

7. Center for Disease Control. 1978. Laboratory methods in clinical bacteriology. CDC, Atlanta, Ga.
8. Baron, Peterson and Finegold. 1994. Bailey & Scott's diagnostic microbiology, 9th ed. Mosby-Year Book, Inc., St. Louis, Mo.
9. Finegold and Martin. 1982. Bailey & Scott's diagnostic microbiology, 6th ed. The C.V. Mosby Company, St. Louis, Mo.
10. MacFaddin. 1985. Media for isolation-cultivation-identification-maintenance of medical bacteria, vol. 1. Williams & Wilkins, Baltimore, Md.
11. Faur, Weisburd and Wilson. 1975. J. Clin. Microbiol. 1:294.
12. Applebaum and Lawrence. 1979. J. Clin. Microbiol. 9:598.

Availability

Difco™ Cystine Tryptic Agar

AOAC

Cat. No. 252310 Dehydrated – 500 g

BBL™ CTA Medium™

AOAC

Cat. No. 211096 Dehydrated – 500 g
 221631 Prepared Tubes, 8 mL (K Tubes) – Pkg. of 10*
 221632 Prepared Tubes, 8 mL (K Tubes) – Ctn. of 100*

BBL™ CTA Medium™ with Carbohydrates

Cat. No.	297731	Prepared Tubes with Arabinose – Pkg. of 10*
	297732	Prepared Tubes with Cellobiose – Pkg. of 10*
	221633	Prepared Tubes with Dextrose – Pkg. of 10*
	221634	Prepared Tubes with Dextrose – Ctn. of 100*
	296001	Prepared Tubes with Fructose – Pkg. of 10*
	221635	Prepared Tubes with Lactose – Pkg. of 10*
	221637	Prepared Tubes with Maltose – Pkg. of 10*
	221639	Prepared Tubes with Mannitol – Pkg. of 10*
	297101	Prepared Tubes with Raffinose – Pkg. of 10*
	297102	Prepared Tubes with Rhamnose – Pkg. of 10*
	221641	Prepared Tubes with Salicin – Pkg. of 10*
	221643	Prepared Tubes with Sorbitol – Pkg. of 10*
	296002	Prepared Tubes with Starch – Pkg. of 10*
	221645	Prepared Tubes with Sucrose – Pkg. of 10*
	297033	Prepared Tubes with Trehalose – Pkg. of 10*
	221647	Prepared Tubes with Xylose – Pkg. of 10*

*Store at 2-8°C.

Campylobacter Agars

Campylobacter Agar Base • Campylobacter Agar with 5 Antimicrobics and 10% Sheep Blood (Blaser) Campy CSM Agar • Campy CVA Agar • Skirrows Medium • Campylobacter Antimicrobial Supplement Skirrow • Campylobacter Antimicrobial Supplement Blaser

Intended Use

Campylobacter Agar Base, when supplemented with blood or other additives and antimicrobial agents, is used for the primary isolation and cultivation of *Campylobacter jejuni* subsp. *jejuni* from human fecal specimens. Several prepared selective media formulations are provided for the same purpose.

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.¹ Subsequently, Skirrow and other investigators reported similar blood-based selective media that differed in the numbers and types of antimicrobics.²⁻⁶ Bolton et al. reported that charcoal can effectively replace the blood in selective media for campylobacters.⁷

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.^{3,4} Subsequently, cephalothin was incorporated to increase its ability to inhibit the normal bacterial flora associated with fecal specimens.⁵

In 1983, Reller et al. introduced an improved selective medium containing cefoperazone, vancomycin and amphotericin B (CVA) for isolation of *C. jejuni*.⁶ They reported that this combination of antimicrobial agents provided better

inhibition of normal fecal flora for easier detection of *C. jejuni* than the selective blood agar plate developed previously.

Karmali et al., in 1986, evaluated a blood-free, charcoal-based selective medium (designated CSM) in parallel with a Skirrow-type selective medium containing lysed horse blood. They reported that the quality of *Campylobacter* growth on CSM (luxuriant growth with smooth and effuse colonies) was similar to that seen on blood-based media and was significantly more selective than Skirrow medium.⁸

Principles of the Procedure

These media support the growth of *Campylobacter* species due to their content of peptones, yeast extract and other digests, extracts and components specific for the individual formulations provided. *Campylobacter* isolation relies, in addition, on a medium's selectivity, which depends on the antimicrobial agents in the medium, a microaerophilic environment and the incubation temperature of 42°C, which suppresses the growth of most normal bacteria.⁹

The antimicrobial agents required to make Skirrow's and Blaser's formulations are provided as Campylobacter Antimicrobial Supplement Skirrow and Campylobacter Antimicrobial Supplement Blaser, respectively.

