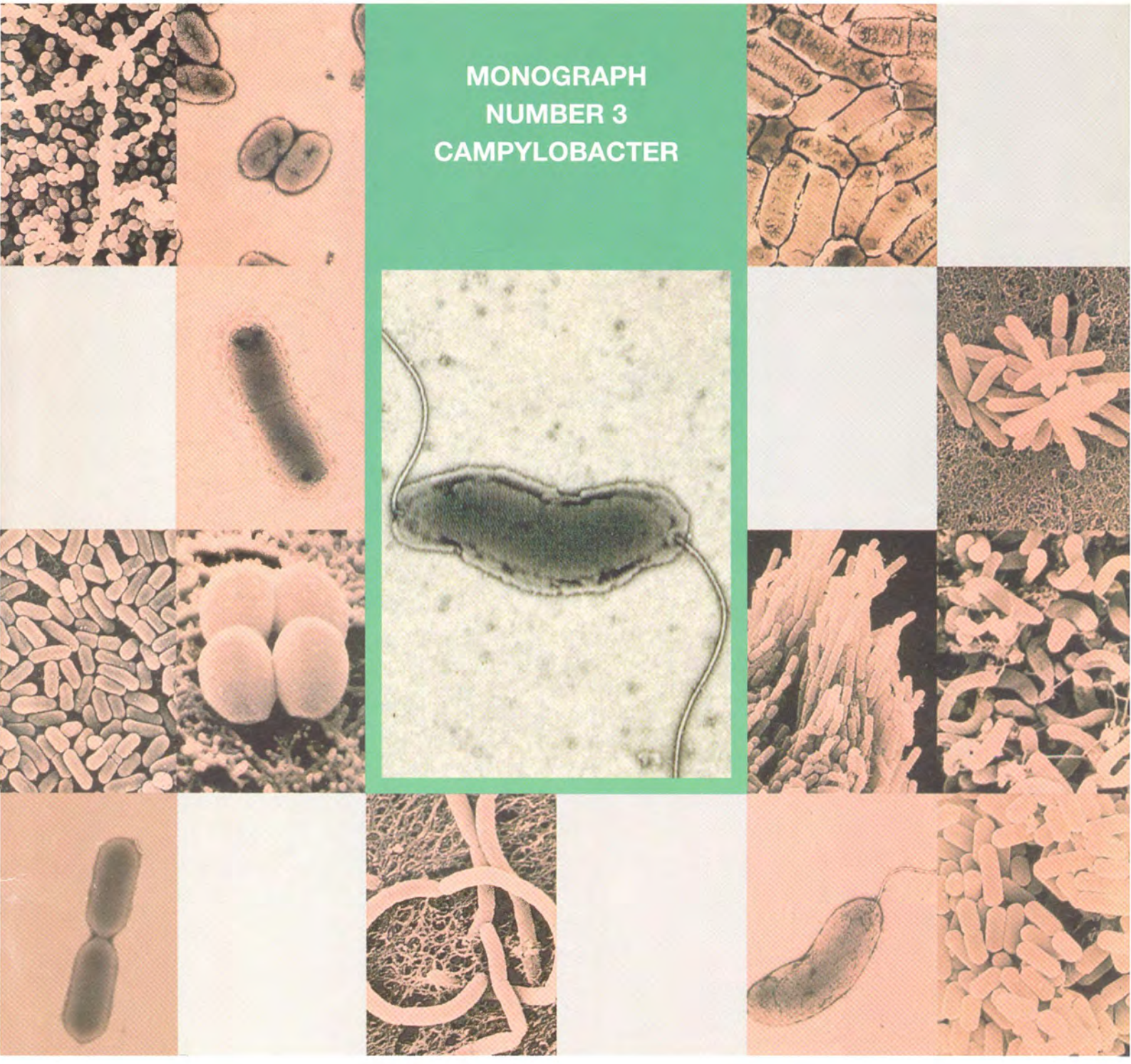
Food-borne Pathogens



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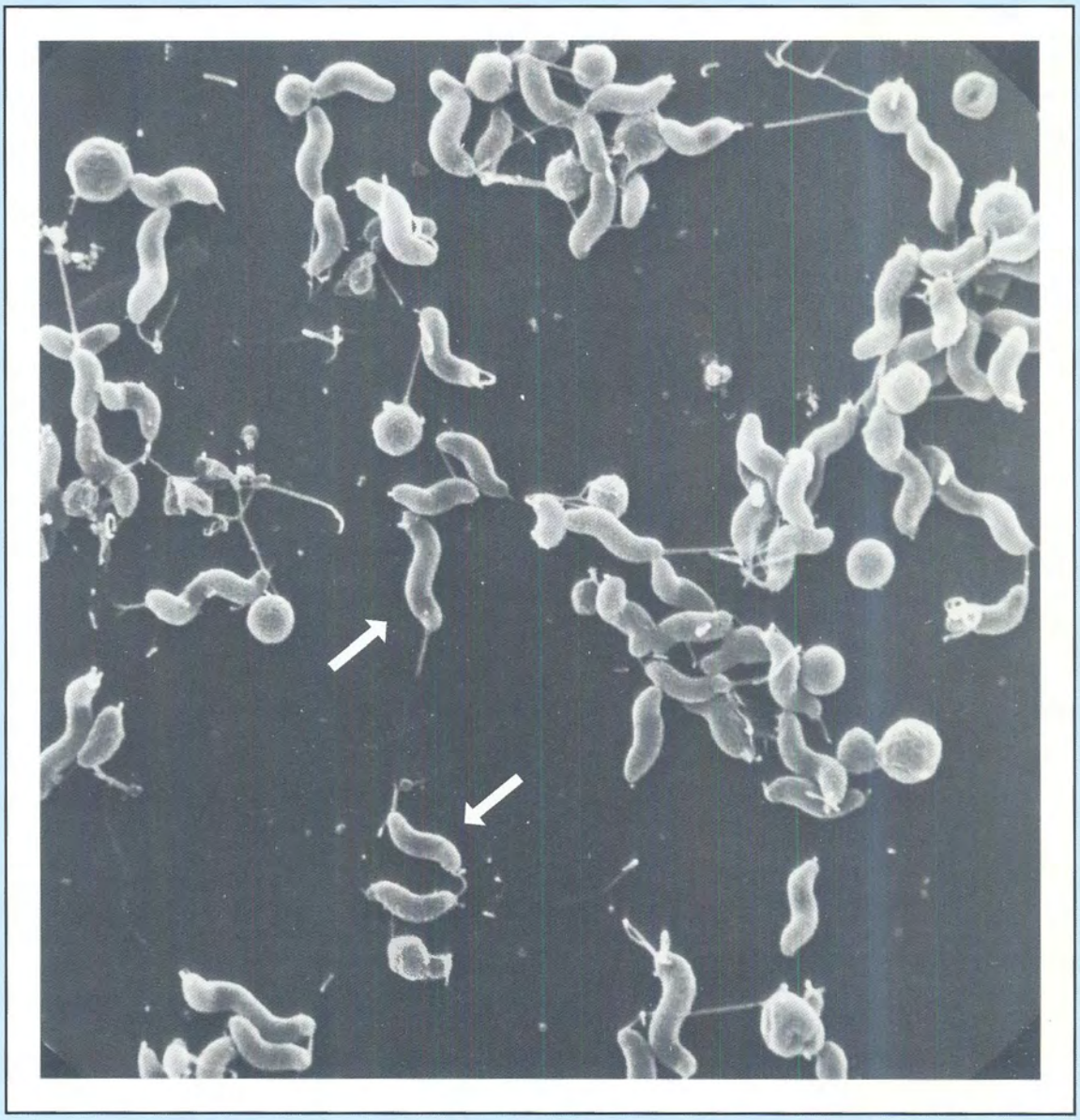

Electron micrograph of Campylobacter showing the characteristic spiral shape. Note the single polar flagellum at one, or both, ends of the cell (arrowed).

Coccoid forms are also present.

Photograph kindly supplied by Professor Diane Newell, Central Veterinary Laboratory, Weybridge, U.K.

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Acknowledgements

Except where otherwise acknowledged, the photographs were produced by Mr Eric Griffin, formerly of the Department of Microbiology, Royal Hampshire County Hospital, Winchester U.K. My thanks to him and the laboratory staff for their assistance.

I am grateful to Mr David Wareing of Preston Public Health Laboratory, U.K. for his assistance and sharing his considerable knowledge of techniques for identifying campylobacters.

My thanks to my secretary Mrs Lynn Hinchliffe for her hard work and seemingly inexhaustible patience.

Introduction

McFadean and Stockman in 1913[1], and Levy in 1946[2], published what are generally assumed to be the earliest descriptions of Campylobacter when they observed spiral curved bacteria in diseased animals and humans.

King, in 1957[3], is thought to have been the first to associate "related vibrios" with diarrhoea in children. In fact, it seems that this association had been made many years earlier by Escherich[4] who, in 1886, described spiral bacteria which he saw in the large intestines of 16 out of 17 children who had died of diarrhoeal disease. Interestingly, he also observed similar organisms in kittens which, too, had died after the onset of diarrhoea. Ehrlich went on to make further observations of spiral organisms in more cases but formed the opinion that their role was prognostic rather than causal. Studies were limited by inability to culture the organisms.

Over the years there were a number of reports linking the presence of spiral bacteria with disease and eventually Sebald and Veron[5] established that the microaerophilic vibrios fundamentally differed from the classical cholera and halophilic groups and proposed the genus name *Campylobacter*.

Veterinarians had known the type species *Campylobacter fetus* (*Vibrio fetus*) for many years as a cause of infectious abortion in cattle and sheep. Other similar organisms were recognised to be associated with disease in domestic animals including enteritis of calves and pigs.

King, in her in-depth study of strains isolated from human patients in whom the most prominent symptom was diarrhoea, found that amongst them were strains that formed a distinct group that grew best at about 42°C. These organisms were isolated from blood; attempts to culture them from faeces were unsuccessful because of overgrowth by contaminants.

The problem of overgrowth by accompanying organisms that prevented isolation from faeces was overcome about 10 years later by Butzler and his co-workers[6]. Their technique used the principle that campylobacters, being small, can pass through a filter that holds back larger organisms. This was a considerable advance and was very successful but the length of time and the manipulation required made the technique impractical for use in routine clinical microbiology. A more effective selective medium was clearly desirable and when one was formulated the filtration process was abandoned. However, methods using filters continue to be used for specialist applications, particularly where existing selective media are inhibitory to more recently discovered species.

Awareness of the apparent role of Campylobacter in human disease was heightened when Skirrow described Campylobacter enteritis as a "new" disease in his 1977 report published in the *British Medical Journal*[7]. In this paper Skirrow draws attention to the suggestion by King that chickens might be the primary source of infection. Skirrow's preliminary results suggested that chicken flocks may commonly harbour Campylobacter, a situation now generally known to be true. The results pointed to the possibility of food being a vehicle of infection.

Another area that brought significant advances was the general recognition that *Campylobacter species* need an oxygen-reduced (microaerobic) atmosphere for growth. The way was now clear for much more work to be done to clarify the role of Campylobacter in enteritis and the place of foods in its transmission.

The adoption of Campylobacter culture in laboratory routines for investigating enteritis has shown *Campylobacter spp.* to be the leading cause of diarrhoeal disease, the number of cases in Britain exceeding those caused by Salmonella. There are still unanswered questions about virulence and epidemiology, but the association of infection with consumption of contaminated water and foods, particularly poultry and unpasteurised milk, is clearly established.

The social and financial costs of Campylobacter infection are enormous. As an example, health officials estimate that more than 2 million cases occur annually in the United States. Typically, symptoms are unpleasant but not life threatening. However, deaths do sometimes occur, most often amongst the elderly or those with underlying disease. The costs of poultry-associated disease alone have been estimated at between 350 million and 700 million U.S. dollars annually.

A great number of culture media have evolved in response to the need to optimise performance. Most of the media were developed for clinical microbiology. The different needs of food microbiologists have necessitated the development of more appropriate formulae. This is particularly noticeable in the field of enrichment culture which generally is still not carried out when examining clinical specimens. This publication will describe a number of media and, where possible, offer guidance on their use. Because of the predominance of *Campylobacter jejuni* in enteric infection most developments in media have been directed towards detection of this pathogen. Other species may possess different antibiotic susceptibilities and current media formulations may not always be optimal for them. For this reason the true incidence of infection by different *Campylobacter spp.* may be understated. It seems likely that new culture media will be developed in future to resolve this question.

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2 Levy, A.J. (1946) A gastroenteritis outbreak probably due to a bovine strain of vibrio. *Yale Journal of Biology and Medicine* **18,** 243-258.

3 King, E.O. (1957) Human Infections with *Vibrio fetus* and a closely related vibrio. *J. Inf. Dis.* **101,** 119-129.

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The Occurrence of Campylobacter in Foods

Campylobacter food poisoning outbreaks occur either sporadically, effecting individuals and small groups such as families, or as larger community outbreaks. In large outbreaks a cause may generally be determined but identification of the infective vehicle in sporadic cases is often much less successful. *Campylobacter jejuni* is generally the most common cause of human enteritis but *C. coli* and, rarely, *C. lari* may also be responsible. *Campylobacter spp.* are present in the intestinal tract of a variety of wild and domestic animals. *C. jejuni* commonly occurs in chicken and bovines, although cattle meat is not nearly as important in transmission as poultry.

C. coli is commonly carried by pigs, but serological studies have shown differences between isolates from pigs and humans indicating that pigs do not appear to be a major source of infection. However, in some countries where large quantities of pork are consumed *C. coli* infection frequently occurs.

C. lari is an uncommon cause of human enteritis. A high proportion of apparently healthy seagulls harbour this species. Isolates have been recovered from asymptomatic humans; however, illness has been reported amongst persons who drank untreated water in areas frequented by gulls.

Under-cooked poultry, in particular chicken, is an important source of Campylobacter infection in cases where a direct food link is established. Cross-contamination from raw poultry to foods which are not cooked before eating is a cause of sporadic cases.

Red meats and meat products may sometimes be implicated. However, their importance is probably secondary to that of poultry.

Milk has frequently proved to be the responsible vehicle in outbreaks ranging in size from incidents involving family groups to several hundreds of persons. Bovine mastitis due to campylobacters has been reported but faecal contamination during milking is a more likely source of the organisms. Most outbreaks have occurred after consumption of raw milk but improperly pasteurised milk, or milk that has become contaminated following pasteurisation, has been implicated. Contamination by birds pecking through milk bottle tops is increasingly thought to be a significant hazard. Infection is associated with liquid milk, not dairy products. Fermented products are unlikely to be a risk because Campylobacter is sensitive to lactic acid.

Various other foods, including seafoods, have been shown to be the source of infection. Seafoods probably acquire Campylobacter from sewage-contaminated water in the rearing beds.

Salads have been implicated in at least one outbreak but probably do not represent an inherent risk.

