

## Comparative Evaluation of Three Selective Media and a Nonselective Medium for the Culture of *Helicobacter pylori* from Gastric Biopsies

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Plating on solid media is the standard technique used in most laboratories for the isolation of *Helicobacter pylori* from gastric biopsies. Recently, various selective media were developed for this purpose. We compared and evaluated three selective media, Skirrow's, Dent's CP, and modified Glupczynski's Brussels campylobacter charcoal media, and chocolate agar medium for the isolation of *H. pylori*. Gastric biopsies taken from a total of 203 patients were plated in parallel on all four media. An isolation rate of 51% (104 of 203) was obtained with a combination of all four media. Of the 104, 92 (88%) were positive with Dent's medium and with modified Glupczynski's medium. Skirrow's medium gave the highest isolation rate, 96% (100 of 104). However, growth of *H. pylori* was scant (only one to five colonies) when growth occurred on Skirrow's medium alone. Overall, modified Glupczynski's medium provided significantly heavier growth. Chocolate agar medium yielded a 76% (79 of 104) positivity rate. We recommend the use of a combination of two selective media for the maximum recovery of *H. pylori* from antral biopsies.

*Campylobacter pylori* has been renamed *Helicobacter pylori* because evidence provided by rRNA sequencing, fatty acid composition, and respiratory quinone profiles and its ultrastructural features have shown that this organism differs considerably from other enteric campylobacters (6, 11). However, *H. pylori* still shares some common biochemical characteristics with the enteric campylobacters, including positive catalase and oxidase reactions, nonfermentation of carbohydrates, and a requirement for microaerobic conditions for growth.

Since Marshall and Warren first described the recovery of a campylobacterlike organism from gastric mucosa of patients with gastritis and peptic ulcer disease (12, 17), many investigators around the world have successfully cultured this organism from the human stomach by adapting the culture method used for enteric campylobacters by using a temperature of 37°C and an extended incubation period (2, 4, 8, 12). Culturing on solid media is the standard technique used in most laboratories for the isolation of *H. pylori* from gastric biopsies. Selective media, such as Skirrow's medium, are required for the isolation of this organism from biopsies in which contaminating bacteria are present. Recently, two additional selective media, Dent's CP medium and Glupczynski's Brussels campylobacter charcoal (BCC) medium, were developed (3, 5). The aim of this study was to compare and evaluate the isolation of *H. pylori* on these three selective media (two of which are commercially available) and a nonselective chocolate agar medium.

### MATERIALS AND METHODS

**Media.** Dent's medium and Skirrow's medium were prepared in accordance with the manufacturer's instructions (Oxoid, Basingstoke, England). The antibiotic supplements

in Dent's and Glupczynski's media are almost identical; each medium contains 5 mg of trimethoprim per liter, 10 mg of vancomycin per liter, 5 mg of cefsulodin per liter, and amphotericin B (5 and 10 mg/liter for Dent's and Glupczynski's media, respectively). The supplements in Skirrow's medium are 5 mg of trimethoprim per liter, 10 mg of vancomycin per liter, and 2,500 IU of polymyxin B. The basal medium used for Dent's medium is 7% sheep blood agar (Columbia); that used for Glupczynski medium is 10% fetal calf serum in brain heart infusion broth containing 40 mg of 2,3,5-triphenyltetrazolium chloride (Sigma, St. Louis, Mo.) per liter, 2 g of activated charcoal per liter, 10 g of yeast extract per liter, and 18.5 g of agar per liter. Since the charcoal-yeast extract agar component used is similar in content to Oxoid Legionella CYE agar, we replaced this component with the commercial product.

