Prospective evaluation of a new “paper urease test” for ultra-rapid detection of Helicobacter pylori

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Summary

Background: The purpose of this study was to determine the sensitivity, specificity, and positive and negative predictive values of a newly developed paper urease test (PUT) for ultra-rapid detection of helicobacter pylori (HP) in gastric mucosa.

Material/Methods: 100 patients (43 men) with a mean age of 57±6.8 years participated. Patients presenting for upper endoscopy with no recent exposure to HP-altering drugs were enrolled. Gastric biopsy specimens were tested by the PUT and histology methods, and then the patients underwent [13C] urea breath tests (UBT). HP was considered positive when either UBT or histology demonstrated it, and negative if HP was not detected in either UBT or histology. The PUT was reported at 15 minutes.

Results: 87 of 100 patients tested positive for HP. The PUT correctly identified 74 of 87 HP-positive and 13 of 13 HP-negative patients, yielding sensitivity, specificity, and positive and negative predictive values of 87%, 100%, 100% and 53.5%, respectively, in this population.

Conclusions: Rapidly available and reliable results from the PUT can facilitate clinical decision prior to patient discharge from the endoscopy suite. We recommend PUT for screening of HP in endoscopy candidates, due to high specificity, rapid reaction, simplicity and low cost. A positive result shows a definite diagnosis, although a negative result needs further diagnostic methods.

key words: endoscopy • fast urease test • Helicobacter pylori • PUT • rapid urease test

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**Background**

There are several methods for diagnosing helicobacter pylori (HP) infection [1]. The biopsy urease test was introduced as a simple and convenient method for diagnosing this infection. The test is based on the presence of large amounts of preformed urease enzymes in HP. Since McNulty and Wise first described the biopsy urease test in 1985 [2], several modifications of this test have been developed and validated [3–4], but they are relatively expensive and may therefore not be available to all clinicians, especially in developing countries [1]. The CLO test is commercially available, but its costs and the time needed for positive results, which may be up to 24 h, limits its usefulness. The length of time it takes for the CLO test still poses a hindrance to those clinicians wishing to treat infected patients while they are still in the endoscopy room [3,5]. On the other hand, there are several locally made urease tests that are very easy to prepare and are inexpensive [1]. In our clinic we have introduced a modified ultra-rapid urease test based on reagent paper, which is extremely simple to prepare and read. We have called this method the “paper urease test” (PUT). The main advantages of this method over the CLO test is the ultra-rapid reaction, simple application, and low price. In a pilot study, its sensitivity and specificity were determined to be 94% and 100%, respectively. Due to the accessibility of the paper, the speed of administration, ease of application, and low cost, it is possible to apply PUT for all endoscopy candidates without further costs. The aim of the present study was to assess prospectively the sensitivity, specificity, and time for positive reaction of PUT.

**Material and Methods**

In this study, 103 consecutive patients referred to the Fatemieh Hospital for endoscopy from March to July 2004 due to a gastrointestinal disorder were included. Patients with upper GI bleeding, history of treatment with proton pump inhibitors in the last 2 weeks, bismuth or antibiotic in the previous month were excluded from the study. The patients underwent esophagogastroduodenoscopy without iv sedation using only lidocaine spray. After full evaluation of the upper gastrointestinal tract three biopsy samples were taken, two from antrum, greater curvature 3 cm from the pylorus, and one from corpus mucosa; lesser curvature near the incisure, with a biopsy forceps (Olympus, FB-25K). After each biopsy, the forceps were washed and placed in a detergent (β-aldehid) for 15 minutes. One of the antrum specimens was assessed using PUT in the endoscopy room, while the two other samples were fixed immediately in 10% neutral-buffered formalin, separately, and stained with hematoxylin and eosin to measure the severity of gastritis according to the Sydney classification [6,7]. Giemsa stain was used to detect HP [8]. The histology was considered positive if either antral or body samples demonstrated HP. The density of HP colonization was graded as:

0 – no bacteria seen;
1 – sporadic bacteria seen;
2 – many bacteria seen in most microscopic fields;
3 – bacteria seen in clusters in all the fields examined [1].