Film-wrapped fresh mushrooms have been implicated epidemiologically.

Contaminated water has been clearly demonstrated as a source of infection. Both large outbreaks and sporadic cases have been attributed to untreated water and contaminated drinking water.

Campylobacter spp. are readily destroyed by temperatures used in pasteurisation and cooking. They may survive for several weeks in a moist environment but quickly die in dry conditions particularly at room temperature. Acidic conditions rapidly destroy them and they show no unusual resistance to disinfectants.

The ability of *Campylobacter spp.* to remain viable in frozen foods varies considerably and freezing cannot be relied upon to ensure food safety.

Fortunately, *Campylobacter spp.* differ from other food poisoning organisms in not multiplying in the contaminated foods.

When assessing the likelihood of Campylobacter infection from foods the comparative sensitivity of the organisms to the usual environmental influences must be balanced against the low infective dose. This is as low as 500 organisms making the risk from even lightly-contaminated foods one that demands attention. Culture media specified by some National bodies are named in Table 1. Procedures specified by various authorities for the examination of foods for Campylobacter are given in the following pages (Tables 2–7).

Reference

Griffiths, P.L. and Park, R.W.A. (1990) *J. Appl. Bact.* **69,** 281-301.

TABLE 1 – Culture media specified by some national bodies for detection of Campylobacter in foods
Oxoid products that may be used are given in parentheses

Country	Organisation responsible	Culture media: enrichment	Culture media: plating	Other media specified in procedure
Australia	Standards Australia Committee FT/4 Food Microbiology	Preston broth (CM67 + SR117 + SR48 + SR84)	(1) Preston agar (CM689 + SR48 + SR117) (2) Skirrow's agar (CM271 + SR48 + SR69)	Nutrient agar Nutrient broth
North America/ Canada	Food and Drug Administration (FDA) Bacteriological Analytical Manual (BAM) 1992	Hunt and Radle broth (BAM M29) (CM67 + L21 + SR84 + SR48) Add antibiotic formulae 1, 2 or 3 (See Table 3)	(1) Isolation agar A (CM739 + L21) (2) Isolation agar B (Campy-cefex)	Blood agar (CM271 + SR48 or SR50) Heart infusion agar Peptone diluent (L34) Brucella semi-solid medium Triple-sugar iron agar (CM277) MacConkey agar (CM115) Cary-Blair medium (CM519)
France	AFNOR: General Guidance for Detection Thermotolerant Campylobacter. Norme Francaise ISO/DIS 10272	(1) Preston broth (CM67 + SR117 + SR48 + SR84) (2) Park and Sanders broth	(1) Karmali agar (CM908 + SR139) (2) Skirrow agar (CM331 + SR48 + SR69) (3) Campylobacter blood-free agar (CM739 + SR155) (4) Preston agar (CM689 + SR48)	Brucella broth Columbia blood agar (CM331 + SR50) Mueller-Hinton blood agar (CM337 + SR51) Triple-sugar iron agar (CM277)
UK	MAFF/DoH Steering Group on the Microbiological Safety of Food	(1) Park and Sanders broth (2) Exeter broth	Campylobacter blood-free agar (CM739 + SR155) Exeter agar	
UK/International	BS 5763: ISO/DIS 10272 Methods for Microbiological Examination of Food and Animal Feeding Stuffs. Detection of Thermotolerant Campylobacters	(1) Preston broth (CM67 + SR117 + SR48 + SR84) (2) Park and Sanders broth	(1) Karmali agar (mandatory) (CM908 + SR139) (2) Skirrow agar (CM331 + SR48 + SR69) or Campylobacter (3) blood-free agar (CM739 + SR155) or (4) Preston agar (CM689 + SR48 + SR117) or (5) Butzler (Virion) agar (CM331 + Virion supplement)	Brucella broth Columbia blood agar (CM331 + SR50) Mueller-Hinton blood agar (CM337 + SR51) Triple-sugar iron agar (CM277)

TABLE 2 – General procedure for FDA BAM method for detecting *Campylobacter spp.* in foods.
The detailed procedures are complex. The Bacteriological Analytical Manual 1992 should be consulted for the complete methodology

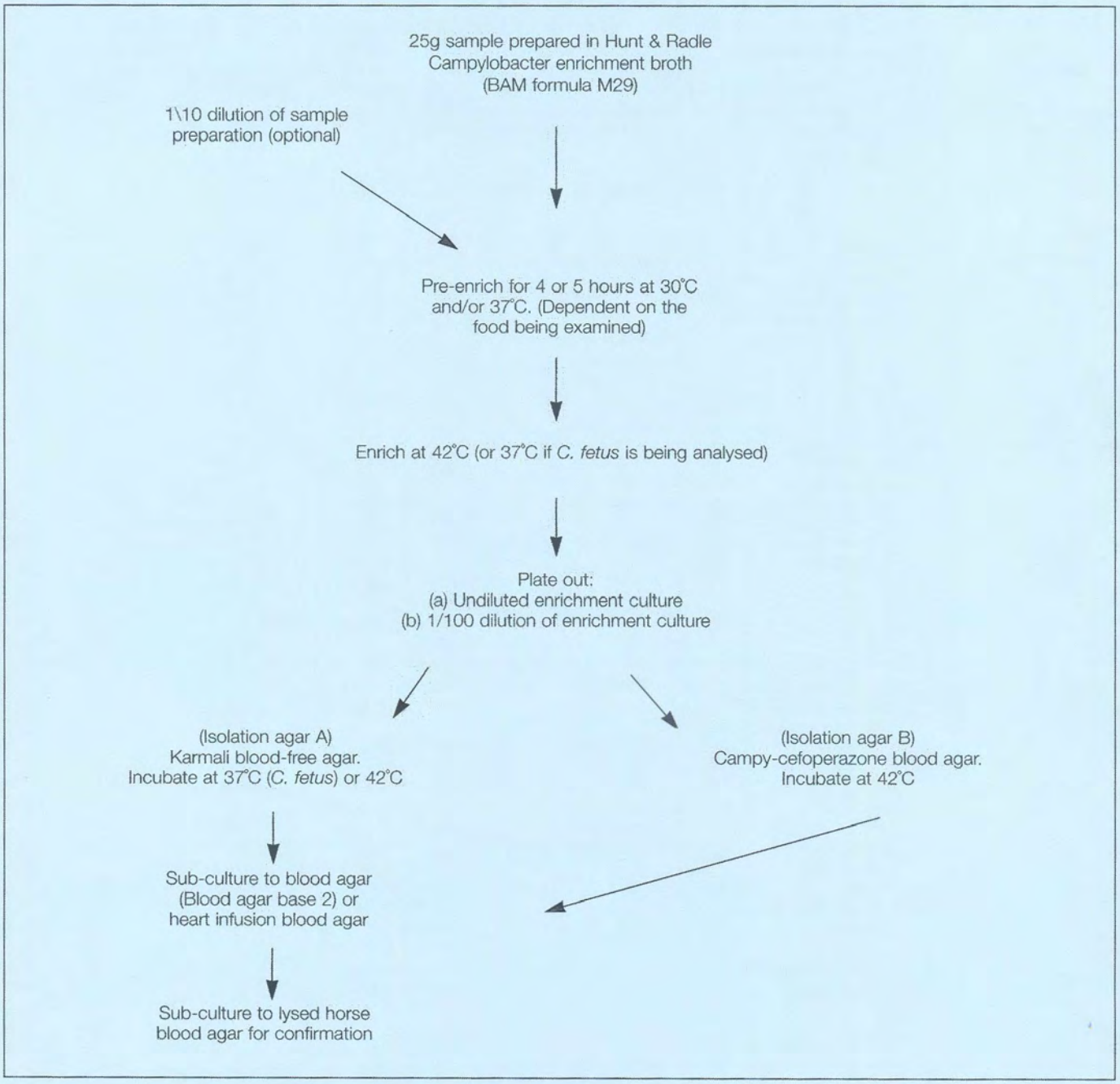


TABLE 3 – Selective agents used in FDA Bacteriological Analytical Manual methods for testing foods, environmental samples and dairy products (1992)

For foods
Antibiotic formula 1. (Modified Park formula)

		mg/litre
First addition:	Cefoperazone	15
	Trimethoprim	12.5
	Vancomycin	10.0
	Cycloheximide	100.0
Second addition:	Cefoperazone	15

For water and environmental swabs
Antibiotic formula 2. (Modified Humphrey formula)

	mg/litre
Cefoperazone	15
Trimethoprim	12.5
Vancomycin	10.0
Cycloheximide	100.0

For dairy products
Antibiotic formula 3. (Modified Preston formula)

	mg/litre
Rifampicin	10
Cefoperazone	15
Trimethoprim lactate	12.5
Cycloheximide	100

TABLE 4 – Australian standard method for detection of Campylobacter in foods

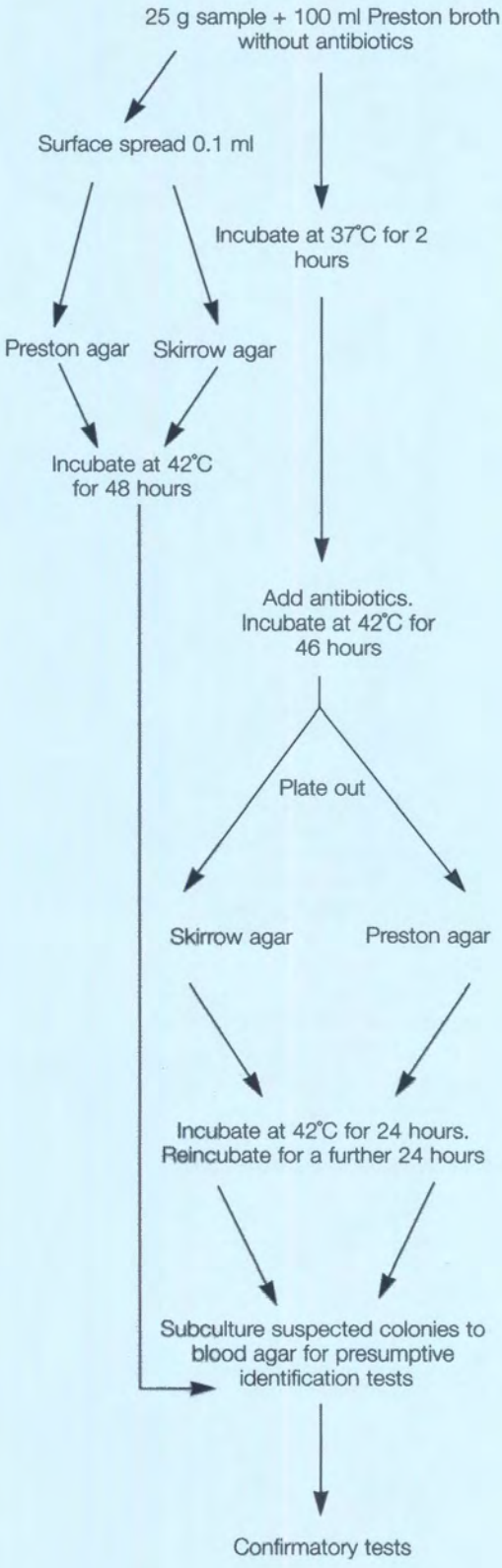


TABLE 5 – General guidance for detection of thermotolerant Campylobacter. Norme Française. ISO/DIS 10272

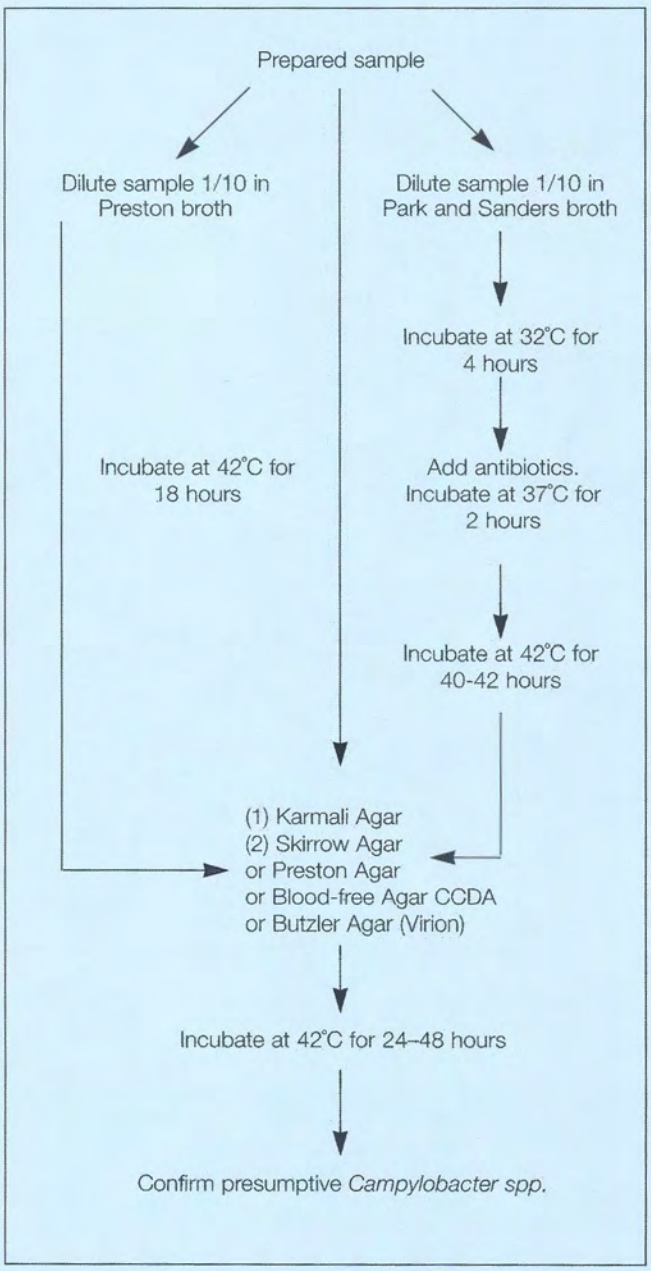


TABLE 6 – UK MAFF/DoH Steering Group on the microbiological safety of food. Validated procedure. Detection of *Campylobacter species:* Exeter method