**Biopsy specimens.** Two antral biopsy specimens were taken from each of a total of 203 patients referred for elective endoscopy with various upper gastrointestinal tract conditions. The biopsy specimens were collected in tubes containing 1 ml of sterile saline and having loosened lids, and the tubes were inserted into Bio-Bag environmental chambers (type cfj; Marion Scientific, Kansas City, Mo.), which were sealed immediately. The gas generator inside the Bio-Bag was activated to provide a microaerobic atmosphere. Because *H. pylori* is sensitive to atmospheric oxygen and because the time from endoscopy, including transportation to the laboratory, to the processing of the biopsy specimen may be more than a few hours, Bio-Bag environmental chambers were used to optimize the survival of the organism in the biopsy specimen prior to culturing. (Our unpublished observations show that in one instance, prior to this study, 4 of 10 biopsy specimens collected were *H. pylori* positive on Gram-stained smears but 10 of 10 were culture negative when tubes of biopsy specimens were left exposed to atmospheric conditions for more than 3 h. This problem has not

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TABLE 1. Results of culturing on selective and nonselective media for the detection of *H. pylori* in antral biopsy specimens

Growth of <i>H. pylori</i> on the following medium <sup>a</sup> :				No. of strains positive <sup>b</sup>
DCP	GBCC	SM	CHOC	
+	-	-	-	2
-	+	-	-	2
-	-	+	-	7
-	-	-	+	0
+	+	-	-	0
-	+	+	-	3
-	-	+	+	0
-	+	-	+	0
+	-	+	-	3
+	-	-	+	0
+	+	+	-	8
+	-	+	+	0
+	+	-	+	0
-	+	+	+	0
+	+	+	+	79

<sup>a</sup> DCP, Dent's CP medium; GBCC, modified Glupczynski's BCC medium; SM, Skirrow's medium; CHOC, chocolate agar. +, growth of *H. pylori*; -, no growth of *H. pylori*. The total numbers (percentages) positive for DCP, GBCC, SM, and CHOC were 92 (88%), 92 (88%), 100 (96%), and 79 (76%), respectively.

<sup>b</sup> Total, 104.

recurred since the use of Bio-Bag environmental chambers for transport.)

**Isolation and identification.** The biopsy specimens were finely minced with a sterile scalpel blade and vortexed at a high speed in 1 ml of saline in a sterile 6-oz (ca. 170-g) bottle containing glass beads. One drop of the tissue suspension was inoculated onto each of the three selective plates and onto a chocolate agar plate. After incubation at 37°C under microaerobic conditions (10% CO<sub>2</sub>, 6% O<sub>2</sub>, 84% N<sub>2</sub>) for 7 days, suspect organisms were identified as *H. pylori* on the basis of rapid urea hydrolysis (within 10 min at room temperature), catalase production, oxidase production, typical colony morphology, negative nitrate reduction, inability to grow in air, and characteristic curved gram-negative bacilli on Gram-stained smears.

## RESULTS

Over the 4-month study period, *H. pylori* was recovered from 104 of the 203 patients who underwent endoscopy, yielding an isolation rate of 51%, with a combination of the four media. Skirrow's medium gave the highest isolation rate, 96% (100 of 104) (Table 1). However, the growth of *H. pylori* was scant (one to five colonies) when growth occurred on this medium alone. Of the 104, 92 (88%) were positive with Dent's medium and with modified Glupczynski's medium (Table 1). The selective media used are highly selective, so endogenous contamination was rare and minimal. Chocolate agar plates yielded a 76% (79 of 104) positivity rate and a contamination rate (when the plates were completely overgrown by other flora) of 17% (18 of 104). Overall, modified Glupczynski's medium provided significantly heavier growth but for fewer specimens.

The seven isolates initially recovered only from Skirrow's medium were later replated on Dent's medium and modified Glupczynski's medium. The positive growth of these isolates on both media suggested that the negative primary cultures were due to sampling variation and an insufficient number of

TABLE 2. Comparison of different combinations of selective and nonselective media for the detection of *H. pylori* in antral biopsy specimens from 203 patients

Medium combination <sup>a</sup>	No. (%) positive
DCP + GBCC.....	97 (93)
DCP + SM.....	102 (98)
DCP + CHOC.....	92 (88)
GBCC + SM.....	102 (98)
GBCC + CHOC.....	92 (88)
SM + CHOC.....	100 (96)
SM + DCP + GBCC.....	104 (100)
SM + DCP + CHOC.....	102 (98)
SM + GBCC + CHOC.....	102 (98)
DCP + GBCC + CHOC.....	97 (88)
SM + DCP + GBCC + CHOC.....	104 (100)

<sup>a</sup> See Table 1, footnote a, for definitions of abbreviations.

organisms present in the biopsy specimen rather than inhibition of the organisms on these two selective media.

