Validation of the String Test for the Recovery of *Helicobacter pylori* from Gastric Secretions and Correlation of Its Results with Urea Breath Test Results, Serology, and Gastric pH Levels

JAVIER TORRES,1,* MARGARITA CAMORLINGA,1 GUILLERMO PÉREZ-PERÉZ,2 GÉRARDO GONZALEZ,1 AND ONOFRE MUÑOZ1

*Unidad de Investigación en Enfermedades Infecciosas, Coordinación de Investigación, IMSS, Mexico City, Mexico,1 and Division of Infectious Disease, School of Medicine, New York University, New York, New York.2*

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The efficacy of the string culture test to isolate *Helicobacter pylori* from gastric secretions of 28 volunteers was studied. With the urea breath test (UBT) as the “gold standard,” the string culture test showed a sensitivity of 75% and a specificity of 100%. The results of string culture did not correlate with the UBT results, with serum antibody levels, or with the pH levels of gastric secretions. The isolation of *Helicobacter pylori* strains from the stomach of persons colonized with this organism allows the monitoring of strain characteristics, the assessment of susceptibility to antimicrobials, and the genotyping of the organisms recovered. In order to culture microbials, and the genotyping of the organisms recovered. In order to culture *H. pylori* from clinical specimens, persons are usually examined by endoscopy and biopsy samples are taken from the stomach. Recently, the isolation of *H. pylori* from vomit and from feces has been reported (5); however, in most cases such samples are not available. Therefore, there is a need for less-invasive, cheaper, but reliable, methods that could be used even for persons infected but not showing clinical symptoms. The aim of this study was to evaluate the sensitivity and specificity of a novel nonendoscopic assay, the string culture test, to determine the presence of *H. pylori* in gastric secretions from individuals with *H. pylori* status defined by the urea breath test (UBT).

We studied 28 adult volunteers (mean age, 35.8 ± 7.6; age range, 24 to 57 years; 1:1 gender ratio). After an overnight fast, persons were subjected to the string test, the UBT, and a serology evaluation. For the string test, the pediatric Entero-test capsule (HDC Corporation, San Jose, Calif.) was used; the capsule contains a 90-cm-long string with absorbent fiber. A 20-cm-long portion of the string was pulled out, and the end was taped to the cheek; the individual swallowed the capsule with water, drinking until the discomfort in the throat vanished. The subject remained seated and still for 1 h and then was asked to look up; the string was then retrieved with a rapid single movement. Minor discomfort (mainly the urge to gag) was reported at the moment that the capsule was swallowed and when the string was retrieved.

After removal, the upper third of the string (30 cm) was cut and discarded to reduce oral and nasopharyngeal contaminants; the remaining length was placed in a sterile petri dish, and the pH along the string was measured using the stick provided by the supplier. The absorbed gastric juice was removed by squeezing the string with a glass coverslip. Usually 100 to 200 μl of gastric juice was obtained by this method, and an aliquot of 15 μl was immediately inoculated onto blood agar plates with antibiotics (nalidixic acid, 10 μg/ml; vancomycin, 3 μg/ml; trimethoprim, 5 μg/ml; and amphotericin B, 2 μg/ml). The plates were then incubated at 37°C in a 9% CO2 atmosphere. The presence of an *H. pylori* isolate(s) was confirmed by urease, catalase, and oxidase activity and by Gram staining. Between 8 and 10 *H. pylori* colonies per person were recovered, expanded for DNA extraction, and studied for vacA alleles using the primers described by Atherton et al. (1).

A sample of serum was tested for the presence of specific immunoglobulin G (IgG) antibodies against *H. pylori* whole-cell antigen and against a recombinant CagA antigen, using enzyme-linked immunosorbent assays (ELISAs) (2). The UBT was performed using [14C]urea capsules (Tri-Med, Charlottesville, Va.).

Of the 28 volunteers studied, 19 (68%) were positive by serology, 16 (57%) were positive by the UBT, and 12 (43%) were positive by string culture. All 12 culture-positive individuals were also positive by both whole-cell ELISA and UBT, and 10 (83%) of them were positive for CagA antibodies. Four persons were positive for IgG and CagA antibodies and positive by UBT, yet negative for culture. In addition, three other persons were positive by serology (for IgG and CagA antibodies) and negative by the other two tests; two of these persons had borderline results for whole-cell ELISA (1.06 and 1.05 ELISA units). Using the UBT test as the “gold standard” test for determining *H. pylori* status, string culture had a sensitivity of 75% and a specificity of 100%.

