INTENDED USE
Difco™ Campylobacter Agar Base, when supplemented with blood and antimicrobial agents, is used for the primary isolation and cultivation of Campylobacter jejuni subsp. jejuni from human fecal specimens. Difco Campylobacter Antimicrobial Supplement Skirrow and Difco Campylobacter Antimicrobial Supplement Blaser are used for preparing Campylobacter Agar according to the formulations of Skirrow1 and Blaser et al.2,3

SUMMARY AND EXPLANATION
In 1972, Dekeyser et al. reported that C. jejuni was isolated from the feces of patients with diarrhea and acute gastroenteritis using a filtration technique and a blood-containing selective medium with antimicrobics to suppress the normal enteric flora.4 Subsequently, Skirrow and other investigators reported similar blood-based selective media that differed in the numbers and types of antimicrobics.1-3,5,6
In 1978, Blaser et al. reported success in isolating C. jejuni with a medium containing four antimicrobics incorporated into Brucella Agar supplemented with 10% defibrinated sheep blood.2,3 Subsequently, cephalothin was incorporated to increase its ability to inhibit the normal bacterial flora associated with fecal specimens.5

PRINCIPLES OF THE PROCEDURE
Difco Campylobacter Agar Base is a nutritionally rich medium based on Blood Agar Base No. 2, rather than on Brucella Agar, to support more luxuriant Campylobacter growth because trimethoprim is more active in Blood Agar Base No. 2. Supplementation of the base with antimicrobial agents as described by Skirrow1 and Blaser et al.2,3 provides for markedly reduced growth of normal enteric bacteria and improved growth and recovery of C. jejuni from fecal specimens. Growth of fungi is markedly to completely inhibited on Campylobacter Agar prepared with Difco Campylobacter Antimicrobial Supplement Blaser due to the presence of amphotericin B.

REAGENTS
Difco Campylobacter Antimicrobial Supplement Skirrow
Formula Per 5 mL Vial
Vancomycin ..................... 5.0 mg
Polymyxin B .................. 1250.0 units
Trimethoprim .................... 2.5 mg

Difco Campylobacter Antimicrobial Supplement Blaser
Formula Per 5 mL Vial
Vancomycin ..................... 5.0 mg
Polymyxin B .................. 1250.0 units
Trimethoprim .................... 2.5 mg
Cephalothin ..................... 7.5 mg
Amphotericin B .................. 1.0 mg

Warnings and Precautions
For Laboratory Use
Use aseptic technique in rehydrating the supplements and in adding the supplements to the basal medium.
Follow proper, established laboratory procedures in handling and disposing of infectious materials.

Storage and Rehydration Instructions
Store Difco Campylobacter Antimicrobial Supplements Skirrow and Blaser at 2–8 °C.
To rehydrate supplements, aseptically add 5 mL of sterile purified water to one vial of supplement. Rotate in an end-over-end motion to dissolve the contents completely. Store the rehydrated vials at 2–8 °C. Use within 24 h after rehydration.
Do not open or rehydrate reagents until ready to use.
The expiration date applies to the products in their intact containers when stored as directed.
SPECIMEN COLLECTION

Fecal specimens should be collected in sterile containers or with a sterile rectal swab and transported immediately to the laboratory. If the specimen cannot be inoculated onto appropriate media within 4 h after collection, the specimen should be maintained or transported in Cary Blair Transport Medium. Food and environmental specimens should be collected in sterile containers and transported to the laboratory in accordance with recommended guidelines.

PROCEDURE

Materials Provided: Difco Campylobacter Antimicrobial Supplement Skirrow or Difco Campylobacter Antimicrobial Supplement Blaser.

Materials Required But Not Provided: Specimen containers or sterile rectal swabs, suitable system for providing a microaerophilic environment (e.g., GasPak™ EZ Campy system), Bunsen burner or incinerator, sterile defibrinated sheep blood or sterile lysed horse blood, inoculating loop, incubator (42 °C), user quality control cultures and sterile Petri dishes.

Preparation of Campylobacter Agar

1. Suspend 39.5 g of the powder in 1 L of purified water. Mix thoroughly.
2. Heat with frequent agitation and boil for 1 minute to completely dissolve the powder.
3. Autoclave at 121 °C for 15 minutes. Cool the medium to 45–50 °C.
4. Aseptically add 5–7% sterile lysed horse blood or 10% sterile defibrinated sheep blood. Mix thoroughly.
5. Aseptically add 1% of the desired antimicrobial supplement (10 mL of supplement to 1 L or 5 mL of supplement to 500 mL of medium base). Mix thoroughly, avoiding the formation of air bubbles and dispense into sterile Petri dishes.
6. Test samples of the finished product for performance using stable, typical control cultures.

