

# Evaluation of a liquid urease test (LUT) for detection of *Helicobacter pylori*

## SUMMARY

The aim of our study was to develop a rapid diagnostic urease test to demonstrate the presence of *Helicobacter pylori* in the Endoscopy room.

**Materials and Methods.** 200 consecutive patients referred to gastroscopy for different indications, were included in this study. One antral biopsy sample was obtained to be immersed in our test. The same sample was used for histological evaluation, considered to be the gold standard method for diagnose of *Helicobacter pylori* infection.

**Results.** 135 patients (67.5%) were found positives and 65 patients (32.5%) were negatives in our test. 128 patients (64%) showed *Helicobacter pylori* on histological examination. Our test showed a sensitivity of 91%, specificity of 88.1%, and positive and negative predictive values of 95% and 80% respectively.

A remarkable correlation between density of *Helicobacter pylori* and reading time was also observed, where a high density of the bacteria reduced the reaction time in this liquid test. Furthermore, an overall accuracy of 90% was shown, which is comparable with other available commercial tests.

**Conclusion.** LUT is easy to handle, cost effective and fast, with a high positive predictive value.

**Index:** *H. pylori*, urease, liquid test, diagnosis.

## INTRODUCTION

It has been demonstrated that there is a causal relationship between *Helicobacter pylori* infection and gastroduodenal disease, representing an association that has contributed to understand the pathogeny of ulcer disease, chronic superficial gastritis, gastric carcinoma and B lymphoma (1,8). Therefore, an ideal diagnostic test to detect *Helicobacter pylori* in the endoscopy room, being rapid, accurate, cost-effective and easy to handle, has turned into a priority.

The urease tests are usually indirect methods to detect *Helicobacter pylori* because they are based on the urease activity of *Helicobacter pylori*. These tests provide a simple and cost-effective tool for the detection of *Helicobacter pylori*. However, the most important practical benefit of these tests depends on their reading time. This may provide an advantage over other tests by allowing the initiation of the therapy before the patient leaves the hospital (1).

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There are several urease tests in the market such as CL.Otest®, Pyloritek®, Hpfast® with variable sensitivity and specificity. The CL.Otest® has shown sensitivity of 75-98.5% and a specificity of 83-100%, on the other hand Pyloritek® has provided a sensitivity of 75-98% and a wide ranging specificity of 93.9-100%. Hpfast® has 82-88% of sensitivity and 99-100% of specificity. In our country the cost of these tests range between 8-10\$ per unit and the reading time varies between 1-24 hours (1,2).

If we consider that the majority of gastroenterologist would start antibiotics therapy to eradicate *H. pylori* infection based on a positive urease test, following international criteria, then a test to be cost-effective, accurate, rapid, easy to read and handily is required.

Therefore, we conducted a prospective study to evaluate the accuracy of a liquid urease test (LUT) which was correlated with histological analysis as a gold standard for the diagnosis of *H. pylori* infection (3-6).

## MATERIALS AND METHODS

Two hundred patients, scheduled for esophago-gastroduodenoscopy (EGD) at the Medical Center, University of Los Andes, Gastrointestinal Endoscopy Unit, were enrolled in the study.

Criteria for exclusion were patients with prior pyloric or gastric resection, those who had been taking antibiotics or bismuth salts, use of nonsteroidal anti-inflammatory drug (NSAID) within four weeks, or proton pump inhibitors for at least two weeks before the procedure. Patients who had received treatment for *H. pylori* eradication, were also excluded. The study was approved by the institutional review board from the University of Los Andes.

EGDs were performed after 6-hr fast using an upper fiberscope Fujinon FQ-100FP. All patients received conscious sedation with intravenous midazolam (Doricum®) and Hyoscina N-metilbromuro (Buscapina®) and topical pharyngeal anesthetic spray [xylocaina Astra (FARMA)®], with the patient lying on a left lateral position.

Maxum reusable biopsy forceps (Wilson-Cook®) were used to obtain a gastric biopsy specimen from an area of inflammation or normal appearing mucosal, at 3 cm from the pyloric orifice on the antral lesser curvature, according to previous publications (7,8). The specimen was removed from the biopsy forceps using a sterile needle and, immersed into our test (LUT), which consisted in 8% (wt/vol) urea, pH 6.5 in sterile distilled water, plus 1% phenol red. The final preparation was filtered through a 0.2 µm millipore membrane, obtaining a sterile solution. After filtration the solution was aliquoted in

1,5 mL polystyrene sterile tubes and, it was prepared within 1-30 days before the procedure and preserved at 4°C. The tube containing the sample, was placed in a rack at room temperature and was examined every five (5) minutes. The last reading was done at thirty (30') minutes. The LUT was considered positive when a color change from yellow to pink or red was present. A single observer, without any previous knowledge of the medical record or diagnosis, performed the reading. The fundamental of the test is to offer a rich medium in urea (substrate) that allows to demonstrate the presence of ureasa (an enzyme produced by *H. pylori*). As a consequence from the chemical reaction, an increase of pH is generated (alkalinization), evidenced through the change of color in the media.

After reading the test and decanting the supernatant, the biopsy specimen from the prepyloric area, was immersed in 10% formalin solution for histopathologic examination. All fixed specimens were stained with hematoxylin-eosin, modified Giemsa staining and were evaluated for two pathologist previously trained in recognizing the bacteria and its density, who were not aware neither of the endoscopy findings nor LUT results. The typical appearance of the bacteria at the pathologist analysis, was considered diagnostic for *H. pylori*. The density of the *H. pylori* organisms in the histologic specimen was graded on a scale from 0 to 3: 0= none, 1= mild, few organisms but present uniformly, 2= moderate density of organisms, 3= dense distribution of organisms, according to previous publication (9), figure 1.A and 1.B.

