JOURNAL OF CLINICAL MICROBIOLOGY, Sept. 1989, p. 2080–2082
0095-1137/89/092080-03$02.00/0

Vol. 27, No. 9

# Optimization of a Medium for the Rapid Urease Test for Detection of *Campylobacter pylori* in Gastric Antral Biopsies

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Received 27 February 1989/Accepted 13 June 1989

**We developed a buffered azide-free urea medium which is sensitive, specific, and nontoxic for rapid detection of *Campylobacter pylori* in gastric biopsies. Detection of urease produced by the organism provides the basis for the test. The substrate is urea in monobasic sodium phosphate buffer, and phenol red provides indication of the pH change that results from urease activity. A rapid change from yellow to red occurs in the presence of *C. pylori*, even at low concentrations of the organism. A slower color change occurs with higher concentrations of other urease producers, such as *Yersinia enterocolitica* and *Proteus mirabilis*. Experience with 51 patients with our medium showed excellent results in detection of *C. pylori* in gastric mucosal biopsies. In clinical research and practice, a rapid bedside test will be helpful for rapid diagnosis of *C. pylori*-positive patients.**

There is a rapidly expanding literature showing a close association between the presence of *Campylobacter pylori* in human stomachs and histologically proven chronic active gastritis (2, 6, 10, 14). The relapse rate of duodenal ulcer may be higher if *C. pylori* is present in the gastric mucosa (5). *C. pylori* is a strong producer of urease (11, 12, 15). Urease activity may create an alkaline environment by hydrolysis of urea and allow the organism to survive in the acid conditions of the stomach.

The current "gold standard" for detection of *C. pylori* is a combination of bacteriological culture and histological examination of gastric biopsies. Of all gastric sites, the antrum has been shown to harbor *C. pylori* most frequently (9).

In clinical practice, a rapid diagnostic test would be helpful in the endoscopy unit, enabling treatment of *C. pylori* before the definitive microbiology and histology reports are available. Rapid methods of diagnosis are based on the preformed urease activity of the organism in gastric biopsies. In a defined medium, hydrolysis of urea by the urease of *C. pylori* can be observed by the red change of the pH indicator. Three media have been developed previously, but each has disadvantages (1, 3, 4, 8, 11); one contains sodium azide, which is potentially toxic and may contribute to hazardous waste (8). This medium also uses a high indicator concentration, which may reduce sensitivity. The second medium is unbuffered and may react with urease from other organisms, leading to a false-positive reaction (1, 3, 11). The third medium is solid and may take 30 min or longer to show a positive change (4).

We developed a medium which is specific, nontoxic, and rapid. We tested the new medium with 51 patients and examined the influence of temperature and storage on the performance of the medium. Preliminary results have been reported elsewhere (7).

## MATERIALS AND METHODS

**Organisms tested.** Five reference strains (obtained from the Laboratory Centre for Disease Control, Ottawa, Ontario, Canada) and 33 recent clinical isolates of *C. pylori* were used. Single strains of the following three groups of organisms were compared with *C. pylori* in each of the three media: strong urease producers (*Yersinia enterocolitica* and *Proteus mirabilis*), weak urease producers (*Pseudomonas aeruginosa*), and urease-negative organisms (*Escherichia coli* and *Aeromonas hydrophila*).

The primary isolates of *C. pylori* were prepared in horse serum to make a dense suspension and frozen in 0.5-ml portions at −70°C. The samples were rapidly defrosted to room temperature and plated for purity on chocolate agar plates. Further subcultures were grown for 2 days on chocolate agar, from which the test suspensions were made in 1% Proteose Peptone water (pH 7.0) (Difco Laboratories, Detroit, Mich.) and adjusted to approximately $10^8$ organisms per ml by using a McFarland 0.5 opacity standard. Further suspensions of test organisms were prepared by a series of 10-fold dilutions to give final concentrations of approximately $10^2$, $10^3$, $10^4$, $10^5$, $10^6$, $10^7$, and $10^8$ organisms per ml. Since opacity standards do not provide an indicator of viability, viable counts were performed on each suspension by using the technique of Miles and Misra (13).

