Influence of transport conditions and media on *Helicobacter pylori* isolation

Helicobacter pylori infection is strongly associated with chronic gastritis, peptic ulcer disease and gastric malignancy (Marshall, 1994). Failure to detect *H. pylori* may be due to inappropriate transport or culture media, sampling error or short length of incubation (van der Hulst *et al.*, 1996). Recovery rates have ranged from 77 to 90.2 % from biopsy specimens transported in Portagerm pylori in cooled containers for 3 days and have reached 97 % from specimens that were stored in glycerol broth at -70 °C (Heep *et al.*, 1999; Grove *et al.*, 2001). *H. pylori* recovery rates from *H. pylori*-positive gastric biopsy specimens stored in selective transport media have been 76–46 % after storage for 5–9 days (Siu *et al.*, 1998). The aim of the present study was to evaluate the influence of short- and long-term freezing of stomach biopsies in Stuart's transport medium and the use of selective and non-selective media on the primary isolation of *H. pylori*.

Between January 1999 and November 2002, 414 dyspeptic patients were investigated (Table 1). None had been treated previously

for *H. pylori* infection. A single antral biopsy specimen was taken from 183 children and two antral biopsy specimens were taken from each of 231 adult patients. The biopsies were taken 3–5 cm from the pylorus. The 294 fresh specimens were transported in Stuart's transport medium (Merck) for less than 5 h, 61 specimens were frozen in Stuart's transport medium at -70 °C for 1–7 days and 59 specimens were frozen in Stuart's transport medium at -70 °C for 8–50 days.

Biopsy specimens were divided into three parts. A smear was prepared from one part by scraping the biopsy on a slide. The smear was used for a modified Gram stain with carbol fuchsin as the counterstain. Another part of the biopsy specimen was placed in urea (10 %) agar medium, as described previously (Boyanova *et al.*, 1996), incubated at 37 °C and observed for colour change after 30 min and 3 h. The remaining part of the specimen was homogenized in 0.1 ml sterile saline with sterile needles and the homogenate was inoculated onto blood agar [Columbia agar base (Oxoid) or

Brucella agar base (Becton Dickinson)] with 10 % defibrinated sheep blood and 1 % Isovitalex (Becton Dickinson), with or without *H. pylori*-selective supplement of Dent (Oxoid). The specimens were inoculated onto one selective and one non-selective plate and incubated for 12 days in a microaerobic atmosphere (Campy Pak; Becton Dickinson). *H. pylori* was identified by Gram staining of suspect colonies, lack of aerobic growth on blood agar and testing for the presence of urease, oxidase and catalase. Frozen specimens were cultured by the same technique after a short period of thawing that did not allow the transport medium to defrost completely. *H. pylori* growth on selective and non-selective media was compared for 102 fresh biopsy specimens. Specimens were considered to be *H. pylori*-positive if either the culture or two of the three diagnostic methods gave positive results. Differences between groups were compared by a χ^2 test without or with Yates' correction factor.

H. pylori isolation rates from the positive specimens from adults were 93.5 % from fresh biopsies and 85.2 % from those that were frozen for 5–50 days ($P > 0.20$), and from paediatric specimens, were 97.7 % from fresh specimens and 90.9 % from long-term-frozen ones ($P > 0.20$) (Table 1). *H. pylori* grew only after incubation for 7–12 days in 11.3 % of the positive fresh specimens but this longer incubation period was required in significantly more (29.7 %, $P < 0.01$) of the positive specimens that had been frozen for 8–50 days (Fig. 1). In 0.5 % of fresh positive specimens and 5.4 % of long-term-frozen positive specimens, *H. pylori* growth was visible only after incubation for 11 days. Of 102 isolates, *H. pylori* grew only on selective medium in 31.4 % and only on non-selective medium in 14.7 % of cases.

Although the best storage media for frozen isolates have been reported to be skim milk/glycerol, Brucella broth/glycerol and Cysteine-Albimi/glycerol (Han *et al.*, 1995),

Table 1. *H. pylori* isolation from fresh and frozen biopsy specimens from non-treated patients

Freezing time (days)	Specimens (n)	<i>H. pylori</i> -positive (%)*	<i>H. pylori</i> isolated (%)	Isolation rate from positive specimens (%)
Adults				
1–7	46	69.6	67.4	96.9
None	147	73.5	68.7	93.5
8–50	38	71.1	60.5	85.2
Children				
1–7	15	53.3	53.3	100.0
None	147	59.2	57.8	97.7
8–50	21	52.4	47.6	90.9
Total				
1–7	61	65.6	63.9	97.5
None	294	66.3	63.3	95.4
8–50	59	62.7	55.9	89.2

*Specimens were considered as *H. pylori*-positive if only culture or two of the three diagnostic methods were positive.

Fig. 1. Length of incubation (days) required for growth of *H. pylori* isolates from fresh biopsy specimens (open bars) and specimens frozen for 1–7 days (hatched bars) or 8–50 days (filled bars).

the present results suggest that Stuart's transport medium can be used for frozen biopsy specimens.

Multiple factors can decrease *H. pylori* isolation rates and could act simultaneously. The present results showed a decrease in isolation rates from long-term-frozen positive specimens by 9.1 % for adults and 7 % for children. The use of single specimens per patient is another unfavourable factor. *H. pylori* has been isolated from only one of two biopsies in 13 % of 39 cases (Kjoller *et al.*, 1991). According to van der Hulst *et al.* (1996), a 10-day incubation was required to detect all *H. pylori* infections with non-selective media, whereas a 7-day incubation was successful with selective media. The present results showed that an 11-day incubation increased the *H. pylori* isolation rate from positive long-term-frozen specimens by 5.4 %. Biopsies that were frozen for 8–50 days required incubation for 7–11 days for the growth of one-third of isolates. This

suggests that *H. pylori* grows slowly in frozen biopsy specimens because of temperature-related stress. It is of diagnostic and clinical importance that *H. pylori* grew on only one type of medium (either selective or non-selective) in 46.1 % of cases.

In conclusion, an 11-day incubation period is helpful for isolating *H. pylori* strains from long-term-frozen specimens. Frozen biopsy specimens provided reliable *H. pylori* isolation, although with a slight decrease in isolation rates from long-term-frozen specimens, and the use of both selective and non-selective media is important for isolation of *H. pylori*.

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