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Media for *Campylobacter jejuni* and other campylobacters

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Despite their recent elaboration and the many variations in antibiotic combinations designed to attain selectivity, highly selective liquid and solid culture media for *Campylobacter jejuni* have proved satisfactory provided they are incubated at about 42°C and in a microaerophilic atmosphere such as a candle jar. Two reservations apply: (i) cephalothin should not be used when *C. fetus* is to be isolated as well as *C. jejuni*; and (ii) typical colonies obtained on selective isolation media should always be checked for failure to grow on blood agar slants in air.

Key words: *Campylobacter jejuni*; *Campylobacter fetus*, Candle jar; Cephalothin

Taxonomic essentials

The examination of foods for Enterobacteriaceae, *Staphylococcus aureus*, Lancefield group D streptococci, moulds, yeasts, etc. has been carried out for over 50 years and considerable experience has consequently been obtained. On the other hand, definite identification of the genus *Campylobacter* as an enteric pathogen of man dates back only to about 1970. Foods have been examined for these organisms for no more than ten years. Nonetheless the procedures adopted from clinical microbiology have been found to work surprisingly well on foods. This does not detract from the fact that here, as in all selective examinations of foods, the Golden Rule applies that the identity of all isolates should be confirmed by verifying the pertinent taxonomic characteristics.

It is generally accepted that of the compylobacters only *C. jejuni* is an enteropathogen transmitted by foods (Butzler, 1984), a situation similar to the yersiniae, where *Yersinia enterocolitica* seems to be the species of major concern. However, some evidence has been obtained that *C. fetus* may not always be innocuous in the context of foods (Harvey and Greenwood, 1983). It is therefore wise not to overlook the fact that the use of cephalothin, which inhibits *C. fetus* in selective media for campylobacters, may lead to a serious isolation bias.

Recommended media

Four combinations of antibiotics have been suggested for inclusion in blood agar to attain a medium that is remarkably selective for campylobacters (Morris et al., 1982). These are the mixtures suggested by Skirrow (Skirrow and Benjamin, 1980), Butzler (Vanhoof et al., 1978; Butzler, 1984), Blaser et al., 1980 and Wang et al., 1982 and more recently by Butzler's team (Butzler et al., 1983; Goossens et al., 1983). Their composition is given in Table I. There are no essential differences in performance between these combinations and it may be assumed that clinicians will soon reach agreement on the most useful one to the benefit of food microbiologists.

The basal medium need not necessarily be blood agar. Blood-free media, containing reducing agents such as ferrous sulphate and sodium pyruvate, also function quite well (George et al., 1978; Mossel et al., 1983). It is of course essential to incubate media under microaerophilic conditions (Hoffman et al., 1979) and candle jars serve the purpose well (Luechtefeld et al., 1982; Mossel et al., 1983).

The minimal infectious dose for *C. jejuni* is low (Robinson, 1981), hence there is a need for enrichment media for this organism. The essential elements of the solid media, a reducing agent and an appropriate combination of antibiotics, will also lead to a successful enrichment medium (Patton et al., 1981; Chan and Mackenzie, 1982; Rothenberg et al., 1984; Hill and Grimes, 1984; Ribeiro and Price, 1984). Incubation at 42°C will increase selectivity, although it will not suppress growth of *Ps. aeruginosa*, since this is also a thermotrophic bacterium. Therefore it is necessary to verify that typical colonies obtained on isolation media will indeed not be *Ps.*

Table I

Antibiotics suggested by various authors for use as selective agents in liquid and solid media for the detection or enumeration of *Campylobacter* spp. in food or water.

Antibiotic		Author's name under which medium is generally known (historic order of description)			
Generic name	Unit in which concentration is expressed	Skirrow	Butzler	Blaser-Wang	Goossens
Amphotericin B	mg l^{-1}	–	–	2	2
Bacitracin	U l^{-1}	–	25000	–	–
Cephalothin	mg l^{-1}	–	–	15	–
Cephazolin sodium	mg l^{-1}	–	15	–	–
Cephoperazon	mg l^{-1}	–	–	–	15
Colistin sulphate	U l^{-1}	–	10000	–	10000
Cycloheximide	mg l^{-1}	–	50	–	–
Novobiocin	mg l^{-1}	–	5	–	–
Polymyxin B	IU l^{-1}	2500	–	2500	–
Rifampicin	mg l^{-1}	–	–	–	10
Trimethoprim	mg l^{-1}	5	–	5	–
Vancomycin	mg l^{-1}	10	–	10	–

aeroginosa but *Campylobacter* by sustantiating their failure to grow on blood agar slants incubated aerobically at 37°C.

It was thought previously that the *Campylobacter* isolation media in current use would also quantitatively recover sublethally stressed populations. Though this has been substantiated for heat injury (Palumbo, 1984) it has been demonstrated since that this certainly does not apply to cold stress (Ray and Johnson, 1984). Moreover, the use of a non-protective diluent could adversely affect colony counts of stressed cells (Abram and Potter, 1985). Consequently, for the assessment of numbers of colony forming units of *Campylobacter* species the same precautions must be taken with respect to resuscitation as with the other Gram-negative rod-shaped bacteria of significance in foods (Mossel and van Netten, 1984).

Monitoring procedures

The procedures used for the monitoring (Mossel, 1982) of liquid and solid selective media for *C. jejuni* are similar to those used for testing Enterobacteriaceae media (cf. Mossel, 1985).

Clearly the test strains are somewhat different and should include (i) four different serotypes of *C. jejuni* and one of *C. fetus*; (ii) *Escherichia coli*, *Salmonella typhimurium*, *Staph. aureus*, *Streptococcus faecalis* and *Bacillus cereus*. Incubation is at 42°C under microaerophilic conditions for 24 to 48 h.

The target values indicated for Enterobacteriaceae, both with respect to colony counting, ecometric monitoring and dilution-to-extinction counts (Mossel, 1985), apply also to solid and liquid *Campylobacter* media.

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