For preparation of paper for the PUT, 3 ml of a 1% solution of red phenol were added to 600 ml of 5% sterile urea solution, and then sterile water was added the solution to reach a pH of 7. Filter papers were soaked with this solution, and after full absorption (10 minutes), the papers were held at room temperature for drying. Then these papers were sliced into small pieces and conserved in a special box in the refrigerator (4°C).

In the endoscopy room, the biopsy sample was placed in the paper, and both were compressed between 2 slides by finger pressure to wet the paper with the secretions. In the presence of HP, urease causes ammoniac formation and pH alteration, which results in the paper changing color from yellow to purple. PUT was defined positive if the paper became purple, while no color change meant a negative result. The slides were saved for 20 minutes and the time of reaction was determined. The speed of color change was classified as:

1 – less than 2 minutes;
2 – 2–5 minutes;
3 – more than 5 minutes.

The results of all three – mentioned tests (histology, PUT and UBT) were recorded separately and blindly. In addition, the density of HP infection in histology was compared with the speed of color change in PUT. The HP was considered positive if UBT and/or histology (antrum or body) showed positive.

All the subjects who agreed to participate signed informed consent. The Digestive Research Center of the Semnan University of Medical Sciences approved the study. Statistical analysis was carried out using the Statistical Package for the Social Sciences (SPSS) program (Chicago version, 11.5). The specificity and sensitivity of PUT were determined, and Spearman correlation was used to compare microbe density in histology with the speed of PUT reaction. P<0.05 was considered statistically significant.

**Results**

103 consecutive patients enrolled in this study, of whom 3 were excluded due to incomplete records. The mean (±SD) age of the patients was 57±6.8 years, and of the 100 patients 45% were males. The prevalence of HP infection was 87% generally. Table 1 shows the results of the three diagnostic tests. The histological study was positive in 71% of cases, of which 40% were positive for infection in both parts of the stomach. The infection rates of corpus and antrum were 40% and 66%, respectively. There were 13% false negative and no false positive results with PUT. The sensitivity and specificity of PUT were 87% and 100%, respectively, and the positive and negative predictive values of PUT were 100% and 53.5%, respectively. Considering UBT alone as the gold standard, the sensitivity and specificity of PUT were 91% and 83%, respectively, while if histology was used as the single gold standard, the sensitivity and specificity of PUT were 87% and 59%, respectively.

There was no association between the speed of paper color change in PUT and UBT results (P<0.05, r=–0.07) and HP density in histology (P<0.1, r=–0.17). In addition, there was no relationship between UBT results and HP density in histology (P<0.88, r:0.018).
In this study, we assessed paper urease test (PUT) based on filter paper as a new, ultra-rapid, simple, home-made and inexpensive biopsy urease test for the detection of HP.

Rapid urease methods are the most commonly used methods for HP detection in endoscopy candidates [5]. These methods are based on bacterial urease. This enzyme converts urea to ammoniac, and ammoniac is detected by buffer color change. Currently there are various kits available, of which the CLO test is the most famous and oldest of them. Despite its high sensitivity, however, it is expensive and is therefore not practical, especially in developing countries. Furthermore, it requires 24 hours for reaction [10]. Although there are two types of rapid urease test available commercially (urea based on agar gel formulation, like the CLO test, and HP Fast and urea based on a reagent strip, such as Pylori Tek), their cost and the time needed for positive results, which may be up to 24 h, limit its usefulness [11]. A slightly modified version of the ultra-rapid urease test (URUT) [12], using a small bottle of urea, is commonly used as a routine method in our country, but its sensitivity and specificity is unknown, and the results in this method are also determined in 24 hours.

In our method, due to compression of the tissue between slides, bacterial urease and tissue extracts wets the urea paper, and in only a few minutes this results in a chemical reaction.