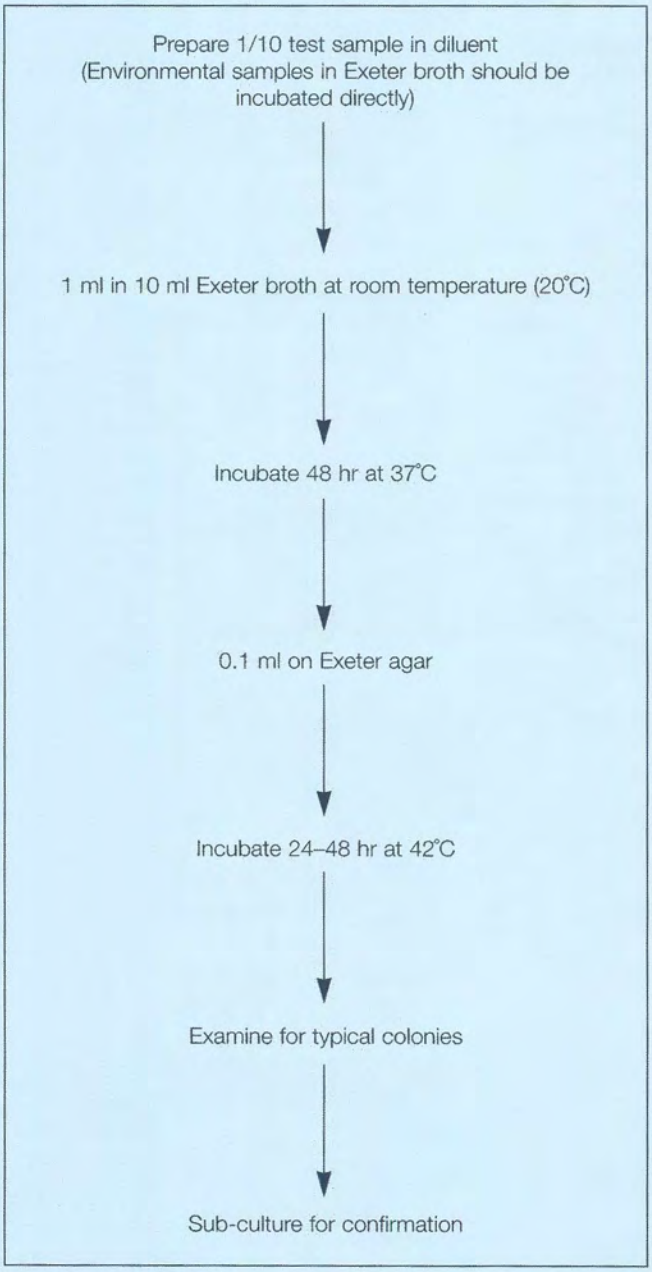
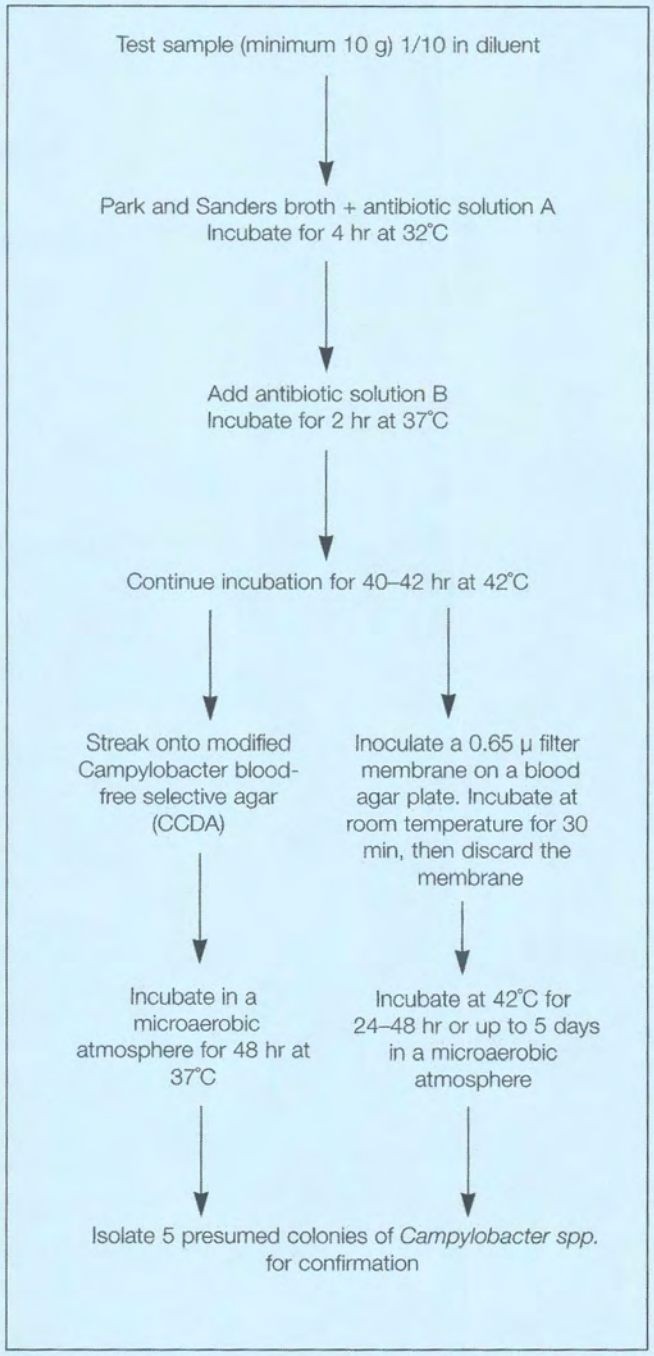


TABLE 7 – UK MAFF/DoH Steering Group on the microbiological safety of food. Validated procedure: Detection of *Campylobacter species:* Park and Sanders method



Enrichment Media

In common with other bacteria important in food-borne disease, *Campylobacter* cells can incur injury from food processing and preservation procedures, making them susceptible to selective agents which are tolerated by undamaged cells. In order to avoid false-negative results when examining food, it is necessary to use culture techniques that minimise the effect of injury and increase the numbers of cells available for culture. This is done by resuscitating the cells initially and then encouraging them to multiply in broth culture using techniques that enable cell repair and growth to occur. Resuscitation can be accomplished by withholding exposure to the selective agents for a period and, during this period, incubating the cultures at temperatures optimal for recovery. After suitable times and temperatures the selective agents are added and the temperature of incubation may then be adjusted to levels that increase the selective pressures on competing organisms. Further advantage may be gained by making additions to the medium such as ferrous sulphate, sodium metabisulphite and sodium pyruvate (FBP). These serve to quench toxic compounds that may have formed in the culture medium and increase the aerotolerance of the culture.

Early media formulated for *Campylobacter spp.* were agar media intended for direct isolation from faecal specimens in which the numbers of Campylobacter cells were generally very considerable. When emphasis began to be placed on detecting sources of infection, attempts were made to modify some of these formulae to create broth versions for use in enrichment culture of food and environmental samples. Problems of inhibitory activity towards campylobacters themselves and inadequate selectivity quickly became apparent. Enrichment broths, e.g. Preston broth, designed for direct exposure of samples to the selective agents were developed, leading to an improvement in isolation capabilities. Further experience showed that increasing the sensitivity of Campylobacter enrichment media demanded modification to the testing methodology. The principles of delaying exposure to selective agents until cell repair has occurred, with use of lower temperatures in the early stages of enrichment are now well established in a number of methodologies (see Tables 2 and 4–7).

Continuing investigation has led to recognition of formulae that possess clear advantages when used in specified methods. Formulae of those in wider use are given on pages 14–15.

Oxoid products that can be used in their manufacture are named alongside.

The Evolution of Media and Methodology for Selective Enrichment of Campylobacter in Foods

The scientific interest in Campylobacter increased very rapidly following the demonstration that selective culture could be used to isolate these demanding organisms from a significant proportion of persons suffering with severe enteritis. The interest of food microbiologists soon matched that of their clinical colleagues when increasing evidence confirmed earlier suspicions that many cases occur following consumption of contaminated food. There was early recognition of the need for enrichment culture when examining food and environmental samples. The large number of culture media formulae reflects the initial urgency to conduct research, some of the researchers probably unaware of simultaneous developments by others. As can be expected, differences existed between some of these parallel approaches to the same needs. Later, developments entered an evolutionary phase, demonstrated by workers improving on their earlier efforts and by others responding to fresh challenges by making subsequent advances on work already published.

This review attempts to put some of the major ideas and advances in sequence to explain how methods for selective enrichment of Campylobacter have arrived at their current situation.

Skirrow's work[1] created intense interest within a very few weeks of publication. Tanner and Bullin[2] reported their search for *Campylobacter spp.* in which they used alkaline peptone water, pH 8.4, for selective enrichment from faeces. Alkaline peptone water was well known for enrichment of *Vibrio cholerae* and presumably was chosen because of similarities of the two genera. The incubation temperature of 43°C used to favour thermophilic campylobacters would have contributed to the selective pressures against accompanying organisms. The success of enrichment was clearly demonstrated because two strains would not have been detected if only direct culture on agar had been used.

Generally, however, the use of alkaline culture medium for campylobacter enrichment has not been developed.

Blaser *et al*[3] in a 1979 report of an investigation into clinical and epidemiological features of Campylobacter enteritis, recognised the microaerophilic nature of the genus in their choice of thioglycollate broth as the basis of an enrichment medium made selective by the addition of vancomycin, trimethoprim, polymyxin B and amphotericin B. This medium was named Campy-thio.

Selective enrichment applied to foods was described in 1981. Park, Stankiewicz, Lovett and Hunt[4] cultured nutrient broth washings of eviscerated whole chicken in brucella broth containing vancomycin, trimethoprim and polymyxin B (VTP). These are the antibiotics Skirrow used for his selective agar. The cultures were incubated under a continuous flow of an oxygen-depleted gas mixture. This enrichment system was reported to recover up to 0.2 cells per gram in the presence of 10^{-4} to 10^{-6} per gram of contaminating organisms.

In the same year Rosef[5] used a basal medium of peptone and meat and yeast extracts to which were added vancomycin, polymyxin B and trimethoprim. This medium was employed to determine the incidence of *Campylobacter jejuni* in the gall bladders of normal pigs at slaughter. Incubation was in a microaerobic atmosphere generated by using a gas generating envelope in an anaerobic jar with the catalyst absent. Resazurin was present in the medium as an Eh indicator. No figures were given for sensitivity but, following enrichment culture, 58% of pigs were shown to be carrying *C. jejuni*. Direct inoculation of the 50 samples on Skirrow selective agar did not yield one positive result, thus providing early confirmation of the value of enrichment culture.

A number of developments were reported in 1982. Christopher and co-workers[6] devised an enrichment broth by omitting agar from Campy-BAP plating medium. It contained cephalothin and amphotericin B in addition to vancomycin, polymyxin and trimethoprim. The possibility that the medium might possess toxic activity was addresed by incorporating pyruvate. Ehlers *et al*[7] further developed this aspect by adding ferrous sulphate and sodium metabisulphite to the pyruvate. This mixture of compounds has become familiar as FBP. The limit of detection in cheese was found to be about 0.3 Campylobacter cells per gram using a Most Probable Number (MPN) method. Acuff *et al*[8] devised a similar medium containing FBP but did not include blood.

Doyle and Roman[9] took a basal medium similar to that of Park *et al* and modified the selectivity by greatly increasing the quantity of polymyxin and adding cycloheximide. Succinate and cysteine hydrochloride were included. 7% lysed horse blood was used as an oxygen-quenching system. The medium when used for examining raw hamburger and milk allowed detection of 0.1 to 4.0 cells per gram but was less effective for chicken, probably because of the different amount and type of flora present.

Park and Stankiewicz[10] modified their original medium by incorporating calf serum and sodium pyruvate. Vancomycin and trimethoprim were retained, polymyxin B was replaced by colistin and the selectivity was increased by the addition of cephalothin. The enrichment culture was filtered through a 0.65 micron membrane to further improve selectivity and the filtrate plated on Skirrow agar and a modified Skirrow agar containing additional polymyxin B. In this way the use of polymyxin B was retained in the overall technique.

Lander[11] encountered problems caused by toxic specimens when attempting to isolate various campylobacters important in cattle disease. The problems were addressed by incorporating charcoal as a detoxicant in a basal medium of veal infusion broth containing lysed horse blood. Selectivity was achieved by using vancomycin, trimethoprim, polymyxin B and two anti-fungal agents, cycloheximide and 5-fluorouracil. The medium could be used without a microaerobic atmosphere and was found to be very versatile, being used not only for selective enrichment, but for specimen transport. Sensitivity of the medium for *C. jejuni* subspecies *fetus* and *C. fetus* subsp. *venerealis* was very satisfactory. Charcoal has not found general application in subsequent selective enrichment broth developments but is incorporated in very successful plating media developed later. However, a broth version of CCDA blood-free agar has been described.

Also in 1982, Bolton and Robertson[12] described Preston agar and a selective enrichment broth version which differed only by the omission of agar. Preston agar was developed as an alternative to Skirrow medium which had been found to be insufficiently selective when culturing from animal and environmental specimens. The liquid medium was based on Oxoid Nutrient Broth No. 2 chosen for its low content of trimethoprim inhibitors but, even so, it was supplemented with lysed horse blood. Trimethoprim and polymyxin B were retained from the earlier formulae but rifampicin replaced vancomycin because of its greater activity against Gram-positive bacteria. Cycloheximide provided antifungal activity. Subsequently, when the effect of phototoxicity was generally known, it became common practice to include FBP supplement.

In the following year, 1983, Park and Stankiewicz[13], with other workers, investigated the effects of temperature, duration of incubation and pH of enrichment culture on the recovery of *C. jejuni* from chicken. They concluded that isolation rates could be

increased greatly by incubating samples in their medium at pH 7.0 for 48 hours at 42°C.

Wesley and co-workers[14] established the general effectiveness of Doyle and Roman broth but were concerned about the difficulties encountered when attempting to detect very low numbers of *C. jejuni* in poultry products. They adopted a fresh approach and devised an alkaline (pH 8.0) Tryptose-yeast extract medium. Oxygen quenching activity was further increased by the addition of haematin to FBP supplementation and there was a change of selective agents. A high level of polymyxin was retained but rifampicin and cefsulodin replaced vancomycin and trimethoprim used in earlier formulations. The new medium was particularly effective for isolating *C. jejuni* in the presence of large numbers of *Pseudomonas aeruginosa*. It made possible the detection of less than 1 cell per gram of chicken when using a 3-tube MPN technique. The procedure and medium were also found to be superior to those of Park *et al* for the investigation of chicken.

Lovett *et al*[15] in 1983 modified the 1981 medium of Park *et al* by reducing the amount of polymyxin it contained following observations that it inhibited many strains of *C. jejuni*. At the same time the levels of the other antibiotics were increased.

1983 also saw the development of a medium by Martin and co-workers intended for enrichment of small numbers of Campylobacter in human, bovine and poultry faecal specimens.[16] The medium was based on brucella broth and contained trimethoprim, cefoperazone and 5-fluorouracil as an anti-fungal agent. Lysed blood to counter trimethoprim-neutralising activity by the medium constituents was not added and oxygen quenching agents were also omitted. The authors used the medium for enriching specimens that had been held for long periods in transport medium.

