

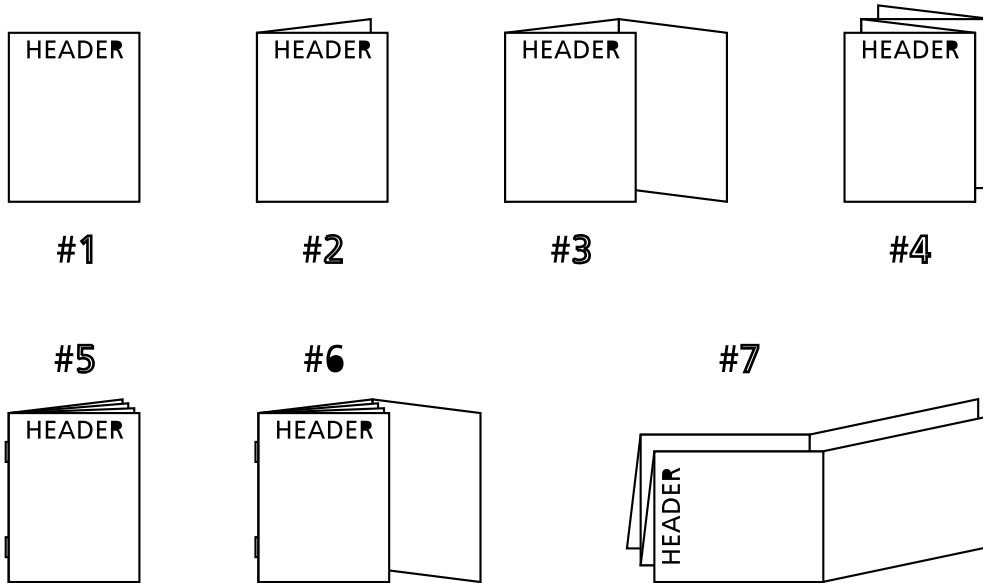
Revisions

SO 0046-2


Rev from	Rev To	ECO #	Date	Appr.
1199	0103	1096-03		

**Notes:**

- BD Cat. Number 214890,214891,214892
- Blank (Sheet) Size : Length: 11.0" Width: 2.75"  
 Number of Pages: 2 Number of Sheets: 1  
 Page Size: Length 11" Width 2.75" Final Folded Size: 2.75" x 2.75"
- Style (see illustrations below): # 1



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- Ink Colors: Printed two sides  Yes  No  
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Part Number: <b>S1350</b>		Category and Description Package Insert, Difco Campylobacter Supplement	Sheet: 1 of 3 <hr/> Scale: NONE	<b>A</b>

**BD Difco™ Campylobacter Antimicrobial Supplement Skirrow**  
**Difco™ Campylobacter Antimicrobial Supplement Blaser**

S1350JAA  
2003/01

**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 Skirrow<sup>1</sup> and Blaser et al.<sup>2,3</sup>

**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 antimicrobials to suppress the normal enteric flora.<sup>4</sup> Subsequently, Skirrow and other investigators reported similar blood-based selective media that differed in the numbers and types of antimicrobials.<sup>1,3,5,6</sup>

In 1978, Blaser et al. reported success in isolating *C. jejuni* with a medium containing four antimicrobials incorporated into Brucella Agar supplemented with 10% defibrinated sheep blood.<sup>2,3</sup> Subsequently, cephalothin was incorporated to increase its ability to inhibit the normal bacterial flora associated with fecal specimens.<sup>5</sup>

**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 Skirrow<sup>1</sup> and Blaser et al.<sup>2,3</sup> 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

**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.

**Product Deterioration:** Do not use the rehydrated supplement if it is contaminated, partially or totally evaporated or shows other signs of deterioration.

**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.<sup>7</sup> Food and environmental specimens should be collected in sterile containers and transported to the laboratory in accordance with recommended guidelines.<sup>8</sup>

**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% CO<sub>2</sub>. 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.<sup>9</sup>
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.

Organism	ATCC™	Recovery Skirrow	Recovery Blaser
<i>Campylobacter jejuni</i> subsp. <i>jejuni</i>	29428	Good	Good
<i>Campylobacter jejuni</i> subsp. <i>jejuni</i>	33291	Good	Good
<i>Candida albicans</i>	10231	Good	Inhibited
<i>Enterococcus faecalis</i>	33186	Inhibited	Inhibited
<i>Escherichia coli</i>	25922	Inhibited	Inhibited

**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.<sup>7,8,10</sup>
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**

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**AVAILABILITY**

Cat. No.	Description
214891	Difco™ Campylobacter Antimicrobial Supplement Skirrow, 6 x 5 mL
214890	Difco™ Campylobacter Antimicrobial Supplement Blaser, 6 x 5 mL
214892	Difco™ Campylobacter Agar Base, 500 g
218201	Difco™ Campylobacter Agar Base, 2 kg

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