Campylobacter Growth Supplement (FBP)

SV61 For the improved isolation of Campylobacter spp. by enhancement of aerotolerance.

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 man¹. 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 microaerophilic, requiring an atmosphere of reduced oxygen for growth and survival, optimal conditions are at 5% O₂ and 10% CO₂. This is due to Campylobacter spp. being more sensitive to reduction products of oxygen such as superoxide anions and hydrogen peroxide than aerobic organisms.

The organisms are often associated with foods that have been stored or treated by chilling, freezing and heating. Such treatment has been shown to cause Campylobacter spp. to become sublethally injured by damaging the outer membrane of the cell. Organisms damaged in this way show an even greater susceptibility to oxygen radicals and are unable to grow in the absence of blood or compounds that quench toxic oxygen derivatives². The use of enrichment supplements such as a combination of ferrous sulphate, sodium metabisulphite and sodium pyruvate, each component at 0.025% (FBP), gives both oxygen radical neutralisation ability and helps enhance the aerotolerance of microaerophilic organisms³. Ferrous sulphate and sodium pyruvate are the active ingredients of the system and sodium metabisulphite acts in synergy with the other components to increase their quenching ability.

Pre-enrichment with broths containing FBP quenching agents, such as Exeter Campylobacter Selective Enrichment Broth, to allow resuscitation and recovery of injured organisms have been shown to significantly increase isolation of damaged campylobacters⁴. The procedure is recommended to enhance the recovery of sublethally injured cells or, with the addition of selective antibiotics as in Mast Campylobacter Enrichment Supplement (Exeter) (SV59), small populations in heavily contaminated samples.

Exeter Campylobacter Selective Enrichment Broth comprising FBP enrichment and selective antibiotic mixture in a nutrient broth base supplemented with 5% lysed blood has been adopted by the FDA and Department of Health for surveillance studies⁵.

Description

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

SV61 Campylobacter Growth Supplement (FBP)

<table>
<thead>
<tr>
<th>Content</th>
<th>Concentration in 1125ml medium</th>
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<tbody>
<tr>
<td>Sodium Pyruvate</td>
<td>281.25mg 250mg/litre</td>
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<tr>
<td>Sodium Metabisulphite</td>
<td>281.25mg 250mg/litre</td>
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<tr>
<td>Ferrous Sulphate</td>
<td>281.25mg 250mg/litre</td>
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Directions

For The Preparation of Exeter Campylobacter Selective Enrichment Broth

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) (SV61) with 10ml of sterile water and 1 vial of Campylobacter Enrichment Supplement (Exeter) (SV59) 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 2ml of reconstituted Campylobacter Growth Supplement (FBP) and 2ml 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.

For General Use

The supplement can be added to any Campylobacter basal medium with or without the addition of selective supplements. Reconstitute one vial as described above and add the contents to 1125ml of sterile molten agar cooled to 50-55ºC. To prepare smaller volumes add the appropriate proportion. Add blood and Campylobacter selective supplements as indicated, mix well and pour into petri dishes. Suggested media and supplements include:- Mast CAMP Selectavial™ (Skirrow) (SV3), CAMP Selectatab™ (Preston Original) (MS28), Mast Blood Agar Base Special (DM101) or Mast Columbia Agar (DM115).

In Use

Exeter Campylobacter Selective Enrichment Broth

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

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