In 1984 Ray and Johnson[17,18] recognised that freezing or refrigeration inflicted sublethal injury on *C. jejuni*. The injured cells became sensitive to polymyxin B used in existing media and probably also to heat. The effect in liquid medium was found to be greater than that in solid medium. Blood included in their selective medium probably afforded some protection but improved detection was achieved by incubating samples in brucella broth supplemented with succinate and cysteine. Four of the five antibiotics were added for an initial incubation period of 6 hours at 37°C. The fifth antibiotic, polymyxin B, was then added and the temperature increased to 42°C for 24 hours.

Also in 1984, Bolton, Coates and Hutchinson[19] reported work done to identify the role of non-selective supplements that had previously been shown to enhance growth and aerotolerance of many Campylobacter strains. They observed that nutrient agar plates in light and air became inhibitory for *C. jejuni*, *C. coli* and what were then known as NARTC strains (now *C. lari*). It was not known whether FBP and horse blood acted as detoxifying agents, growth nutrients, or both. The work established the conditions under which toxicity of medium was induced and demonstrated the ability of blood and other commonly used supplements to neutralise the toxic effects. Both light and air are necessary for toxicity to develop in medium. Once it has developed it cannot be reversed by incubation in anaerobic or microaerobic atmospheres. Blood was shown to be an excellent supplement because it prevents accumulation of photochemically-induced toxicity. It seems unlikely that there is a single compound suitable for supplementing culture media which has the range of detoxifying activities possessed by blood, but charcoal has been found to be very efficient at inhibiting formation of toxic products. The separate components of FBP supplement vary in their effectiveness but unpublished data established that addition of sodium metabisulphite to broth media improved performance. FBP was consequently added to the formula of Preston broth. Results from this enabled further development of charcoal-containing plating media following on from Lander's work. Removal of blood from formulae, desirable for reasons of economy and practicality, was now seen to provide considerable advantages, but broth media containing blood continued to be developed.

Humphrey and Cruickshank,[20] aware of the possibility of selective agents themselves being inhibitory to Campylobacter, investigated the effect of six antibiotics and deoxycholate on uninjured and injured *C. jejuni*. The uninjured cells were affected by rifampicin and the effect on freeze-injured and heat-injured cells was even greater. Deoxycholate was seen to be toxic for some, but not all, injured cells. Preston medium was shown to possess inhibitory activity when compared to Skirrow, Campy-BAP and blood agars.

Further work by Humphrey in 1986[21,22] confirmed the observations of Ray and Johnson that *C. jejuni* recovered better at 37°C than at 42°C even if inhibitors were not present in the medium. This led to the proposal of a method using medium based on nutrient broth containing lysed horse blood and FBP. The method omitted antibiotics for an initial period of incubation at 37°C. The antibiotic mix of trimethoprim, cefoperazone, colistin, amphotericin B and either vancomycin or rifampicin was then added and the incubation temperature raised to 43°C for a period up to 48 hours. Rifampicin continued to show some inhibitory activity. In a paper published three years later, De Boer and Humphrey[23] changed the pre-enrichment period at 37°C to 4 hours. They recommended that the enrichment medium used with the revised methodology should contain rifampicin. Later still,[24] the enrichment medium was again modified to substitute polymyxin B for colistin although this was because of difficulty in obtaining colistin.

The principle of selective isolation by migration of motile campylobacters through a semisolid medium containing selective agents was applied by Goossens *et al* in 1989.[25] The work showed that the choice of agar to make the gel is very important.

Bolton[26] has also developed an enrichment broth for food investigations following on from his earlier work with Preston agar and broth. Full details have yet to be published. The medium is based on a nutrient broth designed especially to aid resuscitation of sublethally injured cells. It contains a number of supplements including FBP, lysed horse blood, haemin and sodium bicarbonate and is designed to avoid the need for a microaerobic atmosphere. A preliminary period of incubation at 37°C is followed by a rise in temperature to 42°C for 14 to 48 hours.

Although the pace of development has slowed, improvements continue to be made. Park and Sanders[27] have developed a medium and method which involves incubation of a broth containing vancomycin and trimethoprim at 31°C to 32°C for 4 hours, followed by addition of cefoperazone and cycloheximide and a rise in incubation temperature to 37°C, followed by another rise to 42°C for 40 to 42 hours. A microaerobic incubation atmosphere is employed. The culture vessels are held static at the two lower incubation temperatures and shaken at 42°C. A variation of Park and Sanders broth devised by Hunt and Radle[28] and cited by Stern and Line[29] is used in a procedure involving ascending incubation temperatures under shaking conditions. The initial temperature is 32°C in a medium containing lysed horse blood, FBP, trimethoprim, vancomycin, amphotericin B and cycloheximide and half the final quantity of cefoperazone. After a further 3 hours the remaining cefoperazone is added and the temperature raised to 37°C.

After another 2 hours the temperature is again raised for the remaining incubation period at 42°C. Incubation is conducted in a flowing microaerobic gas mixture.

Most recently,[30] a blood-free enrichment broth (BFEB) has been developed to replace the blood-containing enrichment broth recommended in the FDA Bacteriological Analytical Manual. No significant differences have been observed in the recovery efficiencies of the two broths but BFEB is less expensive, less time consuming and the method of use is simpler to operate than the BAM procedure.

The essential principles needed for selective enrichment of Campylobacter are probably now understood and further developments are likely to involve fine changes in medium composition and technique. It is possible, too, that optimum conditions will need to be established for the different species because most work so far has been conducted on *C. jejuni*. Enrichment culture media and methodology for investigation of foods has, even so, now reached the point where non-specialist food microbiologists can reasonably expect success. Unipath will maintain its long standing commitment to Campylobacter culture media by introducing selective enrichment broths as preferences become apparent.

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Campylobacter Enrichment Broths

PRESTON BROTH

	grams/ litre	*Suggested Oxoid product*
Lab-Lemco meat extract	10.0	Lab-Lemco powder L29
Peptone	10.0	Peptone bacteriological neutralised L34
Sodium chloride	5.0	
Sodium pyruvate	0.25	Campylobacter growth supplement SR84
Sodium metabisulphite	0.25	
Ferrous sulphate	0.25	
Polymyxin B	5000 i.u.	
Trimethoprim	10 mg	
Rifampicin	10 mg	
Cycloheximide	100 mg	
Water	1000 ml	
Lysed horse blood	50 ml	Laked horse blood SR48

Preston broth may be made as follows from Oxoid culture media:

Nutrient broth Number 2 code CM67	1000 ml

Sterilise, and aseptically add:

Campylobacter Growth Supplement code SR084E	2 vials
Preston Campylobacter Selective Supplement code SR117E	2 vials
Lysed Horse Blood code SR48	50 ml

If the sample has been exposed to chilling, freezing or heat apply a suitable resuscitation technique for the recovery of injured cells. Typically this would delay addition of the selective supplement SR117E until after an initial period of incubation.

Reference

Bolton, F.J. and Robertson, L. (1982) *J. Clin. Pathol.* **35.** 462-467.

PARK AND SANDERS BROTH

	grams/ litre	*Suggested Oxoid product*
Tryptone	10	Tryptone L42
Peptone P	10	Peptone P L49
Glucose	1	
Yeast extract	2	Yeast extract powder L21
Sodium citrate	1	
Sodium chloride	5	
Sodium metabisulphite	0.1	
Sodium pyruvate	0.25	
Water	1000 ml	
Antibiotic solution A		
Vancomycin	0.1 gram	
Trimethoprim	0.1	
Brucella broth	50 ml	
Complete Medium		
Basal medium	950 ml	
Lysed horse blood	50	Laked horse blood SR48
Antibiotic solution A	5	
Antibiotic solution B		
Cefoperazone	0.032 g	
Cycloheximide	0.100 g	
Brucella broth	100 ml	

Antibiotic solution B is added during incubation to a concentration of 5% (50 ml of Antibiotic solution B added to 1000 ml of Complete Medium).

Reference

Park, C.E. and Sanders, G.W. (1991) "A sensitive enrichment procedure for the isolation of *Campylobacter jejuni* from frozen foods". In: Riuz-Palacios, G.M., Calva, F. and Ruiz-Palacios, B.R. (Eds). Campylobacter V. Proceedings of the 5th Int. Workshop on Campylobacter Infection. p102. National Inst. of Nutrition, Puerto Vallarta, Mexico.

EXETER BROTH

	grams/ litre	*Suggested Oxoid product*
Lab-Lemco meat extract	1.0	Lab-Lemco powder L29
Yeast extract	2.0	Yeast extract powder L21
Peptone	5.0	Peptone bacteriological Neutralised L34
Sodium chloride	5.0	
Lysed horse blood	50 ml	Laked horse blood SR48
Ferrous sulphate	200 mg	
Sodium pyruvate	200 mg	
Sodium metabisulphite	200 mg	
Rifampicin	10 mg	
Trimethoprim	10 mg	
Polymyxin B	4 mg	
Amphotericin B	2 mg	
Water	1000 ml	

The basal medium for Exeter broth may be made from:

Nutrient broth CM1	1000 ml
Laked horse blood SR48	50 ml

Reference

De Boer, E. and Humphrey, T.J. (1991) *Microb. Ecol. Hlth. Dis.* **4** (Special issue) S43.

BOLTON BROTH

Basal Medium	*grams/ litre*	*Suggested Oxoid product*
Meat peptone	10	Peptone bacteriological (neutralised) L34
Lactalbumin hydrolysate	5 grams	Lactalbumin hydrolysate L48
Yeast extract	5 grams	Yeast extract powder L21
Sodium chloride	5 grams	
Supplementation		
Haemin	10 mg	
Alpha-ketoglutamic acid	1 gram	
Sodium pyruvate	0.5 gram	
Sodium metabisulphite	0.5 gram	
Sodium carbonate	0.6 gram	
Lysed horse blood	50 ml	Laked horse blood SR48
Cefoperazone	20 mg	
Vancomycin	20 mg	
Cycloheximide	50 mg	
Water	1000 ml	

Final pH 7.4

Reference

Bolton, F.J. (1995) Personal communication.

HUNT AND RADLE ENRICHMENT BROTH (BAM M29)

Base	*grams/ litre*	*Suggested Oxoid product*
Nutrient broth No. 2 (Oxoid)	10	Nutrient broth No. 2 CM67
Yeast extract	6	Yeast extract powder L21
Water	950 ml	
Supplement A		
Ferrous sulphate	0.25 g	Campylobacter growth supplement SR84
Sodium metabisulphite	0.25 g	
Sodium pyruvate	0.25 g	
Lysed horse blood	50 ml	Laked horse blood SR48

Note

Supplement A may be easily and quickly made as follows for 1000 ml of medium.

Campylobacter Growth Supplement SR84	2 vials
Lysed horse blood SR48	50 ml

Supplement B	*mg/litre*
Vancomycin	10 mg
Trimethoprim lactate	12.5 mg
Sodium cefoperazone	15.0 mg
Amphotericin B	2.0 mg

Supplement C	
Sodium cefoperazone	15.0 mg

References

1 Cited in: Stern, N.J. and Line, J.E. (1992) *J. Food Prot.* **55,** 663-666.
2 Hunt, J.M. (1992) Campylobacter. In F.D.A. Bacteriological Analytical Manual 7th Edition 77-94. AOAC, Arlington Va.

DOYLE AND ROMAN BROTH

This medium uses Brucella broth as the basal medium to which is added sodium succinate and cysteine hydrochloride.

Formula	*grams/ litre*	*Suggested Oxoid product*
Casein hydrolysate	15.0	Casein hydrolysate (acid) L41
Peptone P	5.0	Peptone P L49
Yeast extract	2.0	Yeast extract powder L21
Dextrose	1.0	
Sodium chloride	5.0	
Sodium metabisulphite	1.0	
Sodium citrate	0.1	
Sodium succinate	3.0	
Cysteine hydrochloride	0.1	
Water	1000 ml	
Lysed horse blood	70 ml	Laked horse blood SR48

	mg/litre
Vancomycin	15
Trimethoprim	5
Cycloheximide	50
Polymyxin B	20,000 i.u.

Reference

Doyle, M.P. and Roman, D.J. (1982), *Appl. Env. Microbiol.* **43,** 1343-1353.

TABLE 8 – The selective agents incorporated in some Campylobacter enrichment broth media
Concentrations in mg/litre unless otherwise stated

Medium	*Cefoperazone*	*Colistin*	*Cycloheximide*	*Polymyxin B*	*Rifampicin*	*Trimethoprim*	*Vancomycin*
Bolton	20		50				20
Doyle & Roman			50	20,000 i.u.		5	15
Exeter	15	4				10	10
Hunt & Radle:–							
(a) Antibiotic formula 1	30		100			12.5	10
(b) Antibiotic formula 2	15		100			12.5	10
(c) Antibiotic formula 3	15		100		10	12.5	10
Park & Sanders:–							
(1) Antibiotic solution A						10	10
(2) Antibiotic solution B	32		100				
Preston			100	5,000 i.u.	10	10	

Campylobacter Plating Media

Dr. Martin Skirrow, in his paper published in the *British Medical Journal* in July 1977, made it possible overnight for Campylobacter isolation to become routine in ordinary laboratories by describing a simply made selective medium and making generally known the special atmospheric conditions the organism requires. In so doing, the indications that Campylobacter is a significant cause of human disease were almost immediately confirmed. Much information had already been gathered, notably by Jean-Paul Butzler and colleagues, but their method of isolating Campylobacter using filtration culture, although effective, was somewhat restrictive for routine use. The subsequent development by Butzler of his own selective medium and his close friendship and collaboration with Skirrow galvanised activity in Campylobacter research.

Inevitably, more effective selective media became necessary when other researchers extended the horizons beyond the early work. Increased selectivity and sensitivity were demanded and practical and economic problems also had to be addressed, such as the poor availability and high cost in some parts of the world of suitable supplies of blood for incorporation in media. Experimentation began into improving selectivity, sometimes incorporating antibiotics still relatively new in therapy and by applying anti-fungal antibiotics to overcome the problems of fungal and yeast overgrowth in some types of specimens.

Butzler, for his work in Africa, needed a medium possessing greater selectivity than Skirrow's formula. He devised his own, but at some cost to sensitivity. This was later much improved in his Virion formula.

In another development to improve selectivity, the polymyxin B content of Butzler's medium was quadrupled to make BU40 medium.

Blaser started his work with Skirrow medium but quickly modified it when he encountered problems with overgrowth by yeasts and fungi.

Bolton and Roberts, investigating the presence of Campylobacter in abattoirs and the environment, formulated their own medium when they found that Skirrow's medium was insufficiently selective. Later, Bolton addressed the problems caused by the necessity to incorporate blood in existing media and formulated a blood-free medium based on charcoal which was very soon altered slightly to use cefoperazone instead of cephalexin. Subsequently it was modified again by adding Amphotericin B when it became apparent that incubation at 37°C instead of 42°C yielded more isolates of *C. jejuni* but had the undesirable side-effect of allowing yeasts to grow.

