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Transport and Storage of Helicobacter pylori from Gastric Mucosal Biopsies and Clinical Isolates

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Various transport and storage conditions for the recovery of Helicobacter pylori from gastric biopsies were evaluated. Gastric mucosal biopsies from 16 Helicobacter pylori-infected patients were stored in cysteine-Albimi medium containing 20% glycerol in a refrigerator (4°C) for 1 and 2 weeks and in a -20°C laboratory freezer for 4 and 12 weeks. Two clinical isolates were stored in saline, Stuart's transport media, cysteine-Albimi broth with 20% glycerol, brucella broth with 20% glycerol and skim milk with 17% glycerol at room temperature, 4°C, -20°C and -70°C. Storage at 4°C for 1 and 2 weeks resulted in Helicobacter pylori recovery from 81% and 19% of biopsies, respectively. Storage at -20°C yielded Helicobacter pylori recovery from 81% and 19% of biopsies, respectively. Storage at -20°C yielded Helicobacter pylori recovery in 100% and 57% after 4 and 12 weeks, respectively. At room temperature after 6 h, the Helicobacter pylori titer was reduced. The best storage media for frozen isolates were skim milk/glycerol, brucella broth/glycerol and cysteine-Albimi/glycerol (in descending order). Recovery was better at -70°C than -20°C.

Isolation and storage of Helicobacter pylori collected from gastric mucosal biopsies is useful for comparing the original isolate with subsequent isolates from the same patient or from another patient and as a stable source of material for experimental studies. Helicobacter pylori has proven to be sensitive to drying and delays in transport and freezing. Although there have been a number of studies evaluating different storage and transport conditions, there is no widely ac-

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cepted method or methods for either transport or storage of gastric mucosal biopsies for culture (1–14). Lyophilization of isolates has met with some success (6, 8–10). Gastric mucosal biopsies are obtained in an endoscopic unit often far removed from the microbiology laboratory, and specimens obtained by workers in the field or in free-standing endoscopy centers may face long delays before the specimens can be transported or mailed to the microbiology laboratory. Because of the poor recoverability of Helicobacter pylori after drying, the use of a transport medium has been recommended. Various transport media have been used including cysteine-brucella broth (15–17), normal saline (1, 18), glucose (19), milk (20), Stuart’s transport medium (1, 8), semi-solid agar (21), brain heart infusion medium (21), Cairy-Blair medium (1) and urea (3). Most of these transport media are not designed to support the growth of Helicobacter pylori but to maintain its viability. We compared various strategies for transport and storage of gastric biopsies on the recovery of Helicobacter pylori for primary isolation. We also evaluated methods for storing isolates obtained from biopsies.

Materials and Methods. Four large gastric antral mucosal biopsies were obtained from each of 16 men with peptic ulcers. Specimens were individually placed into 0.5 ml of cysteine-Albimi medium containing 20 % glycerol. Two specimens were evaluated for short-term storage (4°C for 1 or 2 weeks), and two were stored at -20°C for 4 or 12 weeks. After these storage periods, biopsy specimens were cultured for Helicobacter pylori. Briefly, specimens were ground between the ground glass ends of two sterile microscope slides and then suspended in 0.5 ml of cysteine-Albimi medium. Approximately 100 μl of the suspension was inoculated on brain heart infusion plates. Approximately 100 μl of the suspension was inoculated on brain heart infusion plates supplemented with 7 % fresh horse blood. Some of these plates were supplemented with 1 ml aliquots, placed in cryovials and stored at: room temperature, 4°C, -20°C or -70°C for 0 (control), 6, 24 or 48 h or 1 week. At the specified interval, samples were taken aseptically and serially diluted in ten-fold increments in physiologic saline for viable cell counting. Diluted bacterial suspensions were inoculated on 5 % sheep blood agar and incubated at 37°C in anaerobe jars with Campy Paks. The storage experiments with mucosal biopsies were scored as the percent of successful culture of Helicobacter pylori from the gastric mucosal biopsy specimens for each group. The Helicobacter pylori isolates were evaluated by colony count compared to zero time.

Results and Discussion. After one week of storage at 4°C, Helicobacter pylori were isolated from 13 (81 %) patients (Figure 1). Recoverability fell to 18 % after two weeks of storage at 4°C. In contrast, Helicobacter pylori was isolated from 100 % of biopsy specimens stored at -20°C for four weeks. Recoverability was only 50 % in

The storage and recovery of two clinical Helicobacter pylori isolates, strains 2765 and 5502, were evaluated using Helicobacter pylori grown on 5 % sheep blood agar for 48 to 72 hours at 37°C in anaerobe jars with Campy Paks (Becton Dickinson, USA). Inocula were prepared by taking colonies from the blood agar plates and adjusting in saline to a 2.0 McFarland standard. A 1 to 100 dilution was made into each type of media tested to achieve an initial inoculum of approximately 10^6 cfu/ml. The storage media tested were 10 % skim milk with 17 % glycerol, brucella broth with 20 % glycerol, cysteine-Albimi media with 20 % glycerol, Stuart’s transport media and physiologic saline. Each type of medium with the desired concentration of inoculum was divided in 1 ml aliquots, placed in cryovials and stored at: room temperature, 4°C, -20°C or -70°C for 0 (control), 6, 24 or 48 h or 1 week. At the specified interval, samples were taken aseptically and serially diluted in ten-fold increments in physiologic saline for viable cell counting. Diluted bacterial suspensions were inoculated on 5 % sheep blood agar and incubated at 37°C in anaerobe jars with Campy Paks. The storage experiments with mucosal biopsies were scored as the percent of successful culture of Helicobacter pylori from the gastric mucosal biopsy specimens for each group. The Helicobacter pylori isolates were evaluated by colony count compared to zero time.

Figure 1: The results of primary isolation of Helicobacter pylori from gastric mucosal biopsies of 16 peptic ulcer patients after storage at 4°C or -20°C.