To our knowledge, this is one of the most rapid diagnostic tests, in comparison with other common kits, which need 24 hours for diagnosis, and so it is preferable. For example, the sensitivity of the CLO test and the Pylori Tek kits at one hour has been reported to be 71% and 89%, respectively [10], but PUT sensitivity was 87% in only 15 minutes. The sensitivity of urease methods in liquid structure in the short term are low, because due to the small biopsy specimen in comparison to the volume of urea-containing liq-

### Table 1. Results of three diagnostic tests for *Helicobacter pylori* detection.

<table>
<thead>
<tr>
<th>Pathology</th>
<th>BUT**</th>
<th>PUT*</th>
<th>Cases</th>
</tr>
</thead>
<tbody>
<tr>
<td>True positive</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>True negative</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>True positive</td>
<td>-</td>
<td>+</td>
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<tr>
<td>True positive</td>
<td>+</td>
<td>-</td>
<td>+</td>
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<tr>
<td>Pseudo negative</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Pseudo negative</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Pseudo negative</td>
<td>-</td>
<td>+</td>
<td>-</td>
</tr>
</tbody>
</table>

* * Paper urease test;
** Breath urea test [13].

### Table 2. The sensitivity of different biopsy urease methods for detection of *Helicobacter pylori*.

<table>
<thead>
<tr>
<th>Original name (Ref)</th>
<th>&lt;15-min %</th>
<th>1-hour %</th>
<th>4-hour %</th>
<th>24-hour %</th>
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<tbody>
<tr>
<td>CLO test [15,17,19,20]</td>
<td>71–95</td>
<td>93</td>
<td>99–93</td>
<td></td>
</tr>
<tr>
<td>HUT [13,15]</td>
<td></td>
<td>80.7–90</td>
<td></td>
<td></td>
</tr>
<tr>
<td>New CLO test [14]</td>
<td></td>
<td>93</td>
<td>100</td>
<td></td>
</tr>
<tr>
<td>Urea Membrane Test [21]</td>
<td></td>
<td>98</td>
<td></td>
<td></td>
</tr>
<tr>
<td>LRUT [22]</td>
<td></td>
<td></td>
<td>92.8</td>
<td></td>
</tr>
<tr>
<td>MRU [20]</td>
<td>93.8</td>
<td>96.6</td>
<td>97.4</td>
<td>88</td>
</tr>
<tr>
<td>Fast test [18]</td>
<td>66–88</td>
<td>88</td>
<td>88</td>
<td></td>
</tr>
<tr>
<td>Polish test [13]</td>
<td></td>
<td></td>
<td>90.3</td>
<td></td>
</tr>
<tr>
<td>URUT [11,23]</td>
<td>92</td>
<td>82.1</td>
<td>96.4</td>
<td></td>
</tr>
<tr>
<td>PUT [present article]</td>
<td></td>
<td></td>
<td>87</td>
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</table>
uid, more time is needed for biochemical reaction. For example, it has been reported that the sensitivity of URUT at 20 minutes and 24 hours are 64.3% and 96.4% respectively [3,11]. In the PUT, due to direct contact between tissues extracts and paper, the chemical reaction occurs very fast and the results are determined in a few minutes.

In this study, as in other studies, there was no association between bacterial density and the speed of the urease test reaction [13]. Table 2 shows the sensitivity of different biopsy urease methods that have been reported in the literature; most of them need 24 hours for a complete response. Although the sensitivity of the urease tests could be improved if we were to read the test results after a longer duration of time, this may also have led to some false positive results [14,15,23]; however, the more rapid reaction of PUT reduces the chances of a false positive result. Because of drying of specimens after 15 minutes, we did not compare the results of PUT with other tests at 1 hour or more.

In this study, due to the use of a single specimen, sensitivity is low; because of segmental involvement of gastric mucosa with HP, it is necessary to provide two samples of gastrotic mucosa for assessment of infection. It has been shown that the sensitivity of the biopsy urease test increases with 2 biopsy samples [13,24].

The other advantages of this method are easy access, conservation and transport, so that it can be used easily in any clinic or private office, and the slides can be used again after washing by detergent. The most important property of the test is rapid reaction, which determines the diagnosis in endoscopy room, so that treatment can be begun immediately.

**Conclusion**

We recommend PUT for screening of HP in endoscopy candidates, due to high specificity, rapid reaction, simplicity and low cost. A positive result justifies a definite diagnosis, although a negative result needs further diagnostic methods to be confirmed.

**Recommendations**

1. Due to segmental involvement of the stomach by HP, the use of two samples instead of one specimen is recommended for increasing sensitivity.

2. In the endoscopy room, the PUT is used as an initial screening test, with an additional sample taken for histology, which is sent only if the PUT is negative and when there is a strong clinical suspicion of HP infection.

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