The medium is claimed to give greater selectivity, particularly where enterococci are present.

All the while during these developments, some workers were paying attention to *C. upsaliensis* and other campylobacters inhibited by the antibiotics in the existing formulae. For this reason it was necessary to use the effective but technically demanding method of culturing filtrates on non-selective medium and this was a disincentive to other workers.

In response, Aspinall and his colleagues have developed CAT medium, a variant of Bolton's CCDA blood-free medium which enables reliable isolation of *C. upsaliensis* on selective medium. CAT medium is very versatile because it is equally as good as CCDA medium for isolating *C. jejuni* and *C. coli*.

As the search for Campylobacter extended to the environment, water and foods, answers had to be found for the new problems caused by differences in the accompanying flora encountered in non-faecal specimens. Veterinarians who had recognised the significance of Campylobacter before the majority of clinical microbiologists, brought their own methods to bear on investigating infections in animals but were also able to adopt many of the advances made in the investigation of human disease.

For working with foods, Humphrey developed Exeter agar made by adding agar to Exeter enrichment broth. Most plating media for Campylobacter were developed for use with faeces and may not always be as effective for isolation from foods although some have been adopted in the standard methods.

The colonial appearance of Campylobacter can be used as a guideline for presumptive identification to species level. *C. jejuni* strains produce grey/moist flat spreading colonies. Some strains may have a green hue or a dry appearance with or without a metallic sheen. *C. coli* strains tend to be creamy-grey in colour, moist, slightly raised and often produce discrete colonies.

Colonies tend to swarm when initially isolated. However, reduction in moisture content of culture media can markedly alter the appearance to round, entire, sometime butyrous, colonies.[1] The extent of this change in colony appearance is variable. The effect may explain differences seen in different laboratories and on different culture media. It cannot be reversed by increasing the moisture in the atmosphere during incubation.

There are still problems with the isolation of some less-commonly occurring campylobacters on current selective media. At present specialised media and/or techniques are required for them, but as the significance of these organisms in disease gradually becomes apparent, pressure will increase for development of simple isolation methods that can be used by non-specialist laboratories. The Unipath commitment to Campylobacter culture will ensure developments are watched closely so that demands for new media can be satisfied.

Plating media currently in the Oxoid range are described on pages 18–24 and the formula of Campy-Cefex agar given on page 25.

Reference

1 Buck, G.E. and Kelly, M.T. (1981) *J. Clin. Microbiol.* **14,** 585-586.

Campylobacter Agar (Skirrow)

Basal medium
Lysed blood agar prepared from Columbia Agar Base code CM331 or Blood Agar No. 2 code CM271 and 5–7% of Laked Horse Blood code SR48.

Campylobacter Selective Supplement (Skirrow)
Code: SR69

	mg/litre
Vancomycin	10
Trimethoprim	5
Polymyxin	2500 i.u.

Directions
Add 2 ml of sterile distilled water to a vial and dissolve the contents.

Add the contents to 500 ml of sterile lysed blood agar cooled to 50–50°C, prepared from Columbia Agar Base code CM331 or Blood Agar Base No. 2 code CM271 with 5-7% of Laked Horse Blood code SR48.

Mix gently and pour into sterile petri dishes.

Plates should be stored at 2–8°C away from light, wrapped, or enclosed in containers with a minimum headspace to delay the formation of toxic oxygen derivatives in the medium.

Description
Campylobacter selective agar (Skirrow) is based on the culture medium described by Skirrow.[1] The medium was devised to overcome practical difficulties associated with the use of the faecal filtrate culture technique described by Butzler.[2] Skirrow did not specify the basal medium for making his blood agar, but Columbia Agar Base code CM331 and Blood Agar Base No. 2 code CM271 are widely used. The medium is made selective using a mixture of vancomycin, polymyxin B and trimethoprim. Lysed horse blood is added to neutralise trimethoprim antagonists present in the basal medium. The incubation temperature of 42°C contributes to the selectivity. Skirrow's medium is intended for the isolation of thermophilic campylobacters. Non-thermophilic strains e.g. *C. fetus* subsp. *fetus* will not grow at 42°C.

Skirrow used his medium for faecal culture in an investigation of 803 unselected patients with diarrhoea. *Campylobacter jejuni* or *C. coli* were isolated from 57 (7.1%) of these patients. There were no isolates from 194 people who did not have diarrhoea. Several patients appeared to have been infected from chickens and in two cases there was evidence that infection was contracted from dogs. Skirrow concluded that the close association between the presence of campylobacters in faeces and the occurrence of a distinctive enteritis was further proof that campylobacters are pathogenic for humans. Numerous reports since Skirrow's work have confirmed the pathogenicity of *Campylobacter spp.* and there is general acceptance of the role of poultry, milk and water as infection sources.

Skirrow's medium is specified in some national methods for detection of *Campylobacter spp.* in foods (Table 1) but is insufficiently selective for many types of sample.

Technique
Techniques involving the use of Skirrow Campylobacter selective agar for the detection of *Campylobacter spp.* in foods are given in Australian and French standard methods (Tables 4, 5). The documents should be read for the detailed methodology.

Incubate cultures in a microaerobic atmosphere at 42°C.

Storage conditions
Store the dehydrated medium in a cool dry place away from bright light. Store the selective supplement at 2–8°C. Prepared plates should be stored at 2–8°C taking care to minimise exposure to air and light.

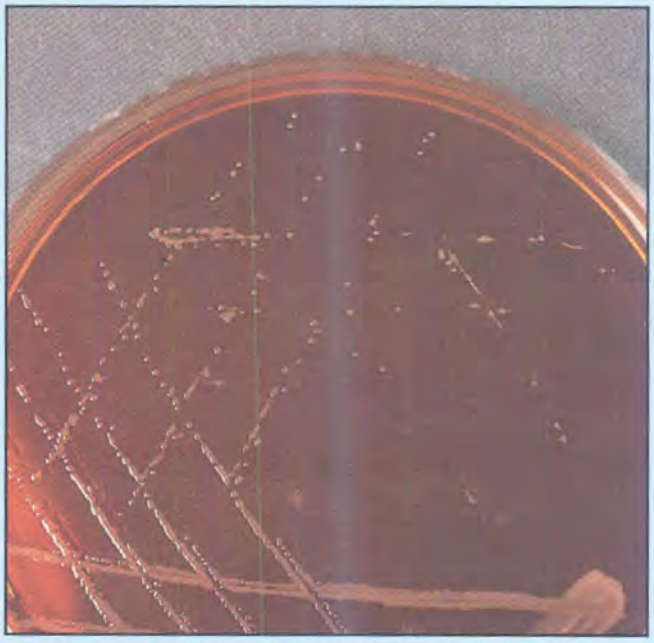
Quality control
Incubation at 42°C.

Positive control
Campylobacter jejuni ATCC 29428

Negative control
Escherichia coli ATCC 25922

Precautions
If plates are first examined after 24 hours incubation, read them immediately and quickly return them to a microaerobic atmosphere to ensure continued viability.

References
1 Skirrow, M.B. (1977). *Br. Med. J.* 2nd July, 9-11.
2 Butzler, J.P. *et. al. J. Paediatrics* (1973) **82,** 493.



Colony appearance of *C. jejuni* on Skirrow's selective agar.

Campylobacter Agar (Butzler)

Formula

Basal medium
Blood agar prepared from Columbia Agar Base code CM331 and 5–7% of defibrinated horse blood.

Campylobacter Selective Supplement (Butzler)
Code: SR85

	mg/litre
Novobiocin	5
Cephazolin	15
Cycloheximide	50
Bacitracin	25,000 i.u.
Colistin	10,000 i.u.

Directions
Prepare Columbia blood agar base according to instructions. Cool to 50–55°C.

Aseptically add 5–7% of defibrinated sheep blood. Horse blood may be used as an alternative.

Aseptically add the contents of Campylobacter Selective Supplement (Butzler) code SR85 rehydrated with 3 ml of 50/50 ethanol/water. Mix well and pour into petri dishes.

Store plates in the dark, preferably wrapped or in a sealed container.

Description
Campylobacter selective agar (Butzler) is based on the formula of Lauwers, De Boeck and Butzler.[1]

The medium was formulated to replace an earlier one used in the technique of filtration and culture on an elective blood-thioglycollate agar medium in the examination of human blood and faecal specimens for "related vibrios".[2]

Addition of cephalothin to bacitracin, novobiocin, colistin and cycloheximide contained in the original formula greatly increased selectivity and made it possible to dispense entirely with filtration.

Butzler's selective Campylobacter agar utilises sheep blood agar as the basal medium. It is incubated at 42°C which decreases the time for *Campylobacter jejuni* to grow but inhibits the growth of *C. fetus* subspecies *intestinalis*.

Goossens and co-workers later formulated a new selective medium (Virion) which has a similar isolation rate to the earlier medium but possesses increased selectivity.[3]

Storage conditions
Store the dehydrated medium in a cool dark place away from bright light.

Store the selective supplement at 2–8°C.

Keep prepared plates at 2–8°C in the dark, preferably sealed to minimise exposure of the medium to the air.

Quality control

Positive control
Campylobacter jejuni ATCC 29428

Negative control
Escherichia coli ATCC 25922

Precautions
Campylobacter Selective Supplement (Butzler) contains cycloheximide and is toxic if swallowed, inhaled or by skin contact.

As a precaution when handling, wear gloves and eye/face protection.

References

1 Lauwers, S., De Boeck, M. and Butzler, J.P. (1978) *The Lancet*, March 18th, 604-605.
2 Dekeyser, P., Gossuin-Detrain, M., Butzler, J.P. and Sternon, J. (1972). *J. Inf. Dis.* **125,** 390-392.
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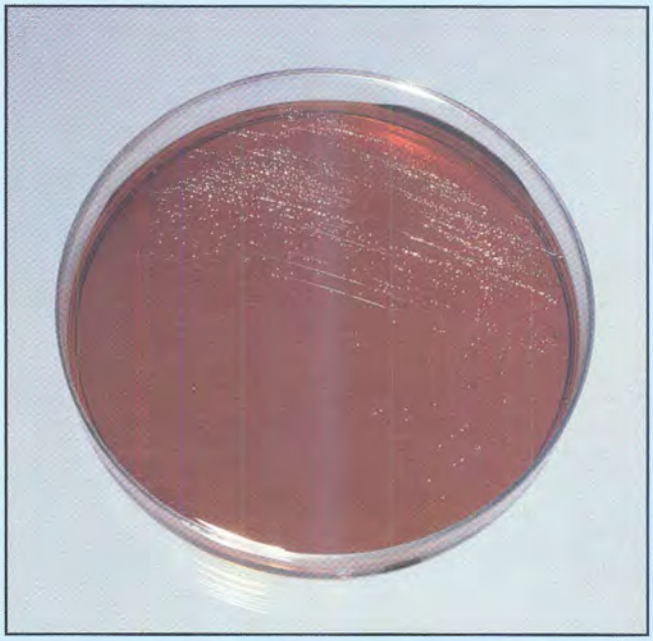

Colony appearance of *C. jejuni* on Butzler selective agar. Unipath Marketing collection.

Campylobacter Agar (Blaser-Wang)

Basal medium
5–10% sheep blood agar prepared from Columbia Agar Base code CM331, or Blood Agar Base No. 2 code CM271.

The medium can also be made using 5–7% Laked Horse Blood code SR48 instead of sheep blood.

Campylobacter Selective Supplement (Blaser-Wang)
Code: SR98

	mg/litre
Vancomycin	10
Trimethoprim	5
Cephalothin	15
Amphotericin B	2
Polymyxin B	2,500 i.u.

Directions
Add the contents of 1 vial, rehydrated in 2 ml of sterile distilled water, to 500 ml of basal medium made from Columbia Agar Base code CM331 or Blood Agar Base No. 2 code CM271 cooled to 50°C. Add 5–10% of defibrinated sheep blood or 5–7% of Laked Horse Blood code SR48.

Mix gently and pour into sterile petri dishes.

Plates should be stored at 2–8°C away from light and preferably wrapped or enclosed in containers to slow down access of air to the medium.

Description
Blaser *et al*[1] took the medium of Skirrow[2] and increased the selectivity initially by the addition of amphotericin B and later cephalothin. Amphotericin B is present to inhibit yeasts and cephalothin has been added to improve inhibitory activity against normal enteric flora.

Trimethoprim is contained in the medium. Neutralisation of trimethoprim antagonists which may be present in the basal medium can be expected to be more effective if lysed horse blood is used instead of sheep blood.

T'he medium was devised for use in a study of the survival of *Campylobacter jejuni* in a variety of biological milieus; hydrochloric acid, human bile and urine, bovine milk and stream water. Significantly long periods of viability were observed in all milieus with organisms recoverable from milk after 3 weeks and from water after 4 weeks. *C. jejuni* survived better in faeces, milk, water and urine at 4°C than at 25°C.

The results confirm the significance of milk and water as food and environmental sources of infection. The tests with hydrochloric acid showed that *C. jejuni* survived a pH of 3.6 although at pH 3.0 viability was much reduced. Acidic conditions in the stomach are an important barrier against ingested pathogens; milk is an effective buffer of gastric acidity and may have a protective effect. The results suggested that even a few *C. jejuni* cells in milk may be enough to survive gastric acidity and initiate infection.

Despite the original use of Blaser-Wang medium to investigate milk and environmental waters it has found little acceptance in food microbiology.

Storage conditions
Store the basal dehydrated media in a cool dry place away from bright light.

Store the selective supplement at 2–8°C.

Prepared plates should be stored at 2–8°C taking care to minimise exposure to air and light.

Quality control
Incubate at 42°C.

Positive control
Campylobacter jejuni ATCC 29428

Negative control
Escherichia coli ATCC 25922

Precautions
If plates are first examined after 24 hours incubation read them immediately and quickly return them to a microaerobic atmosphere to ensure continued viability.

References
1 Blaser, M.J., Hardesty, H.L., Powers, B. and Wen-Lan, L. Wang (1980). *J. Clin. Microbiol.* **11,** 309-313.
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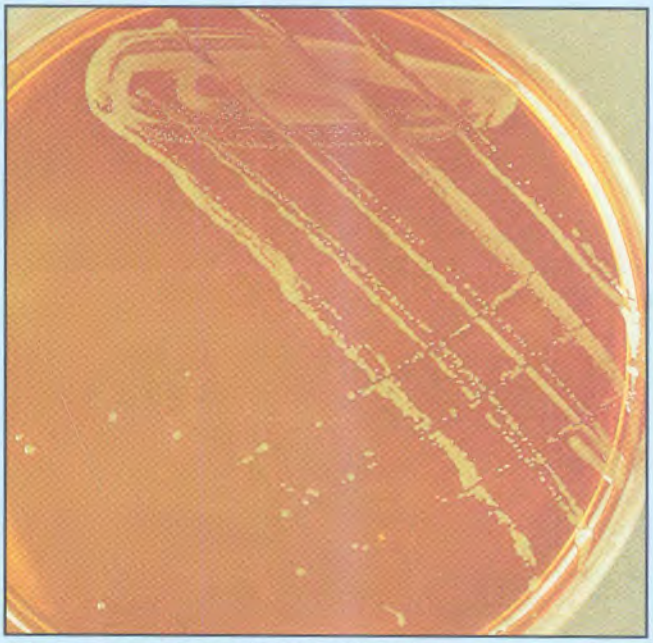

Colony appearance of *C. jejuni* on Blaser-Wang selective agar.

