



## QUALITY CONTROL PROCEDURES

### I INTRODUCTION

Campy CVA Agar is a selective medium for the primary isolation of *Campylobacter jejuni* from stool specimens.

### II PERFORMANCE TEST PROCEDURE

- Inoculate representative samples with the cultures listed below:
  - Prepare the test cultures of the *Campylobacter* strains by swabbing a moderate amount of growth from a 48 h Brucella-BAP or **Trypticase™** Soy Agar with 5% Sheep Blood Agar (TSA II) plate. Transfer to **Trypticase** Soy Broth and mix well. Adjust to a turbidity comparable to a 0.5 McFarland standard. Dilute in **Trypticase** Soy Broth to deliver  $10^3$ – $10^4$  CFU per plate. For the other organisms, use  $10^{-1}$  dilutions of 5-h **Trypticase** Soy Broth cultures. Streak the plates for isolation.
  - Incubate plates at  $42 \pm 2^\circ\text{C}$  in the appropriate **GasPak™** EZ Campy Container System or **GasPak** EZ Campy Pouch System. Minimize the time interval between preparation of dilutions and inoculation/incubation to assure organism viability.
  - Include TSA II plates as nonselective controls for all cultures.
- Examine plates at 42–48 h for growth and selectivity.
- Expected Results

Organisms	ATCC™	Recovery
* <i>Campylobacter jejuni</i>	33291	Growth
<i>Campylobacter jejuni</i>	33292	Growth
* <i>Escherichia coli</i>	25922	Inhibition (partial to complete)
<i>Enterococcus faecalis</i>	29212	Inhibition (partial to complete)
<i>Proteus mirabilis</i>	12453	Inhibition (partial to complete)
<i>Pseudomonas aeruginosa</i>	10145	Inhibition (partial to complete)
<i>Candida albicans</i>	10231	Inhibition (trace to complete)

\*Recommended organism strain for User Quality Control.

### III ADDITIONAL QUALITY CONTROL

- Examine plates as described under "Product Deterioration."
- Visually examine representative plates to assure that any existing physical defects will not interfere with use.
- Determine the pH potentiometrically at room temperature for adherence to the specification of  $7.2 \pm 0.2$ .
- Note the firmness of plates during the inoculation procedure.
- Incubate uninoculated representative plates at  $35 \pm 2^\circ\text{C}$  for 72 h and examine for microbial contamination.

## PRODUCT INFORMATION

### IV INTENDED USE

Campy CVA Agar is a selective medium used in the primary isolation and cultivation of *Campylobacter jejuni* from human fecal specimens.

### V SUMMARY AND EXPLANATION

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.<sup>1</sup> In 1977, Skirrow reported the development of a selective culture medium for *C. jejuni* containing three antimicrobics.<sup>2</sup> In 1978, Blaser et al. reported success in isolating *C. jejuni* using four antimicrobics incorporated into Brucella Agar enriched with 10% sheep blood.<sup>3,4</sup> The addition of a fifth selective agent, cephalothin, to this medium increased the inhibition of the normal flora associated with fecal specimens.<sup>5</sup>

In 1983, Reller et al. introduced an improved selective medium containing cefoperazone, vancomycin and amphotericin B (CVA) for isolation of *C. jejuni*.<sup>6</sup> 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.

For a review of the current taxonomic status of *C. jejuni*, refer to Nachamkin.<sup>7</sup>

### VI 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.

### VII REAGENTS

#### Campy CVA Agar

Approximate Formula\* Per Liter Purified Water

Pancreatic Digest of Casein .....	10.0 g	Agar .....	15.0 g
Peptic Digest of Animal Tissue .....	10.0 g	Cefoperazone .....	20.0 mg
Dextrose .....	1.0 g	Vancomycin .....	10.0 mg
Yeast Extract .....	2.0 g	Amphotericin B .....	2.0 mg
Sodium Chloride .....	5.0 g	Sheep Blood, defibrinated .....	5%
Sodium Bisulfite .....	0.1 g		

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

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

If excessive moisture is observed, invert the bottom over an off-set lid and allow to air dry in order to prevent formation of a seal between the top and bottom of the plate during incubation.

