Evaluation of a Selective Transport Medium for Gastric Biopsy Specimens To Be Cultured for Helicobacter pylori

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Since the means of culturing Helicobacter pylori may not be available in some laboratories, prolonging the survival of this organism during transportation is a major concern in terms of improving detection rates. A selective transport medium was evaluated for the preservation of H. pylori from 254 gastric biopsy specimens collected from a rural area in China where culturing is not feasible. Gastric biopsy specimens were inoculated in sterile broth consisting of brain heart infusion (BHI) broth, horse serum, and yeast extract supplemented with vancomycin, amphotericin B, and nalidixic acid (VAN). Of the 254 biopsy specimens, 238 were identified by histology to have H. pylori infection. Total rates of recovery of H. pylori from the H. pylori-positive gastric biopsy specimens stored in the BHI-VAN broth ranged from 76 to 46% after storage of specimens for 5 to 9 days. In conclusion, the selective medium is useful for prolonging the survival of H. pylori in gastric biopsy specimens for which immediate culture is not feasible.

Although various methods have been developed for detecting Helicobacter pylori infection, bacterial culture remains extremely important. Isolation of H. pylori enables susceptibility testing, which predicts the likelihood of eradication (4, 5, 10). Fingerprinting of different bacterial strains is also crucial in many epidemiological studies (1), such as in the study of reinfection or recrudescence of infection and the mode of transmission. Unfortunately, H. pylori is a fastidious organism (6). Various factors, including bacterial density, transport conditions, culture medium, and microaerophilic condition, directly influence the yield of culture. Since culture for this organism may not be possible for inadequately equipped research centers, prolonging the survival of H. pylori during transportation is a major concern in terms of improving isolation rates. Nevertheless, Roosenaad et al. have suggested that the composition of transport medium is not critical for the survival of H. pylori in gastric biopsy specimens with a delay of up to 24 h (13).

Previous studies on the optimal transport conditions for H. pylori employed pure cultures of H. pylori rather than gastric biopsy specimens (14, 17). In other studies, the short storage time (7, 13, 15) and the small number of gastric biopsy specimens involved (3) made it difficult to generalize the results to clinical practice. In this study, we evaluated the use of a selective medium for storage and transport, for up to 9 days, of gastric biopsy specimens collected from a rural area in China.

The transport medium evaluated in this study was a sterile broth that contained brain heart infusion (Oxoid, Basingstoke, United Kingdom) broth with horse serum (5%), yeast extract (0.25%), 6 mg of vancomycin per liter, 4 mg of amphotericin B per liter, and 20 mg of nalidixic acid per liter (BHI-VAN). After inoculation with biopsy specimens, the broth was stored at 4°C until culture could be performed. In each case, the gastric biopsy specimens alone and the specimens in BHI-VAN broth were cultured separately on 5% horse blood agar under microaerophilic conditions at 37°C for 4 days as previously described (9). Repeated subcultures were performed to obtain pure cultures. Microbiological features used to characterize H. pylori isolates included colony morphology (curved rod), negative Gram staining, and positive catalase, oxidase, and urease tests (6).

Before the large-scale clinical evaluation of the selective transport media, a pilot study was carried out. Fifteen dyspeptic patients undergoing upper gastrointestinal endoscopy in the Prince of Wales Hospital of Hong Kong were enrolled in the pilot study. Two gastric antral biopsy specimens were collected from each patient: one for immediate culture and another for storage in BHI-VAN broth. The second biopsy specimen was cultured after 3, 4, or 7 days of storage at 4°C, with each storage duration represented by five patients.

Results from the pilot study revealed that of the 15 patients, 10 had histological evidence of H. pylori infection. All specimens were positive for H. pylori culture, those cultured immediately and those stored in BHI-VAN broth for 3, 4, or 7 days. Following the pilot study, a large-scale prospective eradication trial was carried out in a rural area of China (Yantai County, Shandong Province) where there is a high prevalence of gastric cancer (8). Asymptomatic volunteers were recruited to undergo endoscopy screening after informed consent was obtained. Those who had histological evidence of H. pylori infection were randomized to receive either a 1-week course of omeprazole, amoxicillin, and clarithromycin or placebo. One year after the initial randomization, a second endoscopy examination was performed to assess the eradication of bacteria and progress of histological changes. Those subjects who had been given placebo were then recruited for the next stage of the study. Extra biopsy specimens were obtained from these patients to evaluate the BHI-VAN transport medium.

Gastric biopsy specimens were obtained for histological examination (two from the antrum and two from the corpus) and for storage in 1 ml of sterile BHI-VAN broth (one antral biopsy specimen). The gastric biopsy specimens inoculated in BHI-VAN broth were stored in Yantai at 4 to 6°C for 4 to 8 days before they were packed in an icebox for delivery to Hong Kong. All specimens were inoculated for culture the day after
arrival at the Prince of Wales Hospital of Hong Kong (i.e., after 5 to 9 days of storage) under conditions described previously (6). For histological examination, all gastric biopsy specimens were fixed in formalin and were examined by a single pathologist who was blinded to the H. pylori status and treatment of the subjects. Identification of spiral-shaped bacilli by Warthin-Starry stain was regarded as the “gold standard” for diagnosis of H. pylori infection.