Campylobacter Agar (Preston)

Campylobacter Agar Base Code: CM689

Formula

	gm/litre
Lab-Lemco powder	10
Peptone	10
Sodium chloride	5
Agar	12

pH 7.5 ± 0.2

Campylobacter Selective Supplement (Preston) Code: SR117

	mg/litre
Polymyxin B	5,000 i.u.
Rifampicin	10
Trimethoprim	10
Cycloheximide	100

Directions
Prepare Campylobacter Agar Base (CM589) according to instructions. Sterilise by autoclaving at 121°C for 15 minutes. Cool to 50°C. Aseptically add 25 ml of Laked Horse Blood code SR48 and 1 vial of Preston Campylobacter Selective Supplement code SR117 reconstituted with 2 ml of 50/50 acetone/sterile distilled water. Mix well and pour into sterile petri dishes. Store plates in the dark and preferably wrapped or in sealed containers.

Description
Preston Campylobacter Selective Agar is based on the formula described by Bolton and Robertson.[1] This medium was specifically formulated to be suitable for isolation of *Campylobacter species* from all types of specimens (human, animal, avian and environmental).

In comparative studies[2] of the selective media of Skirrow, Butzler, Blaser, Campy-BAP and Preston, the Preston medium was found to give the maximum isolation rate of *Campylobacter spp.* from all types of specimens tested and also to be the most selective.

Oxoid Campylobacter Agar Base has been prepared from materials described by Bolton and Robertson[1]. It is also suitable as a basal medium for the selective supplements of Blaser-Wang, Skirrow and Butzler.

Preston Campylobacter Selective Supplement code SR117 can also be used to prepare Preston Campylobacter selective enrichment broth[2] (see page 14).

The selective enrichment technique is recommended for specimens and food samples that are expected to bc heavily contaminated and/or carry small numbers of viable colony-forming units. The Preston Campylobacter selective enrichment broth which is supplemented with the growth supplement code SR84, made to the formulation of George *et al*,[3] effectively quenches toxic compounds which may form on exposure of the medium to light and air.[4]

Humphrey and Cruickshank[5] demonstrated sensitivity of cold-injured and heat-injured Campylobacter cells to rifampicin contained in Preston medium. No other antibiotic tested showed this effect but increased sensitivity to deoxycholate was also shown. Suitable resuscitation techniques must be applied to samples tested using Preston broth and agar. Typically, procedures would involve pre-enrichmcnt at 37°C for at least 2 hours before the addition of selective agents and increase of incubation temperature to 42°C. Microbiologists are advised to consult detailed methodology for appropriate techniques.[5,6,7]

Storage conditions
Store the dehydrated medium in a cool dark place away from bright light.

Store the selective supplement at 2°C to 8°C.

Keep prepared plates at 2°C to 8°C in the dark, preferably wrapped or in a sealed container to prevent formation of toxic oxygen derivatives in the medium.

Store broth medium at 2°C to 8°C in the dark.

Quality control

Positive control
Campylobacter jejuni ATCC 29428

Negative control
Escherichia coli ATCC 25922

Precautions
Campylobacter Selective Supplement (Preston) contains cycloheximide and is toxic if swallowed, inhaled or by skin contact. As a precaution when handling wear gloves and eyes/face protection.

Campylobacter Transport Medium
Campylobacter Selective Supplement (Preston) is incorporated in an improved medium for storage and transportation of thermophilic *Campylobacter spp.*[8]

References

1 Bolton, F.J. and Robertson, L. (1982) *J. Clin. Pathol.* **35,** 462-467.
2 Bolton, F.J. and Robertson, L. (1982) *J. Clin. Pathol.* **35,** 462-467.
3 George, H.A., Hoffman, P.S., Kreig, N.R. and Smibert, R.M. (1979). *Can. J. Microbiol.* **25,** 8-16.
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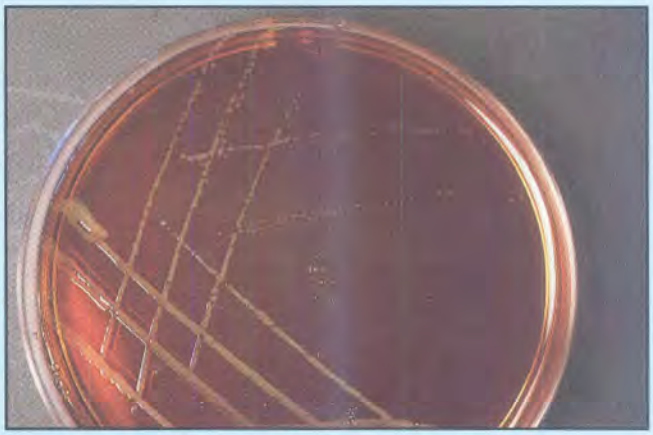


Colony appearance of *C. jejuni* on Preston selective agar.

Campylobacter Agar (Modified CCDA)

Campylobacter Blood-free Agar Base
Code: CM739

Formula

	mg/litre
Nutrient broth No. 2	25.0
Bacteriological charcoal	4.0
Casein hydrolysate	3.0
Sodium deoxycholate	1.0
Ferrous sulphate	0.25
Sodium pyruvate	0.25
Agar	12.0

pH 7.4 ± 0.2

CCDA Selective Supplement
Code: SR155

	mg/litre
Cefoperazone	32
Amphotericin B	10

Directions
Prepare Campylobacter Blood-Free Agar Base (CM739) according to instructions. Sterilise by autoclaving at 121°C for 15 minutes. Cool to 50°C. Aseptically add 1 vial of CCDA selective supplement code SR155 reconstituted with 2 ml of sterile distilled water. Mix well and pour into sterile petri dishes.

Store plates in the dark and preferably wrapped or in sealed containers.

Description
Campylobacter Blood-Free Selective Agar is based on the formula described by Bolton *et al*[1] and subsequently modified to replace cephazolin with cefoperazone to increase the selectivity.[2] It was originally devised for isolation of thermophilic campylobacters from human faecal specimens and is now a specified medium in standard procedures for isolation of *Campylobacter spp.* in foods. (See Tables 1, 5, 7.)

The medium, referred to as Modified Charcoal-Cefoperazone-Deoxycholate Agar (CCDA) was developed to replace blood with charcoal, ferrous sulphate and sodium pyruvate which have been found to enhance the growth and aerotolerance of *Campylobacter spp*. The medium also contains casein hydrolysate which was found to be necessary as it promoted the growth of some environmental strains of *C. lari*. Selectivity is achieved using cefoperazone and sodium deoxycholate.

More recent work has shown an increased isolation rate can be obtained if the plates are incubated at 37°C rather than 42°C.[3] Amphotericin B has been added to the formula to suppress the growth of yeast and fungal contaminants that may occur at 37°C.

Technique
Techniques involving the use of Blood-Free Selective Agar CCDA are given in AFNOR, ISO/DIS 10272 and MAFF/DoH Steering Group methods for the detection of Campylobacter in foods (Tables 5, 7).

The documents should be read for the detailed methodology.

Incubate cultures in a microaerobic atmosphere at 35-37°C.

Storage conditions
Store the dehydrated medium in a cool dry place away from bright light.

Store the selective supplement at 2–8°C.

Prepared plates should be stored at 2–8°C away from light and wrapped or enclosed in containers to minimise access of air.

Quality control
Incubate at 37°C.

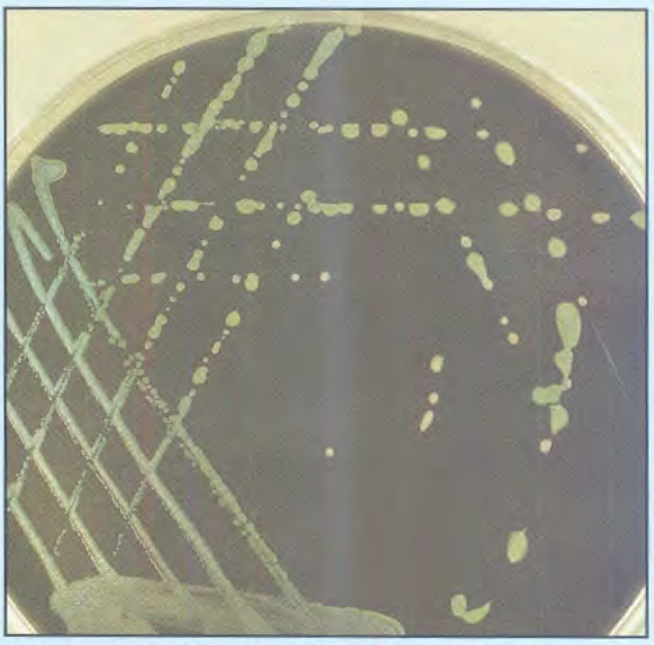
Positive control
Campylobacter jejuni ATCC 29428

Negative control
Escherichia coli ATCC 25922

Precautions
If plates are first examined after 24 hours incubation, read them immediately and quickly return them to a microaerobic atmosphere to ensure continued viability.

References

1 Bolton, F.J., Hutchinson, D.N. and Coates, D. (1984) *J. Clin. Microbiol.* **19,** 169-171.
2 Hutchinson, D.N. and Bolton, F.J. (1984) *J. Clin. Path.* **34,** 956-957.
3 Bolton, F.J., Hutchinson, D.N. and Parker, G. (1988) *Eur. J. Clin. Microbiol. Inf. Dis.* **7,** 155-160.



Colony appearance of *C. jejuni* on CCDA blood-free selective agar.

Campylobacter Agar (Karmali)

Campylobacter Agar Base (Karmali)
Code: CM908

	gm/litre
Columbia agar base	39.0
Activated charcoal	4.0

pH 7.4 ± 0.2

Campylobacter Selective Supplement (Karmali)
Code: SR139

	mg/litre
Haemin	32
Sodium pyruvate	100
Cefoperazone	32
Vancomycin	20
Cycloheximide	100

Directions
Add 21.5 grams of Campylobacter Agar Base (Karmali) code CM908 to 500 ml of distilled water and bring to the boil to dissolve. Sterilise by autoclaving at 121°C for 15 minutes. Cool to 50°C. Aseptically add the contents of 1 vial of Campylobacter Selective Supplement (Karmali) code SR139 reconstituted with 2 ml of sterile distilled water. Mix well and pour into sterile petri dishes. Store the plates at 2–8°C in the dark and preferably wrapped or in sealed containers to minimise access of oxygen to the medium.

Description
Campylobacter medium (Karmali) is based on the formula described by Karmali and co-workers[1] and was designed to overcome perceived deficiencies of Modified CCDA Blood-Free Campylobacter Selective Agar.[2]

Karmali medium uses Columbia agar as a basal medium. The advantages of a blood-free medium were recognised and charcoal was added to the Columbia Agar Base to replace blood used in earlier formulae.

The sodium pyruvate in Campylobacter agar (Karmali) is present in the selective supplement while the other blood-free Campylobacter media in the Oxoid range (Modified CCDA and CAT) contain it in the basal medium. Haemin contained in Karmali medium replaces ferrous sulphate in Modified CCDA and CAT media. Karmali medium contains vancomycin for inhibition of Gram-positive organisms, replacing deoxycholate in the other two media Vancomycin was chosen to eliminate the inherent variability of bile salts and because it has specific activity against enterococci. Cefoperazone in Karmali medium more effectively suppresses *Pseudomonas spp.* and cycloheximide is more effective than Amphotericin B for inhibiting yeasts. Karmali medium should be incubated at 42°C.

The combination of antibiotics contains one predominantly active against Gram-positive organisms, the second predominantly active against Gram-negative organisms and a third active against yeasts, The distinct activities allowed rational assessment of the selective effect of the individual agents when the medium was being developed.

Karmali medium has been compared against Skirrow medium and found to be significantly more selective. However, some *C. coli* strains are susceptible to cephalosporins and Skirrow medium is more satisfactory than Karmali medium for this group because it has little inhibitory activity against them. Karmali concluded that Skirrow medium and Karmali medium used together represents a near optimal combination of selective media for the isolation of thermophilic campylobacters from faeces.

Karmali medium is specified for use as one of a pair of plating media in ISO/AFNOR methods (Table 5). The choice of the second medium is optional.

Technique
Karmali medium is used for plating Campylobacter enrichment cultures as part of a standardised method (see Table 5). The relevant documents should be consulted for details.

Storage conditions and shelf life
Store the dehydrated medium tightly capped in the original container in a cool dry place away from bright light.

Campylobacter Selective Supplement (Karmali) should be stored in the dark at 2–8°C.

Prepared plates should be stored at 2–8°C in the dark and preferably wrapped or sealed in containers to limit access of air.

Quality control
Incubate at 42°C.

Positive control
Campylobacter jejuni ATCC 29428

Negative control
Escherichia coli ATCC 25922

Precautions
If plates are first examined after 24 hours incubation, read them immediately and quickly return them to a reduced oxygen atmosphere to ensure continued viability.

Cycloheximide contained in Campylobacter Selective Supplement (Karmali) is toxic and appropriate precautions should be taken.

References

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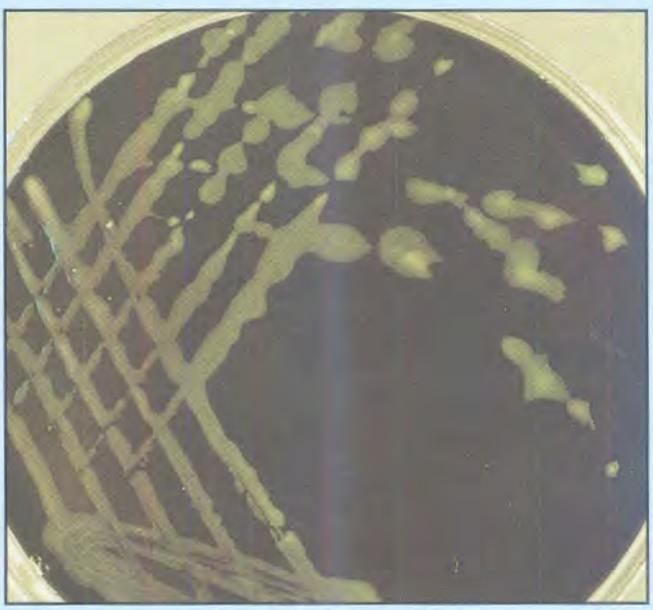

Colony appearance of *C. jejuni* on Karmali selective agar.