**Media.** The following three media were tested: (i) buffered urea medium with azide (sodium azide [20 mg], urea [2 g; Sigma Chemical Co., St. Louis, Mo.], aqueous phenol red [2.5 ml; 0.4%, wt/vol], sodium phosphate [0.14 g; $NaH_2PO_4H_2O$, 10 mmol], deionized water to 100 ml [pH 6.3 to 6.5], Oxoid agar no. 4 [0.4 g]), (ii) unbuffered urea medium with azide (same as i, except that no sodium phosphate was added), and (iii) buffered urea medium without azide (same as i, except that no sodium azide was added).

After preparation, the media were dispensed at 200 μl per well into Linborough flat-bottom, 96-well microtiter trays. Five microliters of each suspension of each organism was inoculated by being stabbed into each well of medium with a multipipette.

**Tests.** Observations for color change were made immediately and after 5 and 30 min and 1, 2, 3, 12, and 18 h at room temperature. Buffered azide-free medium gave the best in vitro results, and because the use of sodium azide is prohibited in our hospital, this medium was further tested. The effects of temperature and storage on the buffered azide-free medium were also studied. The same sets of organisms were tested with this medium, and the results were compared

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TABLE 1. Viable counts of bacterial suspensions

| Organism | No. of viable organisms/ml in suspensions of[a]: | |
|---|---|---|
| | $10^3$ Organisms/ml | $10^2$ Organisms/ml |
| *C. pylori* | $2 \times 10^3$ | $2 \times 10^2$ |
| *E. coli* | Uncountable | $3.4 \times 10^3$ |
| *Y. enterocolitica* | Uncountable | $1 \times 10^3$ |
| *P. mirabilis* | Uncountable | $1.2 \times 10^4$ |
| *A. hydrophila* | Uncountable | $1.4 \times 10^3$ |
| *P. aeruginosa* | Uncountable | $1.8 \times 10^4$ |

[a] At $10^4$ to $10^8$ organisms per ml, the viable organisms were uncountable.

when the medium was refrigerated and left at room temperature for up to 10 days. This medium was also tested at monthly intervals for up to 6 months after storage in a refrigerator. Finally, the sterile buffered azide-free medium was aseptically dispensed into 3-ml sterile bijoux bottles and used in a clinical study of 51 patients who underwent upper gastrointestinal endoscopy.

## RESULTS

The viable counts of the bacterial suspensions are shown in Table 1. At the lower concentrations, the numbers of *C. pylori* colonies were slightly smaller than those of the other organisms.

**Buffered urea medium with sodium azide.** When buffered urea medium with sodium azide was used, immediate reactions were obtained with the higher concentrations of *C. pylori* (Table 2). Other test organisms that produce urease (*Y. enterocolitica* and *P. mirabilis*) were slow to react and gave weaker reactions at 12 h only at the highest concentrations of organisms ($10^7$ and $10^8$/ml). The weak urease producer *P. aeruginosa* and the urease-negative organisms *E. coli* and *A. hydrophila* remained negative throughout the 18 h of incubation.

**Unbuffered urea medium with sodium azide.** When unbuffered urea medium with sodium azide was used, a nonspecific reaction occurred with all of the test organisms, including the urease-negative organisms, with an initial transient red followed by a permanent red reaction throughout the entire series after 12 h (Table 3). *C. pylori* at the highest concentrations ($10^7$ and $10^8$/ml) gave an immediate red, which persisted. A red change developed for the remaining *C. pylori* concentrations ($10^2$, $10^3$, $10^4$, $10^5$, and $10^6$) after 12 h at room temperature.

**Buffered urea medium without sodium azide.** When buffered urea medium without sodium azide was used, *C. pylori* gave an immediate and persistent deep red reaction at the highest concentrations of organisms ($10^7$ and $10^8$/ml) (Table 4).

