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New Plate Medium for Growth and Detection of Urease Activity of *Helicobacter pylori*

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A new medium for detection of urease activity and isolation of *Helicobacter pylori* is proposed. This medium, containing Columbia Agar Base, was supplemented with IsoVitaleX, hemin, urea, and phenol red (nonselective medium [NSM]). Both bacterial growth and color change were evaluated and compared with growth in the same medium supplemented with cefsulodin, vancomycin, polymyxin B sulfate, and amphotericin B (selective medium [SM]). Twenty-five recent clinical isolates and antral biopsy specimens from 33 patients who underwent endoscopy were examined. The isolates showed a rapid color change and good growth at 5 days of incubation with NSM and SM. *H. pylori*-positive biopsies revealed a color change within 36 h, and bacterial growth was better appreciated in NSM, but with more contaminating flora than in SM.

A strong association between *Helicobacter pylori* and chronic active inflammation of the gastric antrum and duodenal ulceration has been recognized, and the duodenal ulcer relapse rate is higher when *H. pylori* is present in gastric mucosa than when it is not (9, 10). Different methods have been proposed to detect this microorganism; however, no unanimity exists among investigators concerning which method represents a "gold standard" (1, 2, 6).

H. pylori can be identified in antral biopsies by bacteriologic and histologic techniques (8, 12). In clinical practice, a rapid diagnostic test would be a helpful adjuvant to endoscopy to allow start of appropriate treatment before definitive histological and bacteriological results are obtained. For microbiological evaluation, most of the media used for isolation of this fastidious microorganism are supplemented with blood (3, 5, 7).

Here, we describe a new plate medium without blood, inexpensive and easy to prepare, which is suitable for direct testing of urease activity and isolation of *H. pylori*.

Twenty-five recently obtained clinical isolates identified by standard methods (8) and *H. pylori* NCTC 11637 were used for this investigation. Stock cultures were stored at −70°C by the method of Drumm and Sherman (4). We also studied 33 patients who underwent endoscopy with symptoms attributable to upper gastrointestinal tract and duodenal ulcers. Three antral biopsy specimens were taken from each subject. One was examined histologically, and the other two were used for bacteriologic examination. The biopsies used for microbiological tests, combined and processed as a single specimen, were maintained in 0.4 ml of brucella broth (Unipath, Ltd., Basingstoke, London, England). They were homogenized and plated within 2 h. In addition, *Proteus vulgaris* ATCC 13315 (a strong urease producer), *Pseudomonas aeruginosa* ATCC 27853 (a weak urease producer), and *Campylobacter jejuni* ATCC 33292 (a urease nonproducer) were cultured in the same conditions as *H. pylori* for comparative studies of urease activity.

This new medium allows isolation of *H. pylori* and at the same time reveals its urease activity. The performance of the medium was studied with and without selective antibiotics, comparing the results obtained with selective (SM) and nonselective (NSM) media. The latter medium consisted of Columbia Agar Base (Unipath) sterilized at pH 6.8, cooled at 50°C, and combined with 10 mg of hemin (Sigma Chemical Co., St. Louis, Mo.) per liter, 2% IsoVitaleX (Becton Dickinson & Co., Cockeysville, Md.), 20 g of urea (Sigma) per liter, and 1.2 mg of phenol red (Sigma) per liter; these last two compounds were sterilized by filtration (dm filter, 0.22-μm pore size; Millipore, Bedford, Mass.). To evaluate the interference of competing flora with the growth of *H. pylori*, we added the following antibiotics to this formula base: trimethoprim (5 mg/liter), vancomycin (10 mg/liter), amphotericin B (5 mg/liter), and cefsulodin (5 mg/liter) to form SM (Table 1).

Isolates were defrosted to room temperature and rapidly plated. Stock isolates and antral biopsies were plated in duplicate and tested on the SM and NSM. Plates were incubated at 37°C in a microaerophilic atmosphere (Campy Pak jar [Unipath]). Bacterial growth was examined after 3, 5, and 7 days of incubation.

