Is enrichment culture necessary for the isolation of *Campylobacter jejuni* from faeces?

DN HUTCHINSON, FJ BOLTON

From the Public Health Laboratory, Preston Infirmary, Meadow Street, Preston, Lancashire, PR1 6PS

SUMMARY The role of enrichment culture for the isolation of *Campylobacter jejuni* from faeces is discussed. It is concluded that enrichment culture is only necessary for those specimens where it is anticipated that the number of organisms is likely to be low. In a trial of a blood free enrichment broth (CCD broth) and the modified Preston enrichment broth the latter gave significantly superior results.

Six years ago Skirrow described the first selective agar for the direct isolation of *Campylobacter jejuni* from the faeces of persons with enteritis.¹ Thus microbiologists, worldwide, were stimulated to recognise this "new" pathogen. Subsequently several other selective media have been described²⁻⁴ which can be used for the isolation of the organisms from faeces and environmental specimens.

Enrichment techniques for the isolation of campylobacters from environmental specimens are almost routine and although the use of enrichment broth for the isolation of the organism from human faeces has been undertaken, it is not widely practised. Tanner and Bullin⁵ advocated the use of alkaline peptone water followed by subculture onto Skirrow's agar.¹ Blaser *et al.*,⁶ Bolton and Robertson,⁴ Bolton *et al.*,⁷ Chan & McKensie⁸ and Martin *et al.*⁹ all found increased isolation rates following selective enrichment culture but Gilchrist *et al.*¹⁰ were unable to demonstrate an advantage over direct plating.

During the trial of a blood-free selective medium (CCD agar) (unpublished data) it was decided to evaluate the medium as an enrichment broth (CCD broth) for faecal culture. A comparison with the modified Preston enrichment broth⁷ was made and the optimal subculture time determined.

This paper presents the findings of these studies and discusses those situations in which enrichment culture of faeces may be of value.

Material and methods

BLOOD FREE SELECTIVE MEDIUM (CCD AGAR)

The following ingredients are added to one litre of

deionised water: Nutrient Broth No 2 (Oxoid) 25 g, New Zealand agar (Davies) 12 g, Bacteriological Charcoal (Oxoid) 4 g, casein hydrolysates (Oxoid) 3 g. Ten millilitres of 10% aqueous sodium deoxycholate (BDH), 5 ml of 5% aqueous ferrous sulphate and 5 ml of 5% aqueous sodium pyruvate are added to the basal medium to give final concentrations of 0.1%, 0.025% and 0.025% respectively. The medium should be adjusted to pH 7.4 and sterilised by autoclaving at 121°C for 15 min. One millilitre of a 10 000 mg/l aqueous solution of cephazolin (Eli Lilly and Co Ltd) should be added to the cooled molten agar to give a final concentration of 10 mg/l.

A selective enrichment broth was also prepared which had a similar formulation except for the omission of agar and the addition of sodium metabisulphite at a final concentration of 0.025%. The broth medium (CCD broth) was distributed in 6 ml volumes in screw capped ¼ oz (7g) bijoux containers.

BLOOD CONTAINING SELECTIVE MEDIUM (PRESTON AGAR)⁴

This was prepared to the following formulation: Nutrient Broth No 2 (Oxoid) 25 g/l, New Zealand Agar (Davies) 12 g/l, pH 7.4 and sterilised by autoclaving at 121°C for 15 min. Saponin-lysed horse blood 5%, polymixin sulphate 5,000 IU/l, trimethoprim lactate 10 mg/l, rifampicin 10 mg/l and cyclohexamide 100 mg/l are added to the cooled molten agar prior to pouring.

The modified Preston enrichment broth contains similar constituents with the exception of agar and with the addition of ferrous sulphate, sodium metabisulphite and sodium pyruvate (FBP supplement)¹¹ each at a final concentration of 0.025%. The broth was distributed in 6 ml volumes in screw capped ¼ oz (7g) bijoux containers.

Table 1 Comparison of campylobacter isolation from direct plating and enrichment culture of faeces

Direct plating on CCD agar and Preston agar	Enrichment CCD broth	Enrichment Preston broth	Total No of isolations
+	+	+	34
+	-	+	2
-	+	+	2
-	-	+	3

FAECAL SPECIMENS

Two hundred and sixty-two faecal specimens, from patients with gastroenteritis, were cultured on the day of arrival in the laboratory. Each specimen was sampled with a cotton tipped swab. This faecal swab was inoculated directly onto both CCD agar and Preston agar and the inoculum was spread out to produce discrete colonies. The plates were incubated at 42°C in a microaerobic atmosphere obtained by evacuating anaerobic jars to 500 mm Hg and refilling with 10% CO₂ in H₂. Plates were examined after 24 h and 42 h incubation.

Enrichment culture was performed using faecal swabs to inoculate faeces directly into CCD broth and modified Preston enrichment broth. These were incubated at 42°C aerobically with the tops tightly closed and subcultured after 24 h and 42 h onto plates of CCD agar and Preston agar. Subculture plates were incubated microaerobically at 42°C for up to 42 h.

