Reduction of incubation temperature from 42ºC to 37ºC has been shown to significantly increase the isolation rate from a variety of samples\(^7\).

Exeter Campylobacter Selective Enrichment Broth comprising FBP enrichment and selective antibiotics in a nutrient broth base supplemented with 5% lysed blood, incubated at the reduced temperature, has been adopted by the FDA and UK Department of Health for surveillance studies\(^8\).

**Description**

Each vial of Campylobacter Enrichment Supplement (Exeter) contains accurately assayed quantities of antibiotics in a soluble, non-interfering carrier substance.

<table>
<thead>
<tr>
<th>Campylobacter Enrichment Supplement (Exeter)</th>
<th>Content</th>
<th>Concentration in 1125ml medium</th>
</tr>
</thead>
<tbody>
<tr>
<td>Trimethoprim</td>
<td>11.25mg</td>
<td>10mg/litre</td>
</tr>
<tr>
<td>Rifampicin</td>
<td>5.625mg</td>
<td>5mg/litre</td>
</tr>
<tr>
<td>Polymyxin B</td>
<td>2812.6iu</td>
<td>2500iu/litre</td>
</tr>
<tr>
<td>Cefoperazone</td>
<td>16.875mg</td>
<td>15mg/litre</td>
</tr>
<tr>
<td>Amphotericin B</td>
<td>2.25mg</td>
<td>2mg/litre</td>
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</tbody>
</table>

**Directions**

1. Sterilise 225ml of Mast Nutrient Broth (DM180) for each sample to be tested. Allow broths to cool to room temperature.
2. Reconstitute 1 vial of Campylobacter Growth Supplement (FBP) with 10ml of sterile water and 1 vial of Campylobacter Enrichment Supplement (Exeter) with 10ml of 50%
methanol. Each vial contains sufficient material for 5 tests. The best method is to aseptically add diluent using a sterile needle and syringe. Draw the diluent into the syringe and after removing the plastic cap of the vials inject through the rubber stopper of the vial. The lyophilised supplement will rapidly dissolve and may be drawn into the syringe.

3. To each 225ml volume of Mast Nutrient Broth prepared add 2mls of reconstituted Campylobacter Growth Supplement (FBP) and 2mls of Campylobacter Enrichment Supplement (Exeter) and discard the needle into an appropriate container. DO NOT TRY TO SHEATH AN EXPOSED NEEDLE. Unused reconstituted supplement should be discarded and not frozen.

4. Mix gently but thoroughly to evenly distribute the selective agents.

5. Aseptically add 5% v/v lysed horse blood. Mix well.

For the preparation of equivalent Exeter Selective Agar plates add 15g/l agar to Mast Nutrient Broth and autoclave at 121ºC for 15 minutes. Cool the medium to 50-55ºC and hold in a water bath at this temperature. Supplement as described above.

In Use

Prepare a $10^1$ homogenate of food sample using either a stomacher or blender by homogenising 25g or 25ml of sample in 225ml of prepared Exeter Campylobacter Selective Enrichment Broth. The broth should be at room temperature before the food sample is added.

Incubate at 37ºC for 48 hours in a tightly closed container. Ensure that the broth is rapidly heated to 37ºC, ideally within 4 hours of inoculation, and that the container used has only minimal head space above the broth surface.

For certain samples e.g. milk or water containing cold injured Campylobacter, incubation at 37ºC for 2 hours in a non-selective broth, using Campylobacter Growth Supplement (FBP) alone, has been shown to improve isolation. After the initial 2 hour period the antibiotic supplement can be added and incubation continued.

Subculture onto a suitable Campylobacter selective agar such as Exeter or Preston (Mast DM251/MS18) medium after 24 and 48 hours. Incubate the plates in a microaerobic atmosphere at 42ºC for 24-48 hours.

Examine the plates for suspect Campylobacter colonies - typically colonies of $C. jejuni$ tend to appear as grey moist flat spreading colonies. On moist agar a thin spreading film may develop. Some strains may produce a green hue or metallic sheen. Colonies of $C. coli$ tend to be creamy grey in colour, moist, slightly raised and often discrete with less spreading than $C. jejuni$.

References

8. Humphrey TJ. Campylobacter. Leatherhead Food Research Association Training Course: Conventional and rapid methods for the detection of food poisoning organisms. 1994: