BBL™ Campylobacter Thioglycollate Medium with 5 Antimicrobics

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QUALITY CONTROL PROCEDURES

I INTRODUCTION

Campylobacter Thioglycollate Medium with 5 Antimicrobics (Campy Thio) is a holding medium for samples suspected to contain *Campylobacter jejuni* subsp. *jejuni* prior to the inoculation of solid medium.

II PERFORMANCE TEST PROCEDURE

A. TEST 1: *Campylobacter jejuni* subsp. *jejuni*

1. Prepare a McFarland #1 standard from a 24- to 48-h **Trypticase™** Soy Agar with 5% Sheep Blood (TSA II) culture of *C. jejuni* subsp. *jejuni*.
2. Dilute standardized culture in **Trypticase** Soy Broth to 10^{-3} concentration.
3. Inoculate one tube of the Campy Thio with 0.1 mL of the diluted culture. Mix by vortexing.
4. From the inoculated Campy Thio tube, immediately inoculate (swab and then streak) a TSA II plate. Label the plate "pre-refrigeration." Incubate plate at 42 ± 2°C using the **GasPak™** EZ Campy System. Read at 36 – 48 h for amount of growth of *C. jejuni* subsp. *jejuni*.
5. Immediately refrigerate the inoculated Campy Thio tubes overnight (16 – 24 h) at 2 – 8°C. DO NOT USE the **GasPak** EZ Campy System.
6. After overnight refrigeration, subculture the inoculated Campy Thio tube. Swab and then streak onto a TSA II plate. Label plate "post-refrigeration."
7. Incubate plate at 42 ± 2°C using the **GasPak** EZ Campy System.
8. Read plate after 36 – 48 h for the amount of growth.

B. TEST 2: Mixed flora

1. Prepare a McFarland #1 standard from an 18- to 24-h mixed-flora culture (consisting of 1:1:1 mixture of *Escherichia coli*, *Enterococcus faecalis* and *Candida albicans*).
2. Prepare 10^{-4} and 10^{-5} dilutions from the standardized mixed flora.
3. Using the 10^{-5} dilution, spread-inoculate 0.1 mL onto a TSA II plate.
4. a. Using the 10^{-4} dilution, inoculate one tube of Campy Thio with 0.1 mL. Mix by vortexing.
   b. Immediately inoculate 0.1 mL from the inoculated Campy Thio onto a Campylobacter Agar with 5 Antimicrobics and 10% Sheep Blood (Campy-BAP) plate and a TSA II plate and spread evenly with a sterile glass spreader. Label the plates "pre-refrigeration."
5. Incubate plates at 42 ± 2°C using the **GasPak** EZ Campy System. Read after 36 – 48 h for amount of growth.
6. Immediately refrigerate the Campy Thio tube overnight (16 – 24 h) at 2 – 8°C. DO NOT USE the **GasPak** EZ Campy System.
7. After overnight refrigeration, subculture by placing the tip of a pipette about 2 cm below the surface of the Campy Thio and continuously withdraw a sample as the tip is slowly drawn to the surface. Inoculate 0.1 mL onto a Campy-BAP and a TSA II plate by spreading with a sterile glass spreader. Label plates "post-refrigeration."
8. Incubate plates at 42 ± 2°C using the **GasPak** EZ Campy System.
9. Read plates after 36 – 48 h for the amount of growth.

C. Expected Results

Organisms	ATCC®	Recovery
**Campylobacter jejuni* subsp. *jejuni*	33291	Recovery of "post-refrigeration" samples on TSA II plate should be no more than one growth score lower than "pre-refrigeration" sample.
Mixed flora culture consisting of 1:1:1 mixture of:		
**Escherichia coli*	25922	The mixed flora should be inhibited (partial to complete) on the Campy-BAP plate. Growth (counts) of mixed culture (10^{-4}) from Campy Thio tube on TSA II must be reduced in comparison to growth (counts) of mixed culture (10^{-5}) from dilution tube on TSA II.
**Enterococcus faecalis*	29212	
**Candida albicans*	10231	

*Recommended organism strain for User Quality Control.

III ADDITIONAL QUALITY CONTROL

1. Examine tubes as described under "Product Deterioration."
2. Visually examine representative tubes to assure that any existing physical defects will not interfere with use.
3. Incubate uninoculated representative tubes at 20 – 25°C and 30 – 35°C and examine after 7 days for microbial contamination.

PRODUCT INFORMATION

IV 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.

V 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 h in thioglycollate broth (0.16% agar) containing the same four antimicrobics.[3,4] A fifth antimicrobic, cephalothin, was later incorporated to inhibit nonpathogenic *C. fetus* subsp. *fetus*.[4]

Campylobacter Thioglycollate Medium has been recommended as a holding medium when facilities for streaking and incubation are not immediately available, when low numbers are expected because of delayed specimen transport to the laboratory or because the acute stage of the disease has passed.[5,6]

For a review of the current taxonomic status, refer to Nachamkin.[6]

VI PRINCIPLES OF THE PROCEDURE

Campylobacter Thioglycollate Medium is a selective holding medium recommended for the isolation of *C. jejuni* subsp. *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* subsp. *jejuni*.