Differences between test methods were analysed statistically using McNemar's test.¹²

Results

Forty-one isolations of *Campylobacter jejuni/coli* were made from 262 specimens following enrichment culture methods compared with 36 isolates obtained on direct plating (Table 1). All campylobacters isolated on direct culture were also isolated following enrichment culture.

The results (Figure) show that all of the 41 isolates were obtained after enrichment in the Preston broth and 36 were recovered from the CCD broth. The number of isolations made from the two

Isolation of campylobacters from enrichment cultures of 262 faecal specimens

Key:

- a Enrichment broths
- b Time of subculture of enrichment broths
- c Subculture medium: CA (CCD agar), PA (Preston agar)

enrichment broths following subculture at two different times is also shown in the Figure. Both the modified Preston broth and the CCD broth gave a significantly greater number of isolations if subcultured after 24 h instead of 42 h.

In all but one sequence subculture onto Preston agar proved more satisfactory than subculture onto CCD agar but in no case was the difference statistically significant ($p < 0.05$). The effectiveness of the various combinations of broth and agars to reduce contamination is given in Table 2. The growth of contaminants was significantly greater in CCD broth subcultured to CCD agar than in the other three combinations ($p > 0.001$).

In this study the modified Preston enrichment broth was significantly ($0.05 > p > 0.01$) superior to the CCD broth but under optimum conditions, ie 42°C incubation and subculture at 24 h, it did not matter which of the two selective agars was used for subculture.

Table 2 No of selective broths which grew contaminating organisms following culture of 262 faeces samples

Incubation temperature	Subculture time	CCD broth Subculture medium		Preston broth Subculture medium	
		CCD agar	Preston agar	CCD agar	Preston agar
42°C	24h	118 (45)	11 (4)	21 (8)	8 (3)
	42h	121 (46)	2 (1)	25 (10)	12 (5)

Figures in parentheses are percentages.

Discussion

It has been recognised for many years that enrichment culture of faeces is necessary for the optimum detection of Salmonella infection whereas in acute Shigella infections the organisms are readily isolated on direct culture. The role of enrichment culture for the routine isolation of campylobacters from faeces has not been clearly defined. The possible value of enrichment is demonstrated in this study by the 14% increase in the number of isolations following enrichment culture.

Of the five specimens positive only after enrichment culture three were convalescent specimens from known positive patients, and one was an "acute" specimen received in the laboratory five days after collection. The other specimen was from a patient whose illness had started when he was abroad and who still had minor symptoms present on his return. It is probable that in all of these specimens the number of viable campylobacter organisms was small and that enrichment allowed them to grow to a detectable level. Evidence to support this surmise was obtained during the initial trials of the Preston medium⁴ when it was found that four known campylobacter positive specimens which were positive only after enrichment and subculture contained < 10⁴ organisms per gram of faeces. It is most likely that a reduction in the number of campylobacters and overgrowth of contaminants accounted for the findings of Martin *et al.*⁹ These workers were able to reisolate campylobacters from only 13/19 direct culture positive human faecal specimens which had been stored at 4°C for 3–4 w. However using brucella broth as the base for a selective enrichment medium they were able to reisolate campylobacters from all 19 specimens. Also using the enrichment broth three additional isolations were made from amongst the batch of direct culture negative faeces similarly stored; a 16% increase in isolation rate.

The value of comparisons between direct plating and enrichment techniques depends upon the selective agar used for direct plating, the type of enrichment medium the subculture medium and other variables including, temperature of incubation, time of reading plates and time of subculture of the enrichment medium. Gilchrist *et al.*¹⁰ concluded that for the isolation of campylobacters from faeces, enrichment in a selective thioglycollate broth was only equivalent to direct plating onto selective agars and in their experience made diagnosis slower. However Blaser *et al.*⁶ using a similar medium found a significant increase in the isolation rate following enrichment whilst Chan and Mackenzie⁸ using a semi-solid enrichment medium achieved a 6% increase in isolation

over direct culture on a modified Skirrow medium. Riberio *et al.*¹³ using Skirrow's agar for both direct inoculation and subculture reported a 74% increase in the number of isolations from faeces following the use of the Preston enrichment broth. This contrasts with our results when using the Preston agar for direct and subculture plating. Using the Preston enrichment broth we found a 15% increase in isolation of campylobacters from human faeces.⁷

Although there is an increasing amount of evidence supporting the value of enrichment culture for the isolation of campylobacters we do not think it should be used routinely. It is our opinion that for the isolation of *Campylobacter jejuni/coli* direct culture onto a good selective agar is adequate for the examination of human faeces collected from subjects with acute diarrhoea. However, enrichment cultures for campylobacter isolation should be considered for those faecal specimens where there has been delay in the transit of the specimen to the laboratory or where the only specimen collected is one taken in the convalescent phase of a diarrhoeal illness.

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Requests for reprints to: Mr FJ Bolton, Public Health Laboratory, Preston Infirmary, Meadow Street, Preston, Lancashire, PR1 6PS, England.