Campylobacter Agar with 5 Antimicrobics and 10% Sheep Blood supports the growth of *Campylobacter* species due to its content of peptones, dextrose, yeast extract and blood. The

User Quality Control

Identity Specifications

Difco™ *Campylobacter* Agar Base

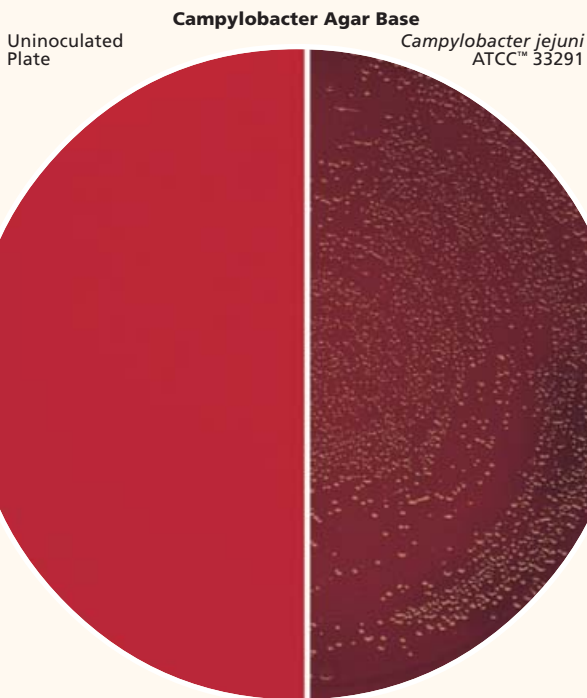
Dehydrated Appearance:	Beige, free-flowing, homogeneous.
Solution:	3.95% solution, soluble in purified water upon boiling. Solution is medium to dark amber, clear to slightly opalescent.
Prepared Appearance:	Plain – Medium to dark amber, very slightly to slightly opalescent. With 10% sheep blood – Cherry red, opaque.
Reaction of 3.95% Solution at 25°C:	pH 7.4 ± 0.2

Cultural Response

Difco™ *Campylobacter* Agar Base

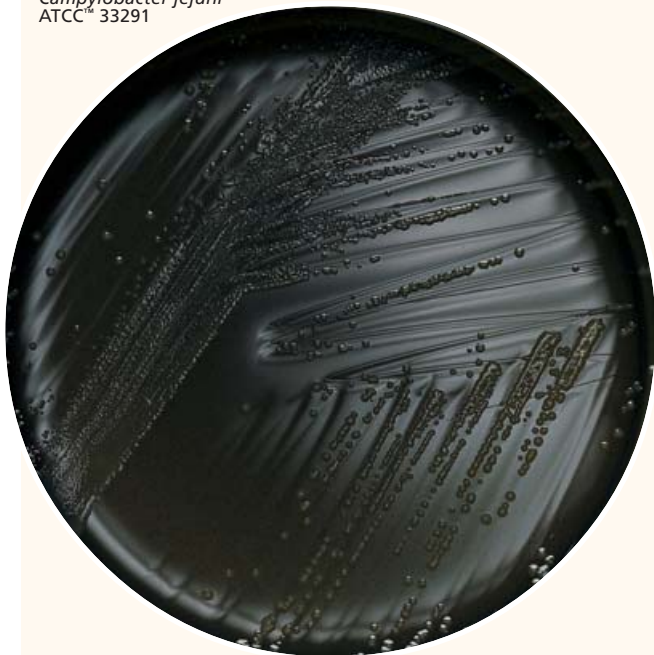
Prepare the medium per label directions; e.g., with 10% sterile defibrinated sheep blood and antimicrobial supplements (Skirrow or Blaser). Inoculate and incubate at 42°C under microaerophilic conditions for 40-48 hours.

ORGANISM	ATCC™	INOCULUM CFU	RECOVERY SKIRROW	RECOVERY BLASER
<i>Campylobacter jejuni</i> subsp. <i>jejuni</i>	29428	10 ² -10 ³	Good	Good
<i>Campylobacter jejuni</i> subsp. <i>jejuni</i>	33291	10 ² -10 ³	Good	Good
<i>Candida albicans</i>	10231	10 ³	Good	Inhibition
<i>Enterococcus faecalis</i>	33186	10 ³	Inhibition	Inhibition
<i>Escherichia coli</i>	25922	10 ³	Inhibition	Inhibition



Campylobacter jejuni
ATCC™ 33291

Campy CSM Agar



Campylobacter jejuni
ATCC™ 33291

Campy CVA Agar



peptones supply nitrogenous compounds, carbon, sulfur and trace ingredients. Yeast extract is a source of the B-complex vitamins. Dextrose is utilized as an energy source. Sheep blood supplies additional nutrients. The incorporation of the antimicrobial agents (amphotericin B, cephalothin, polymyxin B, trimethoprim and vancomycin) suppresses the growth of the normal microbial flora in fecal specimens, thereby facilitating isolation of *C. jejuni*.

Skirrows Medium contains, in addition to the usual nutritional components, laked horse blood, which supplies the X factor (heme) and other growth requirements. Vancomycin inhibits gram-positive bacteria, polymyxin B inhibits most gram-negative bacilli except *Proteus* and trimethoprim is inhibitory for *Proteus* spp.

Campy CSM Agar consists of Columbia Agar Base supplemented with activated charcoal, hematin, sodium pyruvate and three antimicrobial agents (cefoperazone, cycloheximide and vancomycin). The charcoal, hematin and sodium pyruvate improve the aerotolerance of *Campylobacter* species; it has been suggested that these supplements act as quenching agents of photochemically-produced toxic oxygen derivatives.⁸ Cefoperazone is a cephalosporin antibiotic that suppresses the growth of gram-negative enteric bacilli and some gram-positive species. Vancomycin is a glycopeptide antibiotic that inhibits many species of gram-positive bacteria. Cycloheximide is an antifungal agent.

Campy CVA Agar consists of Brucella Agar, a general-purpose medium that supports the growth of *Campylobacter* species. Defibrinated sheep blood provides additional nutrients. Antimicrobial agents are incorporated to suppress the growth of normal fecal flora that could mask the presence of *C. jejuni*. Cefoperazone is a cephalosporin antibiotic that suppresses the growth of gram-negative enteric bacilli and some gram-positive species. Vancomycin is a glycopeptide antibiotic that inhibits many species of gram-positive bacteria. Amphotericin B is an antifungal agent.

Formulae

Difco™ Campylobacter Agar Base

Approximate Formula* Per Liter	
Proteose Peptone No. 3	15.0 g
Liver Digest	2.5 g
Yeast Extract	5.0 g
Sodium Chloride	5.0 g
Agar	12.0 g

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

*Adjusted and/or supplemented as required to meet performance criteria.

Directions for Preparation from Dehydrated Product

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. **To prepare Skirrow's medium:** aseptically rehydrate one vial of Campylobacter Antimicrobial Supplement Skirrow with 5 mL of sterile purified water. Rotate in an end-over-end motion to dissolve the contents completely. Store the rehydrated vials at 2-8°C. Use within 24 hours after rehydration.
To prepare Blaser's medium: aseptically rehydrate one vial of Campylobacter Antimicrobial Supplement Blaser with 5 mL of sterile purified water. Rotate in an end-over-end motion to dissolve the contents completely. Store the rehydrated vials at 2-8°C. Use within 24 hours after rehydration.
- 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.

Procedure

Use standard procedures to obtain isolated colonies from specimens. If immediate inoculation of a *Campylobacter* agar cannot be performed, the use of a suitable holding medium (e.g., Campylobacter Thioglycollate Medium with 5 Antimicrobics) is recommended. Incubate inoculated plates at 42°C in an atmosphere conducive to the primary isolation and cultivation of microaerophilic organisms. 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™, Bio-Bag™ 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.¹⁰

Examine plates at 24 and 48 hours.

NOTE: If plates are to be examined after 24 hours of incubation, treat plates as if they were anaerobic cultures; i.e., examine plates quickly and place them back into a reduced oxygen atmosphere immediately after examination.

Expected Results

Campylobacter jejuni produces two types of colonies. One is small, raised, grayish-brown, smooth and glistening with an entire translucent edge. The other colony type is flat, mucoid, translucent, grayish and has an irregular edge.

A small percentage of strains may appear tan or slightly pinkish.¹¹ Colonies tend to spread, especially when initially isolated from fresh clinical specimens.

Limitations of the Procedure

1. Due to the presence of 15 mg/L of cephalothin, growth of *C. fetus* subsp. *fetus* will be inhibited on Campylobacter Agar with 5 Antimicrobics and 10% Sheep Blood; therefore, this medium is not recommended for the isolation or culture of this subspecies.
2. 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

1. Dekeyser, Gossuin-Detrain, Butzler and Sternon. 1972. J. Infect. Dis. 125:390.
2. Skirrow. 1977. Br. Med. J. 2:9.
3. Blaser, Cravens, Powers and Wang. 1978. Lancet ii:979.
4. Blaser, Berkowitz, LaForce, Cravens, Reller and Wang. 1979. Ann. Intern. Med. 91:179.
5. Wilson and Wang. October 13, 1979. Background and culture techniques for *Campylobacter fetus* subsp. *jejuni*. Information flier, Campylobacter Laboratory, Veterans Administration Hospital, Denver, Co.
6. Reller, Mirrett and Reimer. 1983. Abstr. C274. Abstr. Annu. Meet. Am. Soc. Microbiol. 1983.
7. Bolton and Coates. 1983. J. Appl. Bacteriol. 54:115.
8. Karmali, Simor, Roscoe, Fleming, Smith and Lane. 1986. J. Clin. Microbiol. 23:456.
9. Grasmick. 1992. In Isenberg (ed.), Clinical microbiology procedures handbook, vol. 1. American Society for Microbiology, Washington, D.C.
10. Karmali and Fleming. 1979. J. Clin. Microbiol. 10:245.
11. Kaplan. 1980. In Lennette, Balows, Hausler and Truant (ed.). 1980. Manual of clinical microbiology, 3rd ed. American Society for Microbiology, Washington, D.C.

Availability

Difco™ Campylobacter Agar Base

BS10 **CMPH** **COMPF** **ISO** **MCM7** **SMD** **SMWW**

Cat. No. 214892 Dehydrated – 500 g
218201 Dehydrated – 2 kg

Difco™ Campylobacter Antimicrobial Supplement Skirrow

ISO **SMWW**

Cat. No. 214891 Vial – 6 × 5 mL

Difco™ Campylobacter Antimicrobial Supplement Blaser

SMWW

Cat. No. 214890 Vial – 6 × 5 mL

BBL™ Campylobacter Agar with 5 Antimicrobics and 10% Sheep Blood (Blaser)

BS10 **CMPH** **COMPF** **MCM7** **SMWW**

United States and Canada

Cat. No. 221727 Prepared Plates – Pkg. of 20*
221728 Prepared Plates – Ctn. of 100*

Europe

Cat. No. 254001 Prepared Plates – Pkg. of 20*
254069 Prepared Plates – Ctn. of 120*

Japan

Cat. No. 251727 Prepared Plates – Pkg. of 20*

BBL™ Campy CSM Agar

BS10 **CMPH** **MCM7** **SMWW**

Cat. No. 299614 Prepared Plates – Pkg. of 20*

BBL™ Campy CVA Agar

BS10 **MCM7**

Cat. No. 297246 Prepared Plates – Pkg. of 20*
297713 Prepared Plates – Ctn. of 100*

BBL™ Skirrows Medium

SMWW

Cat. No. 297793 Prepared Plates – Pkg. of 20*

*Store at 2-8°C.

Campylobacter Thioglycollate Medium with 5 Antimicrobics

Intended Use

Campylobacter Thioglycollate Medium with 5 Antimicrobics is recommended as a holding medium for samples suspected to contain *Campylobacter jejuni* subsp. *jejuni* when immediate inoculation of Campylobacter Agar with 5 Antimicrobics and 10% Sheep Blood cannot be performed.

Summary and Explanation

In 1972, Dekeyser et al. reported the isolation of *C. jejuni* from the feces of patients with diarrhea and acute gastroenteritis using a filtration technique and a selective medium with antimicrobics to suppress the normal enteric flora.¹ Skirrow, in 1977, reported a selective culture medium containing three antimicrobics.² Blaser et al. reported success in isolating *C. jejuni* by direct inoculation of stool specimens onto an agar medium containing four antimicrobics and by inoculating this medium with stool swabs held refrigerated for 8 hours in thioglycollate broth (0.16% agar) containing the same four antimicrobics.^{3,4} A fifth antimicrobial, cephalothin, was later incorporated to inhibit nonpathogenic *C. fetus* subsp. *fetus*.⁴

The combined yield using Campylobacter blood agar and Campylobacter Thioglycollate Medium, both containing five antimicrobics, was reported to be 33% higher than when the plated medium only was used and 28% higher than when the broth medium was used alone.⁴ Luechterfeld et al. reported that the number of positives was not substantially increased by holding turkey fecal specimens at 4°C overnight in Campylobacter Thioglycollate Medium.⁵

Campylobacter Thioglycollate Medium has been recommended as a holding medium when facilities for streaking and incubation are not immediately available.⁶

Principles of the Procedure

Campylobacter Thioglycollate Medium is a selective holding medium recommended for the isolation of *C. jejuni* from clinical specimens. The incorporation of antimicrobial agents (i.e., amphotericin B, cephalothin, polymyxin B, trimethoprim and vancomycin) and refrigeration inhibits further multiplication of normal microbial flora in fecal specimens, thus facilitating isolation of *C. jejuni*.