VII REAGENTS

Campylobacter Thioglycollate Medium with 5 Antimicrobics

Approximate Formula* Per Liter Purified Water

Pancreatic Digest of Casein	17.0 g	Sodium Sulfite	0.1 g
Papaic Digest of Soybean Meal	3.0 g	Amphotericin B	2.0 mg
Dextrose	6.0 g	Cephalothin	15.0 mg
Sodium Chloride	2.5 g	Trimethoprim	5.0 mg
Sodium Thioglycollate	0.5 g	Vancomycin	10.0 mg
Agar	1.6 g	Polymyxin B	2500.0 units
L-Cystine	0.25 g		

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

Warnings and Precautions: For *in vitro* Diagnostic Use.

Tubes with tight caps should be opened carefully to avoid injury due to breakage of glass.

Pathogenic microorganisms, including hepatitis viruses and Human Immunodeficiency Virus, may be present in clinical specimens. "Standard Precautions"[7-10] and institutional guidelines should be followed in handling all items contaminated with blood and other body fluids. Prior to discarding, sterilize prepared tubes, specimen containers and other contaminated materials by autoclaving.

Storage Instructions: On receipt, store tubes in the dark at 2 – 8°C. Avoid freezing and overheating. Do not open until ready to use. Minimize exposure to light. Tubed media stored as labeled until just prior to use may be inoculated up to the expiration date and incubated for the recommended incubation times.

Product Deterioration: Do not use tubes if they show evidence of microbial contamination, discoloration, drying or other signs of deterioration.

VIII SPECIMEN COLLECTION AND HANDLING

Specimens suitable for culture may be handled using various techniques. For detailed information, consult appropriate texts.[11,12] Specimens should be obtained before antimicrobial agents have been administered. Provision must be made for prompt delivery to the laboratory.

IX PROCEDURE

Material Provided: Campylobacter Thioglycollate Medium with 5 Antimicrobics

Materials Required But Not Provided: Ancillary culture media, reagents, quality control organisms and laboratory equipment as required.

Test Procedure: Observe aseptic techniques.

1. Sample collection, storage and subculturing to plated medium.[13]

   Place rectal swab about 1 cm into the medium and twirl the swab. Remove the swab or lower it to the bottom of the tube and break the shaft of the swab evenly with the lip of the tube to allow easy access to the shaft.

   With solid stools, prepare a saline suspension, blend in a mechanical mixer (i.e., vortex), and place five drops into the medium about 1 cm below the surface. Alternatively, probe all areas of the stool with a swab and inoculate the medium as described for a rectal swab.

   With diarrheal stools, place five drops in the medium about 1 cm below the surface.

   Refrigerate inoculated Campylobacter Thioglycollate Medium overnight and subculture the next day to Campylobacter Agar with 5 Antimicrobics and 10% Sheep Blood plates using a Pasteur pipette inserted about 2 cm below the surface of the broth to continuously withdraw a sample as the tip is slowly drawn to the surface. Do not subculture onto nonselective media since the normal flora may still be viable.

2. Incubation of plated medium.

   Incubate plated medium at 42°C in a reduced oxygen, increased carbon dioxide atmosphere. This atmosphere can be achieved by 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,[6] or by using the Fortner principle.[14]

User Quality Control: See "Quality Control Procedures."

Quality control requirements must be performed in accordance with applicable local, state and/or federal regulations or accreditation requirements and your laboratory's standard Quality Control procedures. It is recommended that the user refer to pertinent CLSI guidance and CLIA regulations for appropriate Quality Control practices.

X RESULTS

Plates of Campylobacter Agar with 5 Antimicrobics and 10% Sheep Blood inoculated from Campylobacter Thioglycollate Medium with 5 Antimicrobics should be examined for the presence of colonies of *Campylobacter jejuni* subsp. *jejuni*. These colonies on Campylobacter agar will appear as small, mucoid, usually grayish in coloration, flat with irregular edges, and nonhemolytic at 24 and 48 h.[15]

Colonies may be only barely visible in 18 – 24 h. An alternate colonial morphology, which appears to be strain related, consists of round colonies 1 – 2 mm in diameter, which are convex, entire, and glistening.[15] A small percentage of strains may appear tan or slightly pinkish in coloration.[13]

Colonies tend to spread or swarm, especially when initially isolated from fresh clinical specimens. Note: If plates are to be examined after 24 h 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.

XI LIMITATIONS OF THE PROCEDURE

For identification, organisms must be in pure culture. Morphological, biochemical, and/or serological tests should be performed for final identification. Consult appropriate texts for detailed information and recommended procedures.[11,12,16]

XII PERFORMANCE CHARACTERISTICS

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] Luechtefeld 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.[17]

XIII AVAILABILITY

Cat. No.	Description
221747	**BD BBL™** Campylobacter Thioglycollate Medium with 5 Antimicrobics
221748	**BD BBL™** Campylobacter Thioglycollate Medium with 5 Antimicrobics

XIV REFERENCES

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