Campylobacter Agar (CAT)

Basal medium

Blood-free Campylobacter Agar Base
Code: CM739

	mg/litre
Nutrient Broth No. 2	25.0
Bacteriological charcoal	4.0
Casein hydrolysate	3.0
Sodium deoxycholate	1.0
Ferrous sulphate	0.25
Sodium pyruvate	0.25
Agar	12.0

pH 7.4 ± 0.2

Campylobacter Selective Supplement (CAT)
Code: SR174

	mg/litre
Cefoperazone	8
Teicoplanin	4
Amphotericin B	10

Directions
Prepare Campylobacter Blood-Free Agar Base (CM739) according to instructions. Sterilise by autoclaving at 121°C for 15 minutes. Cool to 50°C. Aseptically add 1 vial of CAT Supplement SR174E reconstituted with 4 ml of sterile distilled water. Mix well and pour into petri dishes.

Store plates in the dark and preferably wrapped or in sealed containers.

Description
CAT Medium was devised for the isolation of *Campylobacter upsaliensis* and may be used for the isolation of other thermophilic *Campylobacter spp.* including *C. jejuni*.[1] Prior to the formulation of CAT medium, filtration culture was necessary because of the wide range of antibiotic susceptibilities displayed between strains of *C. upsaliensis* and the consequent unreliability of the existing Campylobacter media formulae. The technical demands and the insensitivity of the filtration method have hitherto made study of *C. upsaliensis* unattractive for many microbiologists.

C. upsaliensis[2] is the species name given to a group of thermophilic catalase-negative or weakly-positive (CNW) campylobacters first isolated from dogs in 1981.[3] Reports have indicated that *C. upsaliensis* is a potential human pathogen associated with enteritis[4-6] and studies have suggested that 3 to 13% of all Campylobacter diarrhoeal disease is associated with this species.[7-8] Routes of transmission are still largely unknown.

CAT Medium contains only 25% of the concentration of cefoperazone present in CCDA Blood-Free Selective Agar. This concentration, based on MIC studies of *C. upsaliensis*, inhibits most Enterobacteriaceae but not enterococci. Inhibition of enterococci is accomplished using teicoplanin. The medium also contains amphotericin B to inhibit yeasts which grow at the recommended incubation temperature of 37°C.

Storage conditions
Store the basal dehydrated medium in a cool dry place away from bright light.

Store the selective supplement at 2–8°C.

Prepared plates should be stored at 2–8°C, wrapped or in sealed containers, to minimise exposure to air.

Quality control
Incubate at 37°C.

Positive control
Campylobacter jejuni ATCC 29428

Negative control
Escherichia coli ATCC 25922

Precautions
If plates are first examined after 24 hours incubation read them immediately and quickly return them to a microaerobic atmosphere to ensure continued viability.

References

1 Aspinall, S.T., Wareing, D.R.A., Hayward, P.G. and Hutchinson, D.N. (1993) *J. Clin. Pathol.* **46,** 829-831.
2 Sandstedt K. and Ursing J. (1991) *Syst. Appl. Microbiol.* **14,** 39-45.
3 Sandstedt, K, Ursing, J. and Walder, M. (1983). *Curr. Microbiol.* **8,** 209-215.
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Growth of *Campylobacter spp.* on CAT medium. Unipath Marketing collection.

Campy-Cefex Agar

Campy-Cefex Agar

	grams/litre
Brucella agar	44
Ferrous sulphate	0.5
Sodium bisulphite	0.2
Sodium pyruvate	0.5
Water	950 ml
Lysed horse blood	50 ml

Antibiotic Supplement

	mg/litre
Sodium cefoperazone	33
Cycloheximide	200

Description

Campy-Cefex agar is isolation agar B specified in the FDA BAM method (see Table 1).

Campy-Cefex agar was formulated by Stern, Wojton and Kwiatek[1] as a selective-differential medium for the isolation of *C. jejuni* from chicken carcasses.

In a comparison against Campylobacter-cefoperazone-deoxycholate agar (CCDA) and Campylobacter brucella agar (Campy-BAP) Campy-Cefex was found to be as productive and selective as the other media.

However, it was easier to differentiate *C. jejuni* from other flora on Campy-Cefex agar than it was on CCDA agar. Selectivity was superior to Campy-BAP. The easier differentiation of *C. jejuni* is attributed to the transparent nature of Campy-Cefex.

The high concentration of cycloheximide in Campy-Cefex medium enables it to more effectively inhibit the growth of moulds and yeasts which is frequently associated with poultry samples than either CCDA or Campy-BAP media.

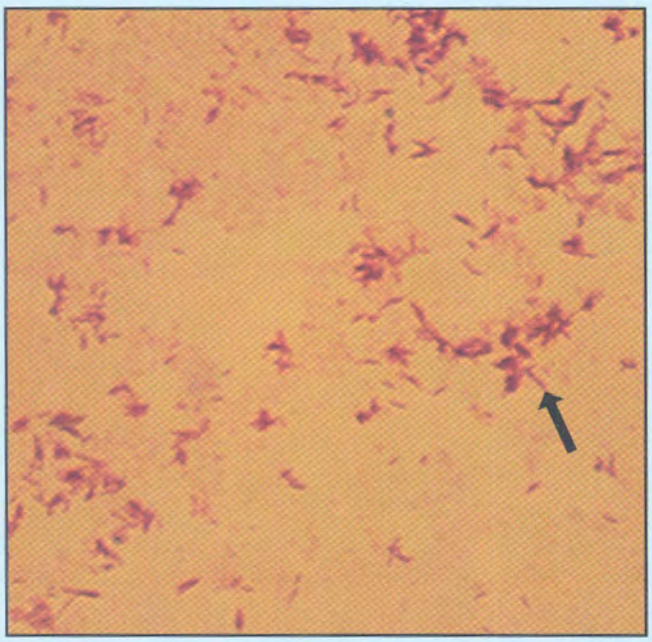
Since the addition of amphotericin B to CCDA medium it can be expected that the performance of CCDA has improved in this respect.

Reference

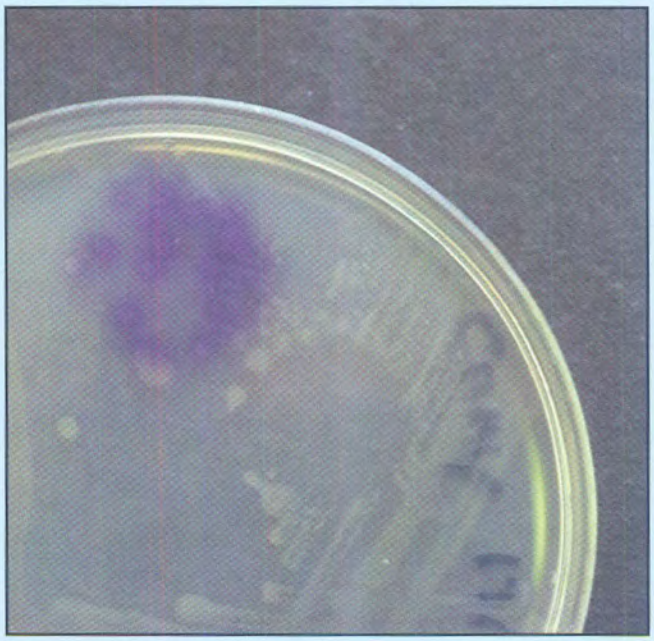
Stern, N.J. and Line, J.E. (1992). *J. Food Prot.* **55,** 514-517.

TABLE 9 – The selective agents incorporated in some Campylobacter plating media
Concentrations in mg/litre unless otherwise stated

Medium	Ampho-tericin B	Baci-tracin	Cefoper-azone	Cephalo-thin	Cepha-zolin	Colistin	Cyclo-heximide	Novo-biocin	Poly-myxin B	Rifam-picin	Teieo-planin	Trime-thoprim	Vanco-mycin	Sodium Deoxy-cholate
Blaser-Wang				15				5	2,500 i.u.				10	
Blood-free CCOA	10		32											1 gram
Blood-free CAT			8								4			1 gram
Blood-free Karmali			32				100						20	
Butzler		2,500 i.u.			15	10,000 i.u.	50	5						
Butzler Virion			15			1,000 i.u.				10				
Campy-Cefex			33				200							
Exeter	2		15						4	10		10		
Preston							100		5,000 i.u.	10		10		
Skirrow									2,500 i.u.			5	10	



Gram-stain of a Campylobacter culture. Note the curved shape (arrowed). The degenerate appearance shown by many of the cells is typical of ageing cultures.



The Oxidase test. Colour reaction of oxidase-positive colonies following application of Kovac's reagent directly to *Campylobacter jejuni* growth on nutrient agar.

Identification of Campylobacter Species

Detailed procedures for identification of campylobacters lie outside the scope of this publication. However, the following brief summary of tests may be helpful. Microbiologists are advised to consult specialised texts for full methodology.

Simple tests to Identify Campylobacter
Colonies that appear to be Campylobacter can be differentiated from other species that are capable of growth on selective media as follows:

1 Gram stain
Use carbol fuchsin for counter-staining as it stains campylobacters more intensely than safranin. Campylobacter cells are Gram-negative and have a spiral or S-shaped appearance. Cells in older cultures change to a coccal appearance.

2 Motility test
Examine a wet film from a broth culture for the characteristic darting, corkscrew-like movement.

3 Catalase test
Add several drops of 3% hydrogen peroxide to 24–48 hours growth on Blood Agar Base

CM55. Production of bubbles indicates a positive result.

Media containing blood may give false-positive reactions.

4 Oxidase test

(a) *Direct plate technique*

Touch a colony with the moistened reagent–impregnated tip of an Oxoid Oxidase Touch Stick code BR64. Observe the tip for a colour change to blue-purple.

Alternatively add 1 drop of freshly-prepared Kovac's reagent directly to the colony to be tested. Development of a dark purple colour indicates a positive oxidase test.

Liquid oxidase reagent is unstable and must be replaced daily or when it develops a pronounced purple colour.

Oxidase test using Oxoid Touchsticks code BR64.
Left: Negative test. Centre and right: Positive test.

(b) *Indirect technique*

Smear a colony onto a paper strip moistened with Kovac's reagent.

Observe for a colour change to blue-purple.

Note – Colonies tested from selective media may show weak or negative reactions. If in doubt, subculture to non-selective medium and re-test.

Growth temperature

The thermophilic campylobacters (*C. jejuni, C. coli* and *C. lari*) grow well at 43°C but not at 25°C.

Differentiation between Campylobacter spp.

C. jejuni, C. coli and *C. lari* may be differentiated by hydrolysis of hippurate, hydrolysis of indoxyl acetate, production of hydrogen sulphide and susceptibility to nalidixic acid and cephalothin (30 µg discs). (See Table 10.)

Hippurate hydrolysis

Grow the strains to be tested for 18 hours on blood agar at 37°C in a microaerobic atmosphere.

Suspend a 2 mm loopful of growth in 2 ml of sterile distilled water. Add 0.5 ml of aqueous solution of sodium hippurate and incubate for 2 hours in a water bath set at 37°C.

Add 1 ml of ninhydrin solution (3.5 gram ninhydrin in 100 ml of a 1:1 mixture of acetone and butanol) and leave for a further 2 hours at room temperature.

The development of a purple colour indicates a positive test. No colour or a pale mauve tint indicates a negative test.

Include negative and weakly-positive control strains in every test.

Indoxyl acetate hydrolysis

Hydrogen sulphide production. Left: Negative test. Right: Positive test showing blackening of FBP medium caused by production of hydrogen sulphide.

Apply growth from a 24 hour culture on CCDA agar base to a moistened paper strip or disc previously impregnated with 10% indoxyl acetate in acetone and air-dried.

The development of a blue/grey colour within 10 minutes at ambient temperature indicates a positive result. Lack of colour development indicates a negative result.

Hydrogen sulphide production

Inoculate a large loopful of overnight growth into a tube of Nutrient broth No. 2 code CM67 containing 1.2 gram/litre Oxoid Agar No. 3 code L13 and FBP. (Oxoid Campylobacter Growth Supplement code SR84, 1 vial in 500 ml of medium.)

Leave at room temperature for 4 hours and examine for blackening around the inoculum. Cultures showing negative results (no blackening) should be re-incubated overnight before discarding as negative.

Include negative and weakly-positive control strains in every test run. H_2S tests may also be conducted in triple-sugar iron agar (TSI) or by using lead acetate test strips. Results may vary according to method.

Susceptibility to nalidixic acid and cephalothin

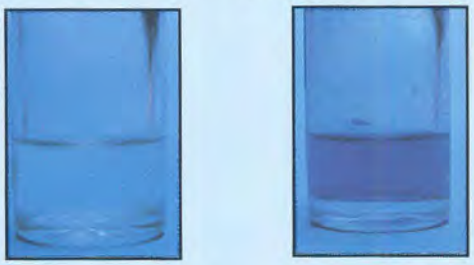
Susceptibility to nalidixic acid and cephalothin is determined using the disc diffusion technique.

Prepare a light suspension of 24 hour growth in 0.1% peptone water. Inoculate a plate of Diagnostic Sensitivity Test Agar code CM261 containing 5% v/v Laked Horse Blood code SR48 over the entire surface using a sterile swab soaked in the culture suspension. Apply 30 µg susceptibility discs of nalidixic acid and cephalothin.

Incubate at 37°C in a microaerobic atmosphere for 48 hours or 72 hours for slowly-growing cultures. Any size zone of inhibition around the discs indicates susceptibility to the antibiotic.

The test should be controlled with known strains of *C. jejuni, C. coli* and *C. lari.*

Note - Nalidixic acid resistance has emerged amongst strains of *C. jejuni*, consequently susceptibility to nalidixic acid is now a less reliable characteristic of this species.



Hippurate hydrolysis. Left: Negative test. Right: Positive test.
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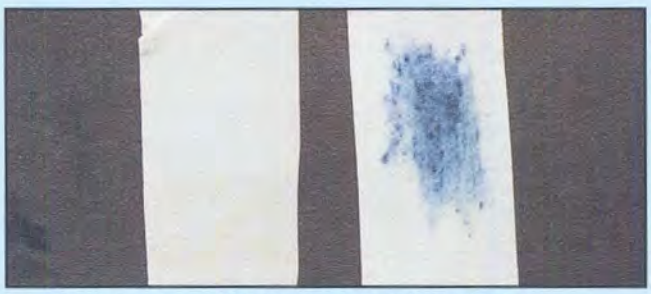


Indoxyl acetate hydrolysis. Left: Negative test. Right: Positive test.

