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# Culture and isolation of Campylobacter species by the Linearcount 3MA system

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**Abstract**

A novel system for the culture and isolation of the micro-aerophilic *Campylobacter* species from foods and the environment is described. It consists of a plastic plate containing some linear wells filled with selective media, where a micro-aerophilic atmosphere can be developed. In this kit, called Linearcount 3MA, various *Campylobacter* species grew well, producing normal-size colonies in 48 h.


*Keywords:* *Campylobacter*; Microaerophilic bacteria; Culture media; Selective media

The thermophilic species of *Campylobacter* are one of the most important bacterial causes of acute diarrhoeal disease in humans [1]. Studies by the WHO have included them among emerging food-borne pathogens; in fact, their incidence in European countries almost doubled between 1985 and 1998 [2]. The most commonly isolated species are *Campylobacter jejuni, C. coli, C. lari* and *C. upsaliensis*, with *C. jejuni* accounting for as many as 95% of all human *Campylobacter* infections [3]. The standard technique for the isolation of *Campylobacter* spp. from either clinical, environmental or food samples is plating on solid media. The aim of the present study is to evaluate a novel system for the isolation of *Campylobacter* spp., in comparison with another commercial standard method.

Several *Campylobacter* strains isolated from clinical and environmental samples and the *C. jejuni* strain ATCC 33291 were used in this study. Strains of *Pseudomonas aeruginosa* (ATCC 10145), *Staphylococcus aureus* (ATCC25932) and *Escherichia coli* (ATCC25922), were used as negative controls. Other species tested were *Helicobacter fennelliae* and *H. cinaedi*.

The Linearcount is a patented diagnostic kit produced by the Microbiol Company (Macchiareddu, Cagliari, Italy), which was originally devised for urineculture and urine microbial counts [4]. The novel Linearcount 3 micro-aero kit (Linearcount 3MA) consists of a transparent plastic plate with 3 linear wells, each containing different culture media (Fig. 1). The new kit is prepared to meet the requirements of *Campylobacter* or other micro-aerophilic species. In the plastic plate there is a linear well, where a hydrosoluble capsule allows the formation of an incubation chamber with a reduced oxygen content (5–7%) and an increased $CO_2$ content (about 8–10%). After seeding the samples, the linear wells are sealed with a transparent plastic strip that adheres to the top of the wells and closes them hermetically. The wells are cultured by standard methods, using 10 μl plastic loops and the plates are protected with a transparent plastic cover.

Either non-selective ( blood agar) or selective media for *Campylobacter*, such as Skirrow agar and Karmali agar [5] were used for culturing the *Campylobacter* strains. All the media, either in Petri dishes or Linearcount plates, were purchased from the Microbiol

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Fig. 1. Representation of the Linearcount 3MA system for *Campylobacter* isolation. The samples are cultured in the linear wells, which contained MacConkey agar, Skirrow and Karmali media; the $CO_2$ generating capsules are dissolved into the reaction chambers and the wells are sealed with the sealing strips; the wells are then protected with the transparent plastic cover and incubated at 37°C for 48 h in a normal-atmosphere incubator.

Company; the culture media were already present in the plates.The Petri dish plates, prepared with standard procedures, were incubated in plastic jars (GENbox microaer system—Biomèrieux) with standard micro-aerophilic conditions [6]. Linearcount plates were seeded and processed according to the manufacturer's instructions. All the collection and clinical strains were suspended in a sterile saline solution with about $10^4$–$10^5$ bacteria/ml and plated in both Petri dishes and Linearcount 3MA. The overall microbial growth in the two systems was checked after 2–3 days and the rate of colony growth and colony size was measured.

All the media tested were almost equally efficient in supporting the growth of the *Campylobacter* colonies, but the Karmali agar appeared to efficiently fit most *Campylobacter* requirements. The overall growth, the rate of growth and the final size of the colonies was similar. By streaking the sample in the linear wells, a dilution factor sufficient to obtain isolated colonies was produced. Even with samples containing up to $10^9$ bacteria per ml (in both pure or mixed cultures), it was possible to obtain clear visible and isolated colonies. Furthermore, the high selective properties of some of the media employed, allowed a clear detection of *Campylobacter* strains even in mixed and overcrowded bacterial cultures, with the possibility of isolating and subculturing the selected colonies for further analyses. Table 1 reports the mean colony size of *Campylobacter* strains grown in Karmali medium in either the Linearcount 3MA or the standard system, with Petri dishes and a jar. All the strains were examined at least 3 times (but even more if necessary). In each culture the diameter of 2 colonies per strain were measured. *C. jejuni* strains show about the same growth rate in Petri dishes and Linearcount 3MA, with a mean colony diameter of $1.4 \pm 0.8$ and $1.5 \pm 0.7$ mm, respectively. Some interesting, but not significant, differences were observed for the species *C. coli*, which appeared to produce greater colonies when grown in the Linearcount 3MA system with a mean of $1.9 \pm 0.9$ mm in comparison to the $1.5 \pm 0.4$ mm of Petri plates. Conversely, minor *Campylobacter* species (*C. lari* and *C. upsaliensis*) were associated with a mean colony size of $1.5 \pm 0.7$ and $1.3 \pm 0.6$ mm on Petri plates and Linearcount, respectively. None of the control species of bacteria grew in the Karmali well of the Linearcount plate even at a concentration of up to $10^7$ bacteria/ml. The Helicobacter strains grew fairly well with both systems, although the colony size appeared a little larger in the Petri dishes than in the Linearcount.

The Linearcount 3MA system is an integrated kit for the selective culture and isolation of Campylobacter species in environmental and food materials. It proved to be at least as reliable as standard Petri dish culture methods in supporting the growth of these bacteria. The real advantage of this novel system is that it allows the growth of aerobic and micro-aerophilic bacteria in different selective media on the same plate, rendering the technical work easier and more practical. It is also convenient for space utilization and culture plate handling since it avoids the use of a jar. Furthermore, it allows the plates to be checked one by one, since each

Table 1
Comparison of *Campylobacter* growth in the Linearcount 3MA and in Petri dishes with the Karmali medium

| Species (number of strains) | Petri dishes[a,b] Mean colony diameter (mm ± SD) | Linearcount 3MA[a] Mean colony diameter (mm ± SD) |
|---|---|---|
| *Campylobacter jejuni* (6) | 1.4 ± 0.8 | 1.5 ± 0.7 |
| *C. coli* (6) | 1.5 ± 0.4 | 1.9 ± 0.9 |
| *Campylobacter* sp.[c] (3) | 1.5 ± 0.7 | 1.3 ± 0.6 |
| *Helicobacter* sp. (3) | 1.6 ± 0.6 | 1.4 ± 0.3 |

[a] With Karmali medium after 48 h of incubation at 37°C.
[b] With a GENbox microaer system.
[c] *Campylobacter* sp.: 1 strain of *C. lari* and 2 strains of *C. upsaliensis* were tested.

culture plate is independent from the others. As regards the cost of the present analysis system, it is lower than the cost of the single plates used for the standard method; costs of around 3.5 euros for the Linearcount 3MA and around 6 euros for the standard method can be estimated [6]. Using other selective media this system could also be suitable for the growth of other microaerophilic bacteria, such as *Gardnerella*, *Neisseriae* and *Haemophilus* species; this possibility deserves further extensive investigation . The use of Linearcount 3MA was shown to be an optimal tool for culturing and growing the majority of C*ampylobacter* species usually found in food and environmental specimens; however, further studies are needed to adapt this method for detecting *Campylobacter* on clinical specimens.

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