BD™ Campylobacter Bloodfree Selective Medium

INTENDED USE
BD Campylobacter Bloodfree Selective Medium is a selective medium for the isolation of Campylobacter species from human fecal specimens.

PRINCIPLES AND EXPLANATION OF THE PROCEDURE
Microbiological method.
Campylobacter has been shown to include species that represent important pathogens causing intestinal infections such as diarrhea and acute gastroenteritis.1-3 In rural areas and in less developed countries, campylobacters outnumber Salmonella as intestinal pathogens. C. jejuni is considered the most common cause of sporadic bacterial enteritis worldwide.1
The most frequently isolated species is Campylobacter jejuni, whereas C. coli, C. lari and other species are rarer.
Several selective media have been developed for the isolation of Campylobacter. The early media all contain blood which is necessary to absorb radicals and peroxides that might be inhibitory to the microaerophilic campylobacters. Later on, replacements for the blood which is not a necessary nutrient for these organisms have been developed.4,5 Comparative studies with bloodfree and blood containing selective media for campylobacters have shown that the bloodfree, charcoal containing media are superior to those containing blood, increasing the aerotolerance of these organisms.4,5 Antimicrobials have been used singly or in combination to inhibit the accompanying flora. Bolton and co-workers developed a combination of desoxycholate and cefoperazone that proved to inhibit most normal flora organisms while it is non-inhibitory to C. jejuni, C. fetus, C. coli, and C. lari.4,5

In BD Campylobacter Bloodfree Selective Medium, beef extract and two different peptones are used as nitrogen sources. The combination of activated charcoal, ferrous sulphate, and sodium pyruvate are used to replace the blood.1,2,7 Charcoal acts as an adsorbent for many toxic compounds, whereas ferrous sulphate and pyruvate act as chemical reductants. Desoxycholate and cefoperazone are used to inhibit the normal intestinal flora.

REAGENTS
BD Campylobacter Bloodfree Selective Medium and BD Campylobacter Bloodfree CTA Agar contain the following base medium:

<table>
<thead>
<tr>
<th>Formula* Per Liter Purified Water</th>
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<tbody>
<tr>
<td>Beef Extract 10.0 g/l</td>
</tr>
<tr>
<td>Peptone 10.0</td>
</tr>
<tr>
<td>Sodium Chloride 5.0</td>
</tr>
<tr>
<td>Charcoal (activated) 4.0</td>
</tr>
<tr>
<td>Hydrolysed Casein 3.0</td>
</tr>
<tr>
<td>Sodium Desoxycholate 1.0</td>
</tr>
<tr>
<td>Ferrous Sulphate 0.25</td>
</tr>
<tr>
<td>Sodium Pyruvate 0.25</td>
</tr>
<tr>
<td>Cefoperazone 32.0 mg/l</td>
</tr>
<tr>
<td>Agar 12.0 g/l</td>
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</tbody>
</table>

pH 7.4+/- 0.2
*Adjusted and/or supplemented as required to meet performance criteria.
PRECAUTIONS

For professional use only.

Do not use plates if they show evidence of microbial contamination, discoloration, drying, cracking or other signs of deterioration.

Consult the GENERAL INSTRUCTIONS FOR USE document for aseptic handling procedures, biohazards, and disposal of used product.

STORAGE AND SHELF LIFE

On receipt, store plates in the dark at 2 to 8°C, in their original sleeve wrapping until just prior to use. Avoid freezing and overheating. The plates may be inoculated up to the expiration date (see package label) and incubated for the recommended incubation times.

Plates from opened stacks of 10 plates can be used for one week when stored in a clean area at 2 to 8°C.

USER QUALITY CONTROL

Inoculate representative samples with the following strains (for details, see the GENERAL INSTRUCTIONS FOR USE document). Incubate the plates at 35 to 37°C in a microaerobic atmosphere (e.g., in a BD GasPak™ jar equipped with BD GasPak™ EZ Campy Container System Sachets) for 42 to 48 hours.