TABLE 10 – Differentiation of thermophilic Campylobacter species

	C. jejuni	*C. coli*	*C. lari*
Growth at 25°C	–	–	–
Growth at 37°C	+	+	+
Growth at 42°C	+	+	+
Catalase	+	+	+
Hippurate hydrolysis	+	–	–
Indoxyl acetate hydrolysis	+	+	–
H_2S production	+*	–	+
Antibiotic susceptibility:**			
Nalidixic acid 30 µg	S	S	R
Cephalothin 30 µg	R	R	R

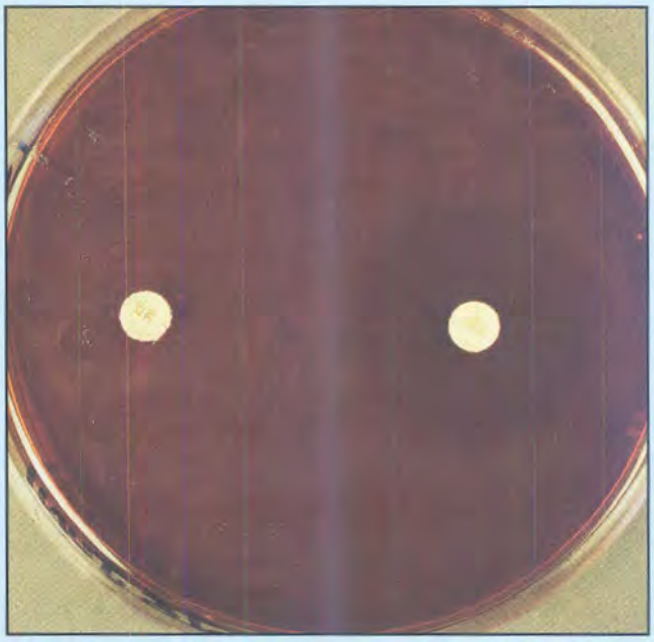
Key

* Negative if tested on TSI Agar.
** S = Sensitive and R = Resistant.

Control strains of Campylobacter spp.

Known positive and negative control strains should be included in all tests. Suitable strains are:

C. jejuni	Biotype 1	NCTC	11168
	Biotype 2	NCTC	11392
C. coli		NCTC	11366
C. lari		NCTC	11352: ATCC 35221



Nalidixic acid and cephalothin susceptibility. Right-hand side: Zone of growth inhibition around a 30µg nalidixic acid disc. Left-hand side: Growth up to the edge of a 30µg cephalothin disc indicates resistance to the antibiotic.

Microaerobic Incubation

Campylobacters are microaerophilic, requiring approximately 6% of oxygen.

In addition to their need for a reduced concentration of oxygen, *Campylobacter spp.* require a greater concentration of carbon dioxide than is present in air. Additionally some species may benefit from the presence of hydrogen but the thermophilic campylobacters will grow satisfactorily without it.

Kiggins and Plastridge[1] in an investigation into gaseous requirements of *Vibrio fetus* (now *Campylobacter fetus*) found that a concentration of 10% carbon dioxide and 5% oxygen was optimal for growth.

Experience with other species has shown that 5–10% carbon dioxide appears to be a general requirement of the genus *Campylobacter*. The correct proportions of gases in the microaerobic atmosphere can be achieved most consistently by drawing a vacuum in the gas jar and replacing the air with a special gas mixture of 5% oxygen, 10% carbon dioxide and 85% nitrogen from a cylinder. However, special gas mixtures are expensive and may not be readily available, cylinders are heavy and not easily manoeuvrable and there is a potential safety hazard from the presence in the laboratory of a highly pressurised vessel containing hydrogen.

A considerable amount of ingenuity has gone into finding economical and effective ways of creating microaerobic conditions. In many cases, methods already known to be successful for other oxygen-sensitive organisms have been used, but less obvious methods have also been tried.

Burning a candle in a closed incubation vessel to reduce the oxygen content of the air has been reported to be successful and particularly appropriate where expense is a major concern.[2] The amount of oxygen consumed in the "candle jar" can be increased by burning methylated spirit instead of candle wax.[3] Further improvements were reported when it was found that increasing the speed and intensity of burning reduces the oxygen level even further.[4]

The Fortner Principle has been applied to isolation of *Campylobacter spp.* from faeces.[5] This is a simple biological technique for reducing oxygen tension which does not need conventional anaerobic isolation equipment. The principle utilises the ability of a rapidly growing facultatively-anaerobic organism to deplete the oxygen contained in a closed system thus making it possible for oxygen-sensitive organisms to grow.

Production of appropriate concentrations of oxygen and carbon dioxide by a method which involves oxidation of steel wool soaked in copper sulphate to reduce the oxygen content of air, with simultaneous production of carbon dioxide from Alka-Seltzer effervescent antacid tablets has been described.[6] The reagents are contained within two cups and sealed with the culture plates in a plastic bag.

Growth of *C. jejuni* and *C. coli* has been reported in an atmosphere of 10% carbon dioxide in moist air in gas jars and CO_2 incubators.[7] Isolation has also been reported in an enriched atmosphere of carbon dioxide produced from dry ice.[8]

Increasing the carbon dioxide concentration without decreasing the oxygen content constitutes a compromise that may prevent the detection of some strains. Improved isolation rates in such sub-optimal conditions may be obtained by incorporating ferrous sulphate, sodium metabisulphite and sodium pyruvate (FBP) in the culture medium. Oxoid FBP Supplement is described on page 30.

Commercial gas generating devices provide a simple and convenient means of producing a reliable microaerobic atmosphere. Water is added to sachets to activate reagents which generate hydrogen and carbon dioxide. Catalysis of the hydrogen and oxygen present in the air sealed into the gas jar produces water, leaving an oxygen-depleted atmosphere and additional carbon dioxide.

A further development is the introduction of an alternative device, CampyGen, which does not produce hydrogen, does not require the addition of water and does not need a catalyst. Exposure of the sachet to air initiates a chemical reaction which simultaneously absorbs oxygen and produces carbon dioxide. This product and Oxoid Gas Generating Kits are described on pages 31 and 32.

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Campylobacter Growth Supplement (FBP) **Code:** SR84

Vial contents
(each vial is sufficient for 500 ml of medium)

Sodium pyruvate	0.125 g
Sodium metabisulphite	0.125 g
Ferrous sulphate (hydrated salt)	0.125 g

Directions
To rehydrate the contents of the vial, aseptically add 2 ml of sterile distilled water and invert to dissolve. Avoid frothing of the solution. Add to 500 ml of culture medium.

Description
Campylobacter species, which require less oxygen (i.e. microaerophilic) are inhibited at the nominal atmospheric oxygen level. The optimum level of oxygen required for growth has been reported to be 6%.[1] The exacting level, together with a carbon dioxide requirement, has made isolation of these organisms from human and animal sources a demanding procedure.

It is postulated that microaerophilic bacteria are more sensitive than other oxygen dependent bacteria to toxic forms of oxygen (superoxide anions, peroxide, etc.) that occur in aerobic culture media. Compounds which enhance the aerotolerance of microaerophilic bacteria do so by quenching these toxic forms of oxygen.[2]

Ferrous sulphate, sodium metabisulphite and sodium pyruvate (FBP)[3] has been found to increase the aerotolerance of *Campylobacter spp.*

Addition of these compounds, presented together as Oxoid Campylobacter Growth Supplement SR84 to the culture medium, will enable *Campylobacter spp.* to be more easily and rapidly isolated on a routine basis.

This means that the probability of isolating strains of *Campylobacter spp.* increases considerably. Those laboratories that can use candle jars only, or use very approximate gas mixtures, will particularly benefit from the use of the growth supplement.

FBP protects medium from toxic compounds that may be formed because of exposure to light and air. Studies have demonstrated the efficacy of FBP in the repair of damaged Campylobacter cells in foods.[4,5,6]

FBP is included in Preston enrichment broth[7,8] and selective enrichment broths based on Nutrient Broth No. 2 specified by the Food and Drug Administration (FDA) for the detection of Campylobacter in foods, milk and water[9] and in Exeter medium specified by the Steering Group on the Microbiological Safety of Foods.[10]

In a modification of the FDA Bacteriological Manual enrichment protocol, FBP as a single supplement was shown to provide better growth compared to the standard enrichment method which utilises blood in addition to FBP. FBP was found to be superior for producing growth in enrichments of both coccal and vibroid forms.

FBP supplement may also be used for detection of hydrogen sulphide production by *Campylobacter spp.* FBP test medium is made by adding FBP and phosphate buffer to Nutrient Broth No. 2 code CM67. Agar is also added to make the medium semi-solid.[12]

A variant of FBP test medium suitable for multipoint inoculation has been described.[13]

FBP supplement is incorporated in an improved medium for storage and transportation of thermophilic campylobacters.[14]

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Campylobacter jejuni showing characteristic micraerophilic growth in deep culture. Note the area of maximum growth below the surface and diminishing towards the base of the bottle.

Campylobacter Gas Generating Kits **Code:** BR56/BR60

Description

The Oxoid Gas Generating Kits for Campylobacter isolation, BR56 and BR60 constitute a reliable and convenient method for producing suitable gaseous conditions, in standard anaerobic jars, for organisms such as *Campylobacter species* which require a reduced oxygen atmosphere.

BR56 and BR60 are disposable gas generating sachets that produce hydrogen and carbon dioxide in sufficient quantity that, after reaction with a palladium catalyst in an anaerobic jar, will produce an optimal gaseous atmosphere for the growth of campylobacters and other microacrophilic organisms. BR56 is designed for 3.0–3.5 litre jars and is suitable for the Oxoid Anaerobic Jar HP11 and for many other jars currently in use in laboratories. When used as directed each sachet will produce about 1,000 ml hydrogen and 350 ml carbon dioxide.

BR60 is designed for 2.5-3.0 litre jars and is for use with the small light-weight plastic anaerobic jar. It is not suitable for use with the Oxoid Anaerojar AG025A which should be used only with Anaerogen and CampyGen sachets. When used as directed, each sachet of BR60 will produce about 700 ml hydrogen and 250 ml carbon dioxide.

In each case a residual concentration of about 6% oxygen and 10% carbon dioxide in the jar is obtained in under 30 minutes.

Some variation in the oxygen level in each jar will occur, depending on the number of inoculated plates.

Use of an anaerobic indicator to check the efficiency of catalysis is inappropriate but catalysis will produce pressure changes which can be observed from the gauge fitted to the Oxoid Anaerobic Jar. After an initial pressure increase of approximately 0.1 bar, catalytic activity will lead to a pressure reduction to zero or even –0.05 bar. Should the catalyst be inactive the pressure will rise to approximately 0.2 bar and be maintained.

Directions

1. Cut off the corner of a sachet as indicated by the broken line.

   Avoid folding or crushing the sachet.

2. Add a measured 10 ml of water.

3. Immediately stand the sachet upright in the anaerobic jar *fitted with an active catalyst* and close the lid according to the manufacturer's instructions. (If preferred, water may be added to sachets already located in the jar.) The use of the Oxoid catalyst is recommended, because of its high efficiency, combined with inbuilt safety features. Activity of the catalyst may be prolonged by ensuring that it is dry on each occasion it is used. Heating to 160°C for 90 minutes after each use is recommended.

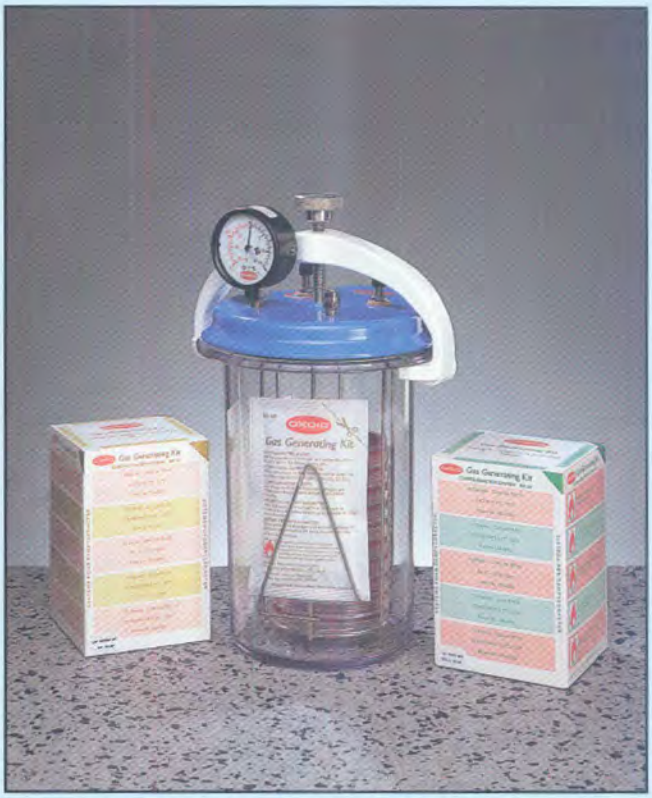
4. *Disposal*

   After opening the jar, the exhausted sachet should be removed without spilling the contents. The solution left in the sachet is mildly acid and may be poured away into a sink and flushed with running water. The empty sachet can then be discarded with normal laboratory litter.

Warning

Gas Generating Kits activated but not in gas jars should be kept away from unguarded flames and sparks. Once the reaction has subsided (after about 30 minutes) the sachet can be discarded as above.

Campylobacter Gas Generating Kit showing the hydrogen and carbon dioxide generating components within the sachet. Unipath Marketing collection.



The Oxoid Anaerobic Jar with Campylobacter Gas Generating Kits for 2.5 litre and 3.5 litre gas jars. Unipath Marketing collection.

CampyGen **Code:** CN025A/CN035A

Description

Oxoid CampyGen creates a suitable atmosphere for growth of *Campylobacter spp.* by decreasing the oxygen content and increasing the carbon dioxide content in air.

It is very easy and safe to use; the sachet is removed from its protective foil pouch and placed in an anaerobic jar immediately before sealing.

It is not necessary to add water to activate CampyGen. Hydrogen is not produced and there is no increase in pressure within the jar. A catalyst is not needed. The reagents contained within the sachet become active on contact with air. Oxygen is rapidly absorbed with the simultaneous generation of carbon dioxide. The chemical reaction is exothermic and it is quite normal for the sachet to become warm to the touch.

CampyGen is supplied in two sizes, for 2.5 litre and for 3.5 litre jars. The smaller size is for use with small anaerobic jars and the Anaerojar, a lightweight gas jar specially designed for use with Oxoid Anaerogen and CampyGen. The larger size is used with the Oxoid 3.5 litre anaerobic jar. Use of the appropriate CampyGen sachet with either of these jars ensures the creation of optimal atmospheric conditions for the growth of *Campylobacter spp.* and other microaerophilic microorganisms.

Directions

Remove the CampyGen sachet from its protective foil pouch and place in the Anaerojar or Oxoid Anaerobic Jar immediately before sealing.

Disposal

CampyGen sachets may be disposed of with normal laboratory dry waste.

Used CampyGen sachets may possess residual activity and become warm to the touch on removal from the jar.

The Oxoid Anaerojar in use with a CampyGen sachet. Unipath Marketing collection.

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