

Enhanced Isolation of *Campylobacter jejuni* by Cold Enrichment in Campy-Thio Broth

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Isolation of *Campylobacter jejuni* from human feces by direct inoculation to Campy-BAP (Scott Laboratories, Inc., Fiskeville, R.I.) was compared with isolation after overnight enrichment at 4°C in Campy-thio broth followed by subculture to Campy-BAP. Of 54 positive specimens, 19 were positive only after enrichment, and 5 were positive only on the direct plate. Among 36 positive patients, 10 were detected by enrichment only and 2 by direct plating only. Laboratories using Campy-BAP should include cold enrichment in Campy-thio broth for optimum recovery of *C. jejuni*.

Campylobacter jejuni is a leading cause of bacterial enteritis. The development of selective plating media, some of which are commercially available, has resulted in isolation rates of *C. jejuni* often equal to those of *Salmonella* spp. Comparative studies of all the selective media have not been done.

Campy-BAP (Scott Laboratories, Inc., Fiskeville, R.I.) is a commonly used medium developed by Blaser et al. (1). It contains sheep blood in a brucella agar base and vancomycin, 10 µg/ml; polymyxin B, 0.25 µg/ml; trimethoprim lactate, 25 µg/ml; amphotericin B, 2 µg/ml; and cephalothin, 15 µg/ml (1). Blaser et al. also described a cold enrichment procedure with thioglycolate medium containing the same five antimicrobial agents (Campy-thio broth; Scott Laboratories, Inc., Fiskeville, R.I.) which increased the recovery of *C. jejuni* from feces.

In a more recent study, Luechtefeld et al. found that *C. jejuni* was consistently recovered from known positive fecal specimens after overnight refrigeration in Campy-thio broth but was rarely isolated if incubation was at 42°C. Although no data were presented, they reported that further studies of both animal and human specimens showed no increase in recovery after enrichment at 4°C (5).

Kaplan et al., however, recovered 17% of isolates only after enrichment in Campy-thio broth (R. L. Kaplan, L. J. Goodman, J. E. Barrett, and W. Landau, Abstr. Intersci. Conf. Antimicrob. Agents Chemother. 21st, Chicago, Ill., abstr. no. 778). Kaplan recommends that enrichment be included for optimum recovery of *C. jejuni* (4).

Recently, Martin et al. (6) described a *Campylobacter* enrichment broth (CEB) consisting of brucella broth medium plus 5-fluorouracil,

333 µg/ml; cefoperazone, 32 µg/ml; and trimethoprim, 32 µg/ml. They found that 69% more *C. jejuni* (13 versus 22) were isolated from human feces after enrichment in *Campylobacter* enrichment broth compared with direct plating on Campy-BAP. However, specimens were held in Cary-Blair transport medium 3 to 4 weeks before testing. The same specimens directly plated on Campy-BAP after a week of storage yielded 19 isolates rather than 13. The authors suggest that the difference between direct plating and enrichment would not be as great as 69% with fresh specimens.

Another approach was examined by Gilchrist et al. (3), who compared isolation on a medium similar to Campy-BAP (C-3 medium; 1 µg of cephalothin per ml instead of 15 µg/ml and no amphotericin B) supplemented with ferrous sulfate, sodium metabisulfite, and sodium pyruvate (FBP) to isolation after enrichment on C-3 medium without FBP. Enrichment was at 4°C in thioglycolate with the same antimicrobial agents as Campy-thio broth (except for a lower concentration of cephalothin). The C-3 agar medium was held at room temperature for up to 8 h before incubation at 42°C. Direct plating on C-3 medium was not done. They found the isolation rate with the FBP-supplemented medium equivalent to that following enrichment and suggested that the FBP-supplemented medium be used.

FBP-supplemented medium is not commercially available, and many, if not most, laboratories, use Campy-BAP (2). Therefore, we compared the isolation of *C. jejuni* from human feces directly plated on Campy-BAP to isolation on Campy-BAP after enrichment at 4°C in Campy-thio broth.

From January 1981 to December 1982, all stool specimens submitted to the Microbiology

Laboratory of St. Francis Hospital and Medical Center, Hartford, Conn., were both plated directly on Campy-BAP (Scott Laboratories Inc., Fiskeville, R.I.) and plated on Campy-BAP after overnight refrigeration in Campy-thio broth (Scott Laboratories Inc.).