<table>
<thead>
<tr>
<th>Strains</th>
<th>BD Campylobacter Bloodfree Selective Medium</th>
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</thead>
<tbody>
<tr>
<td>C. jejuni subsp. jejuni ATCC™ 33291</td>
<td>Growth; grey colonies, may be spreading</td>
</tr>
<tr>
<td>Campylobacter fetus DSM 5361</td>
<td>Growth; grey colonies</td>
</tr>
<tr>
<td>Escherichia coli ATCC 25922</td>
<td>Inhibition partial to complete</td>
</tr>
<tr>
<td>Enterococcus faecalis ATCC 29212</td>
<td>Inhibition partial to complete</td>
</tr>
<tr>
<td>Proteus mirabilis ATCC 14153</td>
<td>Inhibition partial to complete</td>
</tr>
<tr>
<td>Uninoculated</td>
<td>Shiny black</td>
</tr>
</tbody>
</table>

Note that fungi will not be inhibited on this medium.

PROCEDURE

Materials Provided

BD Campylobacter Bloodfree Selective Medium supplied in 90 mm Stacker™ plates. Microbiologically controlled

Materials Not Provided

Ancillary culture media, reagents and laboratory equipment as required.

Specimen Types

Fresh stool specimens or rectal swabs from patients suspected to be infected with Campylobacter species. The medium may also be used for meat and other food samples (see also PERFORMANCE CHARACTERISTICS AND LIMITATIONS OF THE PROCEDURE). Stool specimens, swabs, and food samples should not be older than 24 to 48 hours. Rectal swabs must be inserted into appropriate transport media (e.g., Cary Blair medium). If not processed immediately, store specimens in transport media at 4 to 8°C. Avoid desiccation and exposure to oxygen.

Test Procedure

Streak the specimen for isolation as soon as possible after it is received in the laboratory onto BD Campylobacter Bloodfree Selective Medium. Meat or other foods should first be minced or homogenized and then inoculated directly or after suspension in a small amount of peptone broth onto the medium.

If material is being cultured directly from a swab, roll the swab over a small area of the surface at the edge; then streak from this inoculated area. The application of a special filtration technique for the processing of specimens followed by the inoculation of selective and non-selective media has been described.2,3
Incubate inoculated plates, protected from light, at 35 ± 2°C or 42 ±2°C in a reduced oxygen, increased carbon dioxide (=microaerobic) atmosphere. The incubation at 42°C is inhibitory to Campylobacter jejuni subsp. doylei and a variety of other species. Most organisms isolated on this medium will grow best at 35 ± 2°C. The microaerobic atmosphere can be achieved by using BD GasPak™ EZ Campy Container System Sachets in BD GasPak jars, or using the BD GasPak™ EZ CampyPouch™ System. Alternatively, the atmosphere can be achieved using evacuation of BD GasPak vented jars and replacement with cylinder gases. An incubation period of 2 to 3 days is usually sufficient, but 5 to 7 days of incubation were shown to increase the isolation rates.2

Results
After 42 to 48 hours incubation in a microaerobic atmosphere, the plates are inspected for typical Campylobacter colonies. Fresh isolates, especially of C. jejuni, tend to swarm on these and other media for Campylobacter while other Campylobacter species might produce convex colonies. A positive oxidase test and a Gram stain showing curved to gull wing-shaped Gram negative rods are further hints for the successful isolation. Further tests are necessary for confirmation of the identification.2

PERFORMANCE CHARACTERISTICS AND LIMITATIONS OF THE PROCEDURE
BD Campylobacter Bloodfree Selective Medium is an approved medium for the isolation of Campylobacter species from human stool specimens and for non-clinical materials such as meat and other foods.

BD Campylobacter Bloodfree Selective Medium is inhibitory to Arcobacter cryaerophilus and A. skirrowii.6

This medium must not be used for the subculture from blood cultures suspected to contain Campylobacter. Instead, BD Columbia Agar with 5% Sheep Blood or another non-selective blood or chocolate agar plate must be used and incubated in a microaerobic atmosphere at 32 to 37°C.

The number of species of Campylobacter and related organisms occurring as infectious agents is large, and their sensitivity to antimicrobial agents used as inhibitors in isolation media varies. Additionally, these organisms may need different incubation temperatures for growth. The inoculation of several different selective isolation media and an incubation at different temperatures may be necessary.2 Therefore, before a medium is used for rarely isolated or newly described micro-organisms, its suitability must first be tested by the user by cultivating pure cultures of the organism in question.

REFERENCES

PACKAGING/AVAILABILITY
BD Campylobacter Bloodfree Selective Medium
Cat. No. 254403 Ready-to-use Plated Media, cpu 20
Cat. No. 254095 Ready-to-use Plated Media, cpu 120
FURTHER INFORMATION
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