Reactions which ranged from pale pink to pink after 5 min to 1 h and developed to deep red after 12 to 18 h occurred with suspensions containing $10^4$, $10^5$, and $10^6$ organisms per ml. Suspensions containing $10^2$ and $10^3$ organisms per ml

TABLE 2. Buffered urea medium with sodium azide

| Organism(s) and reaction times | Reaction[a] with the following concn (organisms/ml): | | | | | | |
|---|---|---|---|---|---|---|---|
| | $10^8$ | $10^7$ | $10^6$ | $10^5$ | $10^4$ | $10^3$ | $10^2$ |
| *C. pylori* | | | | | | | |
| Immediate[b]–3 h | 4+ | 3+ | 2+ | 2+ | 1+ | 1+ | 1+ |
| 12 and 18 h | 4+ | 4+ | 4+ | 2+ | 1+ | 1+ | 1+ |
| *Y. enterocolitica* | | | | | | | |
| Immediate–3 h | — | — | — | — | — | — | — |
| 12 and 18 h | 3+ | 1+ | — | — | — | — | — |
| *P. mirabilis* | | | | | | | |
| Immediate–3 h | — | — | — | — | — | — | — |
| 12 and 18 h | 1+ | — | — | — | — | — | — |
| *P. aeruginosa, E. coli, A. hydrophila* | | | | | | | |
| Immediate–3 h | — | — | — | — | — | — | — |
| 12 and 18 h | — | — | — | — | — | — | — |

[a] Reactions: 4+, deep cerise red; 3+, red; 2+, pink; 1+, pale pink; —, no color change.
[b] Immediate, Immediate color change.

TABLE 3. Unbuffered urea medium with sodium azide

| Organism(s) and reaction times | Reaction[a] with the following concn (organisms/ml): | | | | | | |
|---|---|---|---|---|---|---|---|
| | $10^8$ | $10^7$ | $10^6$ | $10^5$ | $10^4$ | $10^3$ | $10^2$ |
| *C. pylori* | | | | | | | |
| Immediate[b]–3 h | 3+ | 3+ | — | — | — | — | — |
| 12 and 18 h | 4+ | 4+ | 4+ | 4+ | 4+ | 2+ | 2+ |
| *Y. enterocolitica* | | | | | | | |
| Immediate–3 h | — | — | — | — | — | — | — |
| 12 and 18 h | 4+ | 4+ | 4+ | 4+ | 4+ | 2+ | 2+ |
| *P. mirabilis* | | | | | | | |
| Immediate–3 h | — | — | — | — | — | — | — |
| 12 and 18 h | 4+ | 4+ | 4+ | 4+ | 4+ | 3+ | 3+ |
| *P. aeruginosa* | | | | | | | |
| Immediate–3 h | — | — | — | — | — | — | — |
| 12 and 18 h | 4+ | 4+ | 4+ | 4+ | 4+ | 3+ | 2+ |
| *E. coli, A. hydrophila* | | | | | | | |
| Immediate–3 h | — | — | — | — | — | — | — |
| 12 and 18 h | 4+ | 4+ | 4+ | 4+ | 4+ | 2+ | 2+ |

[a] For definitions of reactions, see Table 2, footnote *a*.
[b] Immediate, Immediate color change.

TABLE 4. Buffered urea medium without sodium azide

| Organism(s) and reaction times | Reaction[a] with the following concn (organisms/ml): | | | | | | |
|---|---|---|---|---|---|---|---|
| | $10^8$ | $10^7$ | $10^6$ | $10^5$ | $10^4$ | $10^3$ | $10^2$ |
| *C. pylori* | | | | | | | |
| Immediate[b]–3 h | 4+ | 3+ | 2+ | 2+ | 1+ | 1+ | 1+ |
| 12 and 18 h | 4+ | 4+ | 4+ | 4+ | 4+ | 1+ | 1+ |
| *Y. enterocolitica* | | | | | | | |
| Immediate–3 h | — | — | — | — | — | — | — |
| 12 and 18 h | 3+ | 2+ | — | — | — | — | — |
| *P. mirabilis* | | | | | | | |
| Immediate–3 h | — | — | — | — | — | — | — |
| 12 and 18 h | 3+ | — | — | — | — | — | — |
| *P. aeruginosa, E. coli, A. hydrophila* | | | | | | | |
| Immediate–3 h | — | — | — | — | — | — | — |
| 12 and 18 h | — | — | — | — | — | — | — |

[a] For definitions of reactions, see Table 2, footnote *a*.
[b] Immediate, Immediate color change.