Observation of medium color change was made immediately, at 5 and 30 min, and at 1, 2, 3, 6, 24, 36, and 48 h. The first observation of color change was made before the plates were put in an anaerobic jar, and the other readings were made at 37°C in a microaerophilic atmosphere.

To compare the efficacy of growth in the new medium with and without antibiotics, stock isolates and biopsies were also plated in duplicate onto two traditional blood-containing media: chocolate agar plus 1% IsoVitaleX (AC) and Campylobacter selective medium (CP) (Unipath). All of the different plates used for each strain or biopsy sample were always incubated in the same Campy Pak jar. Moreover, for quantitative assessment of bacterial growth among these four media, serial dilutions of 12 stock strains and standard reference strain NCTC 11637 were tested. For this examination, the strains were grown in AC at 37°C for 5 days in a microaerophilic environment. From each strain we prepared a bacterial suspension in 1 ml of brucella broth until it was turbid. In addition, serial 10-fold dilutions were prepared up to 10^{-5} times the original broth concentration. A 10-μl volume of each dilution was spread in duplicate on the new medium with and without antibiotics and on blood-containing media. All of the plates used for this evaluation were

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TABLE 1. Composition of new medium for *H. pylori*

Ingredient (unit)	Amt
Columbia Agar Base (g)	39
Hemin (mg)	10
IsoVitaleX (ml)	20
Urea (g)	20
Phenol red (mg)	1.2
Granulated agar (g)	4
Distilled water (ml)	980
Optional antibiotics (mg)	
Trimethoprim	5
Vancomycin	10
Amphotericin B	5
Cefsulodin	5

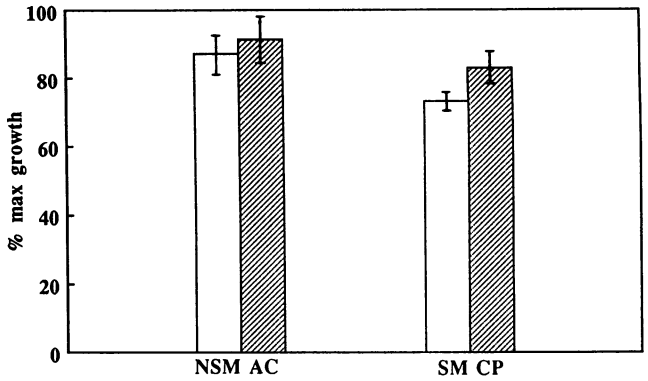

FIG. 1. Mean percentages of maximum growth on the four media studied. The error bars show standard deviations.

incubated in the same Campy Pak jar, and colonies were counted after 3, 5, and 7 days. Student's *t* test was used for statistical analysis.

When microbial growth was macroscopically plain, to determine the CFU, the dilution plates showing between 10 and 99 colonies were read.

For all stock strains and for the *H. pylori*-positive biopsy specimens, maximum growth was obtained after 5 days of incubation. At 5 and 7 days of incubation, the colony counts were the same. Maximum growth was defined as the highest number of CFU counted on the plate medium. The strains isolated were identified as *H. pylori* by standard methods (8).

Concerning the urease activity of our stored and *H. pylori* NCTC 11637 strains, a rapid change from yellow to red was observed. Of 26 plates containing *H. pylori*, 21 showed an appreciable color change immediately at room temperature; the remaining 5 plates displayed a red reaction after 1 h in the anaerobic jar at 37°C. The medium became completely red within 3 h with all of the stock strains plated.

Proteus vulgaris ATCC 13315 and *Pseudomonas aeruginosa* ATCC 27853 revealed a color change of the medium at 24 and 48 h, respectively, whereas *Campylobacter jejuni* ATCC 32292 did not reveal urease activity within 48 h. These weak and slow color changes allow elimination of false-positive results.

Concerning bacterial growth, SM and NSM displayed vigorous growth macroscopically, as confirmed by phase-contrast microscopy and modified Gram staining (0.3% carbol fuchsin was the counterstain), evidencing curved and spiral-shaped bacteria.