Table 2 summarizes the results obtained with various combinations of two media. A combination of Skirrow's medium and either modified Glupczynski's medium or Dent's medium yielded 98% positive specimens. These two combinations appeared to be the most effective in the primary isolation of *H. pylori*.

Table 3 shows comparisons of percent yields of *H. pylori* on various media in this study and in previous studies.

## DISCUSSION

Over the years, a number of different media, i.e., nonselective, selective, or a combination of both, have been proposed for the isolation of *H. pylori* (1, 3, 7, 9, 14-16). The most frequently used nonselective medium is chocolate agar. The results of the present study confirmed the finding in other studies that chocolate agar has the lowest rate of isolation of *H. pylori* (Table 3). Overgrowth of contaminants obscuring the growth of *H. pylori* on this nonselective medium probably accounts for the low yield. For selective media, Skirrow's medium is preferred, probably because it has been commercially available and has been commonly used by many laboratories for the detection of *Campylobacter* species, negating the need for introducing another medium. Our results (percent yields) with Skirrow's medium are comparable to those of previous studies (Table 3). Recent publications, including that of Dent and McNulty, have revealed that some isolates of *H. pylori* are susceptible

TABLE 3. Comparisons of percent yields of *H. pylori* on various media in this study and in previous studies

Study	Medium	Yield (%)
Dent and McNulty (3)	Dent's CP	100
	Chocolate	77
	Skirrow's	94
Krajden et al. (9)	Skirrow's	97
	Chocolate	77
Glupczynski et al. (5)	Glupczynski's BCC	94
	Glupczynski's BCC (modified)	89
Present	Chocolate	76
	Dent's CP	89
	Skirrow's	96

to polymyxin (3, 9), resulting in a lower yield of *H. pylori* on Skirrow's medium. However, this finding was not confirmed in our study of 104 isolates from the Melbourne population in Australia. In contrast, Skirrow's medium provided the highest isolation rate, as compared with Dent's and modified Glupczynski's media, which contained cefsulodin but not polymyxin B.

This comparative study of Dent's and modified Glupczynski's media revealed similar isolation rates. Nevertheless, growth on modified Glupczynski's medium was more confluent, and *H. pylori* was present in greater numbers. Since these two media possess identical antibiotic supplements, the difference may be attributable to the richer basal medium of charcoal-yeast extract agar incorporated with 10% fetal calf serum in modified Glupczynski's medium. Furthermore, the charcoal may bind toxic or interfering moieties which could otherwise affect bacterial growth. The addition of 2,3,5-triphenyltetrazolium chloride to the medium also enables easier detection of *H. pylori* colonies because of their golden appearance. Other, less commonly used selective media contain nalidixic acid which, according to some reports, may inhibit some strains of *H. pylori* (3, 5, 7, 10).

The uneven distribution of *H. pylori* in the human stomach has been observed by various investigators (1, 7, 13). Variations in bacterial numbers within antral biopsy specimens may explain why, on several occasions, scant growth of *H. pylori* (only one to five colonies) was detected by Skirrow's medium alone but subculturing demonstrated that the isolates were able to grow on Dent's and modified Glupczynski's media. Another interpretation is that Skirrow's medium may be better able to support the growth of a small inoculum when the concentration of bacteria in a biopsy sample is low.

We found that one medium used alone will not result in a maximum yield of *H. pylori*. A combination of two selective media is required for the maximum recovery of *H. pylori* from antral biopsy specimens. We recommend the combined use of Skirrow's medium and either Dent's or modified Glupczynski's medium for culturing stomach biopsy specimens when it is important to recover living organisms. Because of the material and labor costs involved in producing these media and the fastidious nature of *H. pylori*, which requires prompt processing of biopsy samples, the use of a combination of more than two media is not advisable for laboratories doing routine procedures. This recommendation is particularly relevant considering that two combinations (Dent's plus Skirrow's media or modified Glupczynski's plus Skirrow media) yielded a 98% positivity rate.

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