Inoculation and Incubation

1. Inoculate the specimen directly onto the surface of a Campylobacter Agar plate and streak for isolation.
2. Incubate inoculated plates at 42 °C under a microaerophilic atmosphere containing 5–6% oxygen and 3–10% CO2. This atmosphere can be achieved by using one BBL™ CampyPak™ or CampyPak™ Plus disposable gas generator envelope in a GasPak™ 100 jar, three envelopes in a GasPak 150 jar or using the BBL CampyPouch™, BioBag™ Type Cfj or GasPak EZ Campy systems. Alternatively, the atmosphere can be achieved using evacuation of GasPak vented jars and replacement with cylinder gases, or by using the Fortner principle.
3. Examine plates for growth after 24 and 48 h incubation.

EXPECTED RESULTS

C. jejuni colonies on Campylobacter Agar appear nonhemolytic, flat and gray with an irregular edge or raised and round with a mucoid appearance; some strains may appear tan or slightly pink. Swarming or spreading may be observed on moist surfaces. Plates examined after 24 h incubation should be examined quickly and reincubated under microaerophilic conditions to maintain the viability of the more oxygen sensitive strains. Growth of normal enteric bacteria is markedly to completely inhibited. Growth of fungi is markedly to completely inhibited on Campylobacter Agar prepared with Campylobacter Antimicrobial Supplement Blaser.
USER QUALITY CONTROL

1. Examine the agar base for color and texture. The powder should be beige, free-flowing and homogeneous.
2. Determine the pH of the medium after preparation and cooling to 25 °C. The pH should be 7.4 ± 0.2.
3. Examine the lyophilized and rehydrated antimicrobial supplement for evidence of deterioration as described under “Product Deterioration.”
4. Check the performance of the base and antimicrobial supplement by testing in the complete medium. Plates should be inoculated with approximately 100–1,000 colony forming units (CFUs) of the test cultures and incubated at 42 °C in a reduced oxygen atmosphere. Examine plates for growth after 24 and 48 h incubation.

<table>
<thead>
<tr>
<th>Organism</th>
<th>ATCC®</th>
<th>Recovery Skirrow</th>
<th>Recovery Blaser</th>
</tr>
</thead>
<tbody>
<tr>
<td>Campylobacter jejuni subsp. jejuni</td>
<td>29428</td>
<td>Good</td>
<td>Good</td>
</tr>
<tr>
<td>Campylobacter jejuni subsp. jejuni</td>
<td>33291</td>
<td>Good</td>
<td>Good</td>
</tr>
<tr>
<td>Candida albicans</td>
<td>10231</td>
<td>Good</td>
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</tr>
<tr>
<td>Enterococcus faecalis</td>
<td>33186</td>
<td>Inhibited</td>
<td>Inhibited</td>
</tr>
<tr>
<td>Escherichia coli</td>
<td>25922</td>
<td>Inhibited</td>
<td>Inhibited</td>
</tr>
</tbody>
</table>

LIMITATIONS OF THE PROCEDURE

1. Campylobacter Agar Base and Campylobacter Antimicrobial Supplements Skirrow and Blaser are intended for use in the preparation of Campylobacter Agar. Although these agars are selective primarily for Campylobacter, biochemical testing using pure cultures is necessary for complete identification. Consult appropriate references for further information.7,9,10
2. Due to the selective properties of the complete media and the organisms themselves, some strains of C. jejuni may be encountered that fail to grow or grow poorly on these media. Similarly, some strains of normal enteric organisms may be encountered that are not inhibited or only partially inhibited on these media.
3. Since C. jejuni is thermophilic, it is important to incubate the plates at 42 °C; otherwise, growth will be delayed. Also, the higher temperature improves selectivity by inhibiting the normal flora.

REFERENCES


Technical Information: In the United States, contact BD Technical Service and Support at 800-638-8663 or www.bd.com/ds.

AVAILABILITY

<table>
<thead>
<tr>
<th>Cat. No.</th>
<th>Description</th>
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<tbody>
<tr>
<td>214891</td>
<td>Difco™ Campylobacter Antimicrobial Supplement Skirrow, 6 x 5 mL</td>
</tr>
<tr>
<td>214890</td>
<td>Difco™ Campylobacter Antimicrobial Supplement Blaser, 6 x 5 mL</td>
</tr>
<tr>
<td>214892</td>
<td>Difco™ Campylobacter Agar Base, 500 g</td>
</tr>
<tr>
<td>218201</td>
<td>Difco™ Campylobacter Agar Base, 2 kg</td>
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</tbody>
</table>

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