Among the 600 H. pylori-infected subjects who were randomized to receive treatment, 515 (86%) returned for endoscopy in the second year. Two hundred fifty-four subjects were in the placebo group and were recruited for further study. The mean age of these subjects was 53 (range, 29 to 76), and there were 139 women and 115 men. Histology identified H. pylori in 238 (94%) subjects in the placebo group.

The rates of recovery of H. pylori from gastric biopsy specimens alone and from those in BHI-VAN broth were correlated with the storage time prior to culture (Table 1). The rate of recovery of H. pylori by culturing either the biopsy specimens alone or the specimens in BHI-VAN broth fell gradually as the number of days of storage increased and ranged from 76% (5 days of storage) to 46% (9 days of storage). The overall recovery rate was 61%. Culturing the specimens stored in BHI-VAN broth in addition to the gastric biopsy specimens alone did not offer any obvious advantage. There was only one case each on days 5, 6, 8, and 9 where a positive broth culture result was accompanied by a negative biopsy specimen culture result. Four of the 254 bottles of BHI-VAN broth were contaminated by other bacteria.

One of the major limitations of H. pylori culture is the transit time before the arrival of the biopsy specimen at the laboratory. Previous studies suggested that culture of such specimens should not be delayed for more than 4 to 24 h (7, 16). Various transport and storage systems for H. pylori have been described. Conventional media such as nutrient broth (14), thiglycolate broth (12), normal saline (11), hypertonic glucose (2), and Stuart’s transport medium (14) could prolong the survival of this organism by only 5 to 48 h. A previous study showed that brain heart infusion broth with horse serum is superior to that which exists under controlled laboratory conditions rather than that which exists under microaerophilic conditions (18). How-ever, data were not provided for the use of selective transport media for primary isolation of H. pylori.

Besides a suitable medium, the storage temperature is also important in maintaining the viability of H. pylori. It has been shown that H. pylori was unable to survive at temperatures above 15°C for more than 6 h (14). On the other hand, Han et al. were able to isolate H. pylori from 13 of 16 gastric biopsy specimens that had been stored in cysteine-Albini medium with 20% glycerol at 4°C for 1 week (3).

In the present study, we evaluated BHI-VAN selective transport medium in a real clinical situation involving a large number of gastric biopsy samples, the largest of such studies to date. Endoscopic biopsy specimens were obtained from a rural area where basic culturing and specimen storage facilities were not readily available. Bacteriological culture was performed in a laboratory several thousand miles away. The use of the selective transport medium in this study enhanced the recovery of H. pylori from gastric biopsy samples. Our results showed that in over 70% of the cases, H. pylori could still be recovered after 5 days of storage in the transport medium. As expected, the detection rate gradually decreased after prolonged storage and dropped to 46% after 9 days of storage. Our pilot study yielded excellent results, with all biopsy specimens being positive for H. pylori culture even after up to 7 days of storage. The slightly lower recovery rates in our subsequent study could be accounted for by several factors. First, only a single biopsy specimen was used for culture, increasing the chance of sampling error, especially for specimens with low bacterial density. Second, there were unpredictable delays in refrigerating and shipping the gastric biopsy specimens. The specimens were stored in a foam box with ice packs for 2 days before delivery and were exposed to undesirably high temperatures, jeopardizing the recovery rate. Our results reflect the “real life” scenario rather than that which exists under controlled laboratory conditions for evaluating the transport medium. We believe that the recovery rate would be higher if the temperature were kept at 4°C during transport and two or more gastric biopsy specimens were cultured.

In conclusion, the selective medium BHI-VAN is useful for prolonging the survival of H. pylori in gastric biopsy specimens when immediate culture is not feasible. In fact, we have been using this transport medium for storing gastric biopsy specimens whenever there is an anticipated delay in specimen inoculation, such as during long holidays when the laboratory is not in service.

**REFERENCES**


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**TABLE 1. Rates of recovery of H. pylori**

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<thead>
<tr>
<th>Culture</th>
<th>Recovery rate (%)&lt;sup&gt;a&lt;/sup&gt;</th>
</tr>
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<tbody>
<tr>
<td>Biopsy specimen</td>
<td>Day 5</td>
</tr>
<tr>
<td>BHI-VAN broth</td>
<td>(n = 41)</td>
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<tr>
<td>Biopsy specimen in BHI-VAN broth</td>
<td>18 (44)</td>
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<tr>
<td>Total&lt;sup&gt;b&lt;/sup&gt;</td>
<td>31 (76)</td>
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<sup>a</sup> Numbers before parentheses indicate the number of specimens from which H. pylori was recovered, and numbers within parentheses indicate the recovery rate. Recovery rate was calculated by using histology results as the standard.

<sup>b</sup> Number of H. pylori-infected subjects.

<sup>c</sup> Positive culture result from either biopsy specimens alone or biopsy specimens in direct broth culture.