No reliable differences in the growth of isolates were noted between SM and NSM, and the isolates were successfully maintained in the same media by passage every 3 days. The growth obtained with this new proposed medium with and without antibiotics and that obtained with traditional media with blood (AC and CP) were comparable. In fact, the CFU were counted for each of the 13 strains studied at the same dilution on a plate containing the four media. The maximum growth for each strain on any of the media was calculated. The maximum CFU of one strain were determined on a medium, and the colony counts on the other three media were expressed as percentages of this maximum value (100%). Figure 1 shows the mean percentages (± standard deviation) of maximum growth obtained with the four media compared. The differences between the values obtained with NSM and AC (87.1 ± 13.7 and 91.3 ± 16.3, respectively) were not significant ($P = 0.48$). Similar results were obtained when SM and CP were compared (73.1 ± 6.4 and 82.8 ± 9.5, respectively, of the average percentage; $P = 0.09$). The advantages of easy preparation and reduced costs support the use of this new medium instead of blood-containing media.

SM and NSM also produced good results with plated biopsy specimens. Table 2 summarizes the results obtained with 33 antral biopsies plated onto SM and NSM compared with those obtained by other methods.

Twenty-two specimens were positive for *H. pylori* by the modified Gram stain and histological techniques. A specimen was defined as histologically positive for *H. pylori* when a section stained with Warthin-Starry stain showed curved, spiral-shaped bacteria. With NSM, the number of *H. pylori*-positive plates was in agreement with the above-mentioned methods. In all cases, curved and spiral-shaped bacteria were observed with the modified Gram stain but *H. pylori* was identified and stored in only 20 of the 22 cases. In fact, the contaminating flora in two cases did not allow isolation of the microorganism. Also, in the corresponding NSM containing blood, 22 positive cases were determined by microscopic examination but only 19 *H. pylori* isolates were identified and stored. AC exhibited more contaminating microorganisms than NSM (three cases). With SM there was less incidence of contamination; however, the presence of antibiotics in the medium inhibited the growth of *H. pylori* when its concentration in the antral biopsy was low (one case). CP revealed the same sensitivity. The percentage of

TABLE 2. Numbers of *H. pylori*-positive antral biopsies detected by using different methods and the new proposed medium

Method	No. of *H. pylori*-positive biopsies	%[a] of total
Histological techniques	22	100
Modified Gram stain	22	100
Rapid urease test[b]	20	91
AC	19 (3)[c]	100
CP	21	95
NSM	20 (2)[c]	100
SM	21	95

[a] Percentage of true-positive results with respect to the number positive by histologic techniques.
[b] Reading obtained at 3 h.
[c] Number of cases in which competing flora did not allow isolation of *H. pylori* observed by microscopic examination.

H. pylori isolation with these last two selective media was 95%.

Concerning the urease activity of *H. pylori*-positive specimens, the color change was appreciable at 24 to 36 h. In fact, 22 plates of NSM and 21 of SM showed redness in the microaerophilic environment within 36 h. When a complete color change was produced, macroscopically apparent colonies were not present in NSM and SM; bacterial growth became visible only after 3 days of incubation.

By using this method to reveal the urease activity of *H. pylori*, a therapeutic approach is made possible in a shorter time, reducing the false-negative results obtained with the rapid urease test (Table 2). This method is also suitable for exclusion of false-positive results.

In fact, among the other strains isolated in gastric tissue, urease producers, such as *Proteus* spp. and *P. aeruginosa*, occasionally occur in this environment and their concentration is always low (11). Furthermore, *H. pylori* expresses higher urease activity than these strains. Consequently, when *Proteus* spp. and *P. aeruginosa* are found in a biopsy specimen their urease activities do not achieve levels sufficient for a medium color change within 48 h.

These observations suggest that in *H. pylori* isolation by antral biopsy, parallel use of the proposed SM and NSM could produce the highest sensitivity and specificity. Furthermore, our results show that NSM and SM could be used for rapid screening and isolation of *H. pylori*. In fact, the color change revealing urease activity would lead to a first-hand result for a therapeutic approach and cultivation using this medium would lead to correct identification of *H. pylori*. In conclusion, these findings indicate that NSM and SM are suitable for a rapid detection and isolation of *H. pylori*.

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