We sought to correlate the magnitude of the UBT results and the intensity of the immune response, as indicated by the serology values, with the achievement of positive results by string culture (Table 1). Among the UBT-positive cases, no correlation was found between the magnitude of the UBT response and the results of culture for either culture-positive or culture-negative cases. Similarly, no difference was found in
the magnitude of levels of antibodies against either *H. pylori* whole-cell or CagA antigens between culture-positive and culture-negative cases. We analyzed whether the pH of the gastric secretion correlated with the recovery of *H. pylori*; of the 16 UBT-positive persons, 9 had a pH of ≤7 and 7 (78%) of these were culture positive; 7 had a pH of 7, and 5 (71%) of these were culture positive (Table 1). Thus, there was no correlation between the pH of the biopsy sample and positive culture. The lack of correlation between string culture test results and the magnitude of the response to the above-mentioned tests might be attributable to the fact that gastric juice sampling does not reflect the degree of colonization or the histological changes that occur in the vicinity of the gastric epithelium.

We recently documented a high frequency of mixed infection in our population by studying vacA alleles in *H. pylori* isolates from gastric biopsy samples (3). In this study we obtained multiple single-colony isolates from gastric secretions of 8 of the 12 culture-positive persons; we found that all isolates from the same person had the same vacA s and m alleles. This result suggests either that a homogeneous *H. pylori* population is colonizing the gastric juice or that only a predominant strain is shed into the gastric milieu.

Previous studies have reported the use of the string test; however, only PCR and rapid urease tests are customarily used for the detection of *H. pylori* (8), despite the fact that these tests offer only indirect evidence for the presence of the bacteria. Few studies have reported the use of the string test to culture *H. pylori*, and these have been reported in abstracts or with minimal description of the technique (4, 6). Recently, Samuels et al. (7) reported a string test protocol that allowed the recovery of *H. pylori* in up to 97% of infected patients. To achieve this, a complex scheme of dilutions and selective media was used. In the present study, a sensitivity of 75% was reached for the string culture test; although this rate of recovery is lower than that obtained by the method of Samuels et al., it was obtained with minimal manipulation of the gastric secretion and using only one agar plate with antibiotics per sample. This study further suggests that the string test is a reliable method for culturing *H. pylori* from gastric secretions without the need of endoscopic examinations. The string method is also suitable for epidemiological studies of asymptomatic individuals. A further potential use is in the assessment of antibiotic susceptibility of *H. pylori* isolated from patients for whom treatment failed to eradicate the infection; these patients can then be treated appropriately without the need for endoscopy.

In conclusion, this study confirms that the string test is an easy and fast method for culturing *H. pylori* from gastric secretions. If a simple and less costly protocol is needed, the protocol described in this study, despite its low sensitivity (75%), can be used, especially in communities where there is little technical support.

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REFERENCES


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**TABLE 1. Recovery of *H. pylori* with the string test and correlation with values of UBT, serology, and gastric pH**

<table>
<thead>
<tr>
<th>Test or gastric pH*</th>
<th>No. of subjects</th>
<th>No. of subjects for whom culture result was#:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Positive</td>
</tr>
<tr>
<td>UBT</td>
<td>16</td>
<td>12 (1,185 ± 725)</td>
</tr>
<tr>
<td>Serology</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Whole-cell</td>
<td>19</td>
<td>12 (5.4 ± 3.7)</td>
</tr>
<tr>
<td>CagA</td>
<td>16</td>
<td>12 (4.20 ± 7.03)</td>
</tr>
<tr>
<td>Gastric pH of:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>7.0</td>
<td>9</td>
<td>7</td>
</tr>
<tr>
<td>7.0</td>
<td></td>
<td>5</td>
</tr>
</tbody>
</table>

* CagA serology and gastric pH levels were correlated with culture results only for the 16 UBT-positive subjects.

# Values in parentheses are test results in disintegrations per minute (for UBT) or ELISA units (for serology).