Pathogenic microorganisms, including hepatitis viruses and Human Immunodeficiency Virus, may be present in clinical specimens. "Standard Precautions"<sup>8-11</sup> and institutional guidelines should be followed in handling all items contaminated with blood and other body fluids. After use, prepared plates, specimen containers and other contaminated materials must be sterilized by autoclaving before discarding.

**Storage Instructions:** On receipt, store plates in the dark at 2-8°C. Avoid freezing and overheating. Do not open until ready to use. Minimize exposure to light. Prepared plates stored in their original sleeve wrapping at 2-8°C until just prior to use may be inoculated up to the expiration date and incubated for recommended incubation times. Allow the medium to warm to room temperature before inoculation.

**Product Deterioration:** Do not use plates if they show evidence of microbial contamination, discoloration, drying, cracking 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.<sup>12,13</sup> Specimens should be obtained before antimicrobial therapy has been administered. Provision must be made for prompt delivery to the laboratory.

## IX PROCEDURE

**Material Provided:** Campy CVA Agar

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

**Test Procedure:** Observe aseptic techniques.

The agar surface should be smooth and moist, but without excessive moisture.

Streak the specimen as soon as possible after it is received in the laboratory. Alternatively, inoculate the specimen, by means of a swab, directly onto the agar surface and streak the plate for isolation.

Incubate inoculated plates, protected from light, at 42°C in a reduced oxygen, increased carbon dioxide atmosphere. This atmosphere can be achieved by using the **GasPak EZ** Campy Container System with sachets or the **GasPak EZ** Campy Pouch System with sachets. Alternatively, the atmosphere can be achieved using evacuation of **GasPak** vented jars and replacement with cylinder gases,<sup>7</sup> or by using the Fortner principle.<sup>14</sup>

Examine plates at 24 and 48 h.

**NOTE:** *C. jejuni* and *C. coli* grow well on selective media at 42°C. However, for optimal recovery of *Campylobacter* spp. and *Arcobacter* spp. the use of two selective agars is recommended along with incubation at 37°C as well as 42°C. Plates should be incubated for 72 h before reporting as negative.<sup>7,13</sup>

**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 (formerly NCCLS) guidance and CLIA regulations for appropriate Quality Control practices.

## X RESULTS

*Campylobacter jejuni* will appear as small, mucoid colonies, usually grayish in coloration, flat with irregular edges, and nonhemolytic at 24–48 h.<sup>7</sup> 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.<sup>7</sup> 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

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.

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.<sup>12,13,15-18</sup>

A single medium is rarely adequate for detecting all organisms of potential significance in a specimen. It should be recognized that organisms generally susceptible to the antimicrobial agent in a selective medium may be completely or only partially inhibited depending upon the concentration of the agent, the characteristics of the microbial strain and the number of organisms in the inoculum. Organisms that are generally resistant to the antimicrobial agent should not be inhibited. Cultures of specimens grown on selective media should, therefore, be compared with specimens cultured on nonselective media to obtain additional information and help ensure recovery of potential pathogens.

## XII AVAILABILITY

Cat. No.	Description
297246	<b>BBL™</b> Campy CVA Agar, Pkg. of 20 plates
297713	<b>BBL™</b> Campy CVA Agar, Ctn. of 100 plates
260671	<b>GasPak™</b> EZ Standard Incubation Container
260673	<b>GasPak™</b> EZ Standard Rack
260680	<b>GasPak™</b> EZ Campy Container Systems Sachets (20 sachets per box)
260685	<b>GasPak™</b> EZ Campy Pouch System (containing 20 sachets and 20 resealable pouches)

### XIII REFERENCES

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