Specimens were plated on routine enteric agars as soon as they arrived in the laboratory and then were held at 4°C until late in the afternoon, when all direct Campy-BAP and Campy-thio broths were inoculated. Two to three drops of liquid stool were inoculated directly to Campy-BAP, and five drops were inoculated just below the surface of the Campy-thio broth with a plastic transfer pipette (American Scientific Products, McGaw Park, Ill.). Solid stool specimens were probed in several areas with a cotton swab which was then rolled over about one-quarter of a Campy-BAP. The stool was sampled again in the same manner, and the swab was rotated several times in the upper part of the broth to disperse the specimen. Infrequently, rectal swabs were submitted. They were twirled in 0.5 ml of thioglycolate broth and then were treated as liquid stool. Campy-BAP were incubated in a GasPak jar with a CampyPak gas generator (BBL Microbiology Systems, Cockeysville, Md.) at 42°C and were examined after 24 and 48 h.

The Campy-thio broths were placed at 4°C until the following afternoon, when all were subcultured. A disposable transfer pipette tip was placed 2 cm below the surface of the broth, and about 1 ml was withdrawn. The pipette was inverted to mix the specimen, and then three drops were inoculated onto Campy-BAP, streaked for isolation, and incubated and examined in the same manner as the direct Campy-BAP. Colonies morphologically suggestive of *Campylobacter* spp. were confirmed by the methods described in the *Manual of Clinical Microbiology* (4).

There were 2,178 fecal specimens examined, and 59 specimens from 39 patients were positive for *C. jejuni* (Table 1). Five specimens positive on direct Campy-BAP were not inoculated into Campy-thio broth and are not included in Table

1. Three of these were the only positive specimens from three patients. There were also 33 patients positive for *Salmonella* spp., 20 positive for *Shigella* spp., and 1 patient each with stools positive for *Yersinia enterocolitica* and *Vibrio parahaemolyticus*. Among the 54 positive stool specimens, 19 (35%) were positive only after enrichment. Five were positive only on direct plating (9%). If any of several specimens from a single patient was positive for *C. jejuni* by direct plating, the patient was classified in Table 1 as direct positive. Ten patients would have been missed if enrichment had not been done. This difference is significant (34 in Campy-thio broth versus 26 on the direct Campy-BAP; $P = 0.01$ by χ^2 analysis). Ten patients had two or three positive specimens. Two were detected only by enrichment, and four others had only a single specimen positive by direct plating. Among all the specimens positive by direct plating, 27% were positive at 24 h, whereas 57% of those positive after enrichment were detected on the Campy-BAP subculture after 24-h incubation.

Thus, we found that after enrichment with Campy-thio broth, the number of isolates increased from 35 to 54, and the number of positive patients increased from 26 to 36. Frequently, the direct Campy-BAP had both fewer colonies of *C. jejuni* and larger amounts of other bacteria than the plates subcultured from Campy-thio broth. This enhancement is probably source and technique dependent. For example, Martin et al. (6) found no increased isolation with *Campylobacter* enrichment broth when poultry feces were tested but almost twice as many isolates after enrichment of bovine specimens. *Campylobacter* enrichment broth does not appear to increase isolation rates as much as Campy-thio broth if fresh human specimens are tested, but a direct comparison has not been done. Another example is the medium that Gilchrist et al. (3) used for enrichment and subculture. It varied slightly in antimicrobial agent content and concentration from Campy-BAP and Campy-thio broth. Also, the inoculated plates were held at room temperature in air for several hours before incubation. This may be why 57% of our positive Campy-thio broth subcultures were detected at 24 h compared with only 7% as reported by Gilchrist et al.

Although Kaplan (4) suggests that only 4 h at 4°C is necessary for concentration of *C. jejuni* in Campy-thio broth, the 24-h period we used may have contributed to the enhanced recovery. Further studies to determine the optimum time of subculture after enrichment, the best enrichment medium and technique, and the effectiveness of the FBP supplement are needed. In the meantime, we believe that laboratories using Campy-BAP for isolation of *C. jejuni* should, for

TABLE 1. Isolation of *C. jejuni* on Campy-BAP before and after overnight refrigeration in Campy-thio broth

Isolation from:	No. positive	No. detected by:		
		Both	Campy-thio broth only	Direct Campy-BAP only
Patients	36	24 (66.7) ^a	10 (27.9)	2 (5.6)
Specimens	54	30 (55.6)	19 (35.1)	5 (9.3)

^a Numbers in parentheses are percentages.

maximum recovery, include enrichment in Campy-thio broth at 4°C. The direct plate should be included because about 9% of isolates were detected only on the direct plate. It is not known if cold enrichment will enhance isolation if other *Campylobacter*-selective media are used.

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