As previously mentioned, histologic evaluation was considered as the gold standard for diagnosing *H. pylori* infection.

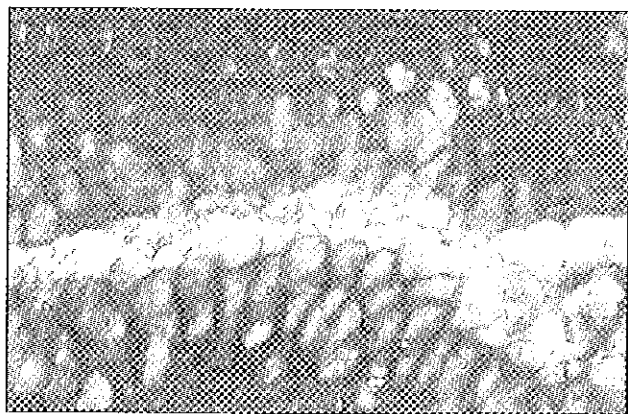
Sensitivities, specificities, positive and negative predictive values and overall accuracy were determined.

## RESULTS

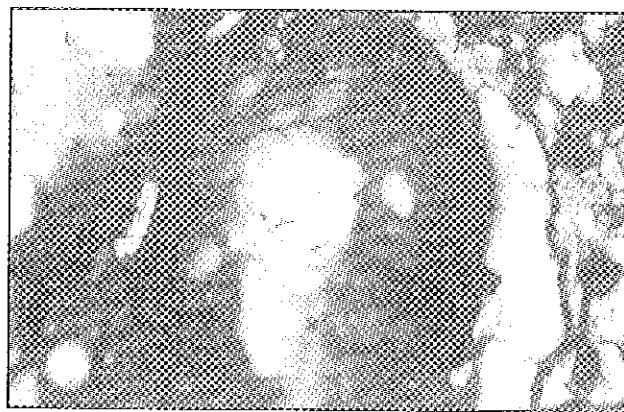
In this study 200 patients, 76 male and 124 females with a mean age of  $45.08 \pm 17.17$  years were included. Major indications for upper endoscopy were no ulcer dyspepsia and dyspepsia type reflux disease as shown in table I.

Fifty four percent (54%) of the patients displayed gastritis as seen by upper endoscopy, 40% had findings compatible with reflux disease and 35% had hiatal hernia (table II). This high incidence of gastritis may be related to the high prevalence of *Helicobacter pylori* infection in our population (10), also the high frequency of hiatal hernia and reflux disease (35% and 40%) is comparable with other western countries.

One hundred and twenty eight patients (64%) were



**Figure 1 - A:** Gastric antral mucosa specimen stained with modified Giemsa witch shows a density equal to 3 (dense distribution of organisms).



**Figure 1 - B:** Gastric antral mucosa specimen staining with modified Giemsa showing a density of 1 (few organisms).

found to show biopsy specimens positive for *H. pylori* on histological examination, which represents an incidence comparable with other reports in our country (10,11).

135 patients (67.5%) were found positives and 65 patients (32.5%) were negatives in the LUT. Our test showed a sensitivity of 91%, specificity of 88.1%, and positive and negative predictive values of 95% and 80% respectively and, an overall accuracy of 90% (Table III and IV). A reliable interpretation of these is that the test can detect the presence of *Helicobacter pylori* with a prediction of 95%, in unique samples obtained from the antrum.

These results also demonstrated that an unique sample of gastric mucosa is suitable for a diagnostic urease test as previously mentioned but, our technique has an additional advantage: we have used the same sample

applied to the liquid media to detect urease activity, to show *Helicobacter pylori* density by histologic analysis (if the outcome of the LUT was negative), which represents a simplification of this invasive procedure (8).

A remarkable correlation between density of *H. pylori* and reading time was also observed, where a high density of the bacteria reduced the reaction time in this liquid test (Table V). In 82% of positive tests, the reaction time was between 1-10 minutes and, almost 50% of the total positives samples reacted before 5'. Thus, our LUT offers faster results because the reaction time has been shortened.

No correlations were observed between density of *H. pylori* and the presence of inflammatory atypias or dysplasia.

**Table I:** Indications for upper endoscopy

Indications	N° of patients	%
Epigastric pain	6	3
Ulcer dyspepsia	19	9.5
No ulcer dyspepsia	63	31.5
Dyspepsia GERD	87	43.5
Dyspepsia dysmotility	3	1.5
History of duodenal ulcer	2	1.0
History of duodenitis	1	0.5
Upper gastrointestinal bleeding	1	0.5
Alarm symptoms	2	1.0
Other	16	8.0
Total	200	100

**Table II:** Endoscopic findings

Endoscopic findings	Frequencies	%
Gastritis	107	54
Gastric ulcer	10	5
Duodenal ulcer	2	1
Reflux esophagitis	79	40
Normal	10	5
Carcinoma of stomach	2	1
Duodenitis	6	3
Hiatal hernia	70	35

**Table III:** Results of liquid urease test (LUT) compared with histology evaluation.

	Histology positive	Histology negative	Total	%
LUT positive	128	7	135	67.5
LUT negative	13	52	65	32.5
TOTAL	141	59	200	100

**Table IV:** The sensitivity, specificity, and positive and negative predictive values of the liquid urease test (LUT).

Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)	Overall accuracy
90.8	88.1	95	