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Use of Tri-Gas Incubator for Routine Culture of Campylobacter Species from Fecal Specimens

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We evaluated a tri-gas incubator for Campylobacter isolation to be used instead of an anaerobic jar. Fecal specimens were cultured in duplicate onto charcoal selective medium and incubated at 43°C for 48 h in two different environments: a tri-gas incubator (Forma Scientific) adjusted to provide an atmosphere of 10% CO2, 10% O2, and the balance N2; and evacuated anaerobic jars with a replacement gas mixture of 10% CO2, 5% O2, and 85% N2. A total of 106 Campylobacter jejuni and 8 Campylobacter coli isolates were obtained from 2,348 stool specimens. Of the positive specimens, 113 isolates came from the incubator and 111 isolates came from the anaerobic jars. An additional 32 previously positive specimens were replated onto charcoal selective medium and restested by both methods. We recovered 27 C. jejuni isolates, 26 isolates by each method. The isolates from the incubator typically produced discrete colonies, while the isolates from the anaerobic jar showed some degree of swarming in colony formation. The tri-gas incubator provided a cost-effective method for culturing Campylobacter spp.

Culturing for Campylobacter spp. has always been cumbersome. In fact, until it was apparent that an appropriate microaerophilic atmosphere could be provided by a candle jar or by an anaerobic jar evacuated and refilled with mixed gases, Campylobacter culture simply could not be performed in routine diagnostic laboratories.

Since 1978, routine culture for Campylobacter spp. has become increasingly available in diagnostic medical laboratories. As a result, it is now recognized that in most areas of the world, Campylobacter spp. rival Salmonella spp. as the most common enteric bacterial pathogens.

This study takes an important step toward making Campylobacter culture as simple to perform as other routine culture procedures. We accomplished this using a tri-gas incubator and a relatively new selective medium, charcoal selective medium (CSM) (3).

This paper was presented in part at the 89th Annual Meeting of the American Society for Microbiology, New Orleans, La., 14 to 18 May 1989 (J. S. Thompson, D. S. Hodge, D. E. Smith, and Y. Au Yong, Abstr. 89th Annu. Meet. Am. Soc. Microbiol. 1989, C233, p. 432.)

Fecal specimens (n = 2,348), received in Cary-Blair transport medium for routine enteric culture, were batched and then cultured in duplicate on CSM and immediately incubated for 48 h at 43°C. One set of inoculated media was incubated in anaerobic jars which were evacuated and in which the atmosphere was replaced by a commercial gas mixture of 10% CO2, 5% O2, and 85% N2. The second set of media was incubated in a tri-gas incubator (Forma Scientific, Div. of Mallinckrodt, Inc., Marietta, Ohio). The incubator was set to provide an atmosphere of 10% CO2 and 10% O2. The gas content was confirmed by using two Fyrite gas analyzers containing either CO2- or O2-testing fluids (model 10-5001; Baccarach, Inc., Pittsburgh, Pa.). We selected 10% O2 on the basis of studies by Bolton and Coates showing this to be the optimum oxygen content for culturing Campylobacter jejuni (1). With this setting for the incubator, less N2 gas was required to displace the atmospheric O2 than would have been required to obtain an atmosphere of 5% O2.

We cultured 2,348 fecal specimens in parallel, from which we obtained 106 C. jejuni and 8 Campylobacter coli isolates for an isolation rate of 4.9%. Of the 114 positive specimens, 113 were isolated by the incubator method and 111 were isolated by the anaerobic jar method. We also cultured an additional 32 previously positive specimens and recovered 27 C. jejuni isolates, 26 isolates by each method. The identification of Campylobacter isolates was confirmed by microscopic appearance; catalase and oxidase tests; hippurate hydrolysis; growth at 25, 37, and 43°C; resistance to cephalothin; and susceptibility to nalidixic acid (4, 5).

Colonial characteristics of the Campylobacter isolates were somewhat different by each method, with isolates from the anaerobic jars showing some degree of swarming, which has been an important characteristic for recognition of Campylobacter spp. in many laboratories. Isolates from the incubator invariably produced discrete colonies because the humidity in the incubator was lower than that in the smaller anaerobic jar. The appearance of discrete colonies provided the opportunity to recognize mixed infections with two Campylobacter species. Although not as a part of this study, our laboratories have isolated mixed growths of C. jejuni and C. coli and of C. jejuni and Campylobacter lari dis from single specimens.

The use of the tri-gas incubator obviates the requirement for anaerobic jars with attendant possibilities of jar failure. Cultures can be examined at 24, 48, and 72 h in the incubator without an increase in gas usage. A laboratory could culture

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up to 40,000 specimens annually, approximately 150/day, in one incubator. Opening the incubator three times daily, 6 days/week, requires changing the CO₂ tank every 6 to 8 weeks and changing the N₂ tank weekly. Although absolute costs vary with labor and material costs, we found this method cost-effective in providing an appropriate microaerophilic atmosphere for culturing Campylobacter spp. Over a 2-year period, taking into account the cost of gas, the technical and materials cost of culturing control organisms, and the technical time required to set up anaerobic jars, we found the cost of providing the atmosphere for culture for 17,000 specimens to be less than the cost of the evacuation and replacement method and approximately one-half the cost of using gas generator envelopes. Although we restricted the opening of the incubator to three times daily during the study, we have not adhered to that guideline in 2 years of routine use subsequent to the study. We have continued to encourage batching of procedures to keep the number of openings of the incubator to a minimum; however, no increase in gas costs has been evident over this period.

The comparison served also to highlight the efficiency of CSM as a selective medium. More than 84% of specimens cultured showed no growth. Very few organisms other than Campylobacter spp. grew on the medium, making it very easy to screen plates. Gun-Munro et al. (2) and Karmali et al. (3) have already shown that growth of normal fecal flora from specimens is significantly less on CSM than on Skirrow medium. One of these studies showed a 75% reduction of fecal flora in 57% of specimens (n = 1,587), while the other showed no growth for 57% of specimens tested (n = 1,227) for plates incubated for 48 h.

In summary, the use of a tri-gas incubator was found to be a cost-effective, efficient method for isolation of C. jejuni and C. coli as well as an attractive labor-saving maneuver.

LITERATURE CITED