TABLE 5. Results of rapid urease tests of 51 patients

| Result of rapid urease test | No. of results of culture and histology | | |
|---|---|---|---|
| | Positive | Negative | Total |
| Positive | 26 | 1 | 27 |
| Negative | 2 | 22 | 24 |
| Total | 28 | 23 | 51 |

gave only pale pink reactions after 12 to 18 h of incubation. *Y. enterocolitica* and *P. mirabilis* gave only 3+ or 2+ reactions (defined in Table 2, footnote *a*) at the highest concentration of organisms at 12 and 18 h. In contrast to the buffered medium with sodium azide, suspensions of *C. pylori* containing $10^4$ and $10^5$ organisms per ml gave deep red reactions at 12 and 18 h.

Tests were performed on the buffered azide-free medium after it was left at room temperature in a cupboard to exclude light, since media exposed to light may develop peroxide, which could interfere with the urease reaction. Tests after 1, 5, and 10 days demonstrated no color change in the medium. When challenged with the same set of organisms in suspensions similar to those described in Materials and Methods, the medium gave results similar to those previously reported. The same results were obtained when this medium was tested every month for a total of 6 months after it had been stored in a refrigerator.

Media at refrigerator temperature compared with media brought to room temperature showed a reduction of reaction speed of 5 to 10 min for color development when tested with lighter inocula (i.e., $10^5$ and $10^6$ CFU/ml) of pure cultures of recent clinical isolates of *C. pylori*. A heavy inoculum (i.e., $10^8$ CFU/ml) still gave a deep red reaction within 1 to 2 min. At room temperature, the greater the inoculum the faster the red reaction occurred (Tables 2 to 4). With pure jackbean urease, color development was immediate (1 to 2 s).

The buffered azide-free medium was studied with 51 nonconsecutive patients who underwent upper gastrointestinal endoscopy. Three endoscopic antral biopsies were taken: one for histology, one for culture, and one for testing the buffered azide-free medium. Patients were considered to be infected with *C. pylori* if the results of culture and/or histology were positive. The results are summarized in Table 5. Twenty-six *C. pylori*-positive patients and 22 *C. pylori*-negative patients were correctly identified by the medium. Two false-negative results and one false-positive result occurred. Sensitivity was 93%, and specificity was 96%.

## DISCUSSION

Our results show that the buffered azide-free medium was superior in vitro to both the unbuffered and buffered azide-containing media. The fact that viable counts of *C. pylori* at the lower concentrations were slightly lower than those of the other organisms did not influence its performance. Since use of sodium azide is prohibited in our hospital, only the buffered azide-free medium was studied further and tested clinically in the endoscopy unit.

After storage at room temperature, this medium remained stable for at least 10 days. However, since urea is known to undergo autohydrolysis, it is advisable to store the medium in a refrigerator at 4 to 8°C. Although the color change may take slightly longer at refrigerator temperature, this is probably of little significance. An important aspect of our study are the results of prolonged incubation. Gastric biopsies obtained in the endoscopy unit may arrive late in the day at the microbiology laboratory, and results of the rapid urease medium may therefore be read only on the following day. More importantly, when the total number of organisms is low (which can also occur after treatment that is only partially successful), a late-occurring color change can correctly indicate the presence of *C. pylori* organisms.

Clinical experience with our buffered azide-free medium and biopsies from 51 patients showed excellent sensitivity and specificity. Currently used rapid urease tests are potentially toxic (8), are unbuffered (which makes them potentially less specific [1, 3, 11]), or are solid (which makes them slower to respond [4]). We showed that buffered, azide-free urea medium is sensitive, specific, and nontoxic for rapid detection of *C. pylori* in gastric biopsies. We believe that use of this medium avoids the potential lack of specificity of the previously described *Campylobacter*-like organism test, as well as the toxicity of sodium azide-containing media.

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