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TECHNICAL PRODUCT INFORMATION

CAMPY-BLASER Catalog No. P1120

CAMPY-THIO BROTH Catalog No. T1200

INTENDED USE:

Campy-Blaser is a selective media used to isolate *Campylobacter jejuni* from human fecal specimens. Campy-Thio is recommended as a holding medium for samples suspected to contain *Campylobacter jejuni* when immediate inoculation cannot be performed.

HISTORY/SUMMARY:

In 1972 Dekeyser reported that *C. jejuni* was isolated 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]. In 1977, Skirrow reported the development of a selective culture medium for *C. jejuni* containing three antimicrobics[2]. In 1978, Blaser et al. reported success in isolating *C. jejuni* using four antimicrobial agents incorporated into Brucella Agar enriched with 10% sheep blood[3, 4]. The addition of a fifth selective agent, cephalothin, to this medium increased the inhibition of the normal 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.

FORMULA:

CAMPY AGAR (BLASER)

Component (per liter of purified water)	Amount
Brucella Agar Base	43.0 g
Defibrinated Sheep Blood	100 mL
Vancomycin	0.010 g
Amphotericin B	0.002 g
Cephalothin	0.015 g
Polymixin B	2500 units
Trimethoprim	5.0 mg

Final pH: 7.2 ± 0.2 at 25°C

CAMPY-THIO

Component (per liter of purified water)	Amount
Pancreatic Digest of Casein	17.0 g
Pancreatic Digest of Soybean Meal	3.0 g
Dextrose	6.0 g
Sodium Thioglycollate	0.5 g
Sodium Chloride	2.5 g
Agar	1.6 g
L-Cystine	0.25 g
Amphotericin B	0.002 g
Cephalonthin	0.015 g
Trimethoprim	0.005 g
Vancomycin	0.010 g
Polymixin B	2500 units

Final pH: 7.0 ± 0.2 at 25°C

PRINCIPLES OF THE PROCEDURE:
This medium 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.

PRECAUTIONS:
This medium is for In Vitro Diagnostic Use and should be used by properly trained individuals. Precautions should be taken against the damages of microbial hazards by properly sterilizing specimens, containers and media after their use.

PRODUCT DETERIORATION:
This medium should not be used if (a) there is evidence of dehydration, (b) contamination, (c) color change, or (d) expiration date has passed.

SPECIMEN COLLECTION, STORAGE AND TRANSPORTATION:
Specimens should be transported to the laboratory without delay and protected from excessive heat and cold. If there is any delay in processing, a swab inoculated with the specimen should be placed in a suitable transport medium, such as Amies or Stuarts.

PROCEDURE:
Preparation of Specimens and Inoculation:

A. Direct Inoculation:
1. Swab - Inoculate a Campy Agar plate and streak for isolation.
2. Diarrheal Stool - Inoculate 3 drops onto a Campy Agar plate and streak for isolation. Make a direct smear and look for fecal leukocytes and small, curved gram negative rods.
3. Solid Stool - Prepare a 1:10 suspension of stool by placing a pea sized portion into 5ml of saline. Vortex. Inoculate 3 drops onto a Campy Agar plate and streak for isolation.

B. Indirect Inoculation:
1. Swab - Place into Campy-Thio. Refrigerate overnight. **DO NOT INCUBATE**.
2. Diarrheal Stool - Place 5 drops in Campy-Thio. Refrigerate overnight.
3. Solid Stool - Prepare a 1:10 suspension of stool by placing a pea sized portion into 5ml saline. Vortex. Place 5 drops in Campy-Thio. Refrigerate overnight.

To subculture Campy-Thio:
With a Pasteur pipette placed 2 cm below the surface of the thio, continuously withdraw toward the surface.
Place 3 drops onto a Campy Agar plate and streak for isolation.

C. Incubation:
Incubate the Campy Agar plate at 42°C. The higher temperature allows for better growth at 24 hours and suppresses the growth of most normal bacteria. Cultures of Campylobacter should be incubated as indicated and observed at 24 and 48 hours. An atmosphere of 10% CO_2 is required.

QUALITY CONTROL:
Inoculate this medium with *Escherichia coli*, *Candida albicans* and *Campylobacter fetus subsp. jejuni*. Incubate *Escherichia coli* and *Candida albicans* at 42°C and observe for (partial) inhibition at 24 and 48 hours. Cultures of Campylobacter should be incubated as indicated and observed at 24 and 48 hours.

IDENTIFICATION:
The morphological appearance of *Campylobacter fetus subsp. jejuni* is that of spirally-curved rods with "S" and gull-wing shapes. Organisms exhibit darting and corkscrew like motility.
Campylobacter jejuni produces two types of colonies. One is flat, grayish to tan and translucent with an irregular edge.
The second type is round, raised, glistening, translucent at the edge and grayish-brown at the center. Colonies may swarm on a moist surface. *C. fetus* does not hemolyze blood.

Blaser et al[4] consider organisms to be *C.fetus ssp. jejuni* when they meet the following criteria:

1. Vibrio forms by Gram stain or darting motility when wet preps are examined by microscopy.
2. Non-fermenters.
3. Grows in Brucella Broth at 37°C and 42°C but **NOT** at 25°C.
4. Does not grow in 3.5% and 6.5% sodium chloride.
5. Grows in broth containing 1% glycine.
6. Produces H2S in Kliger Iron Agar with lead acetate paper.
7. Sensitive to nalidixic acid.

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5. Wilson, N.A., and W-L.L. Wang. October 13, 1979. Background and culture techniques for Campylobacter Laboratory, Veterans Administration Hospital, Denver.
6. Reller, Mirrett, and Reimer. 1983. Abstr. C274. Abstr. Annu. Meet. Am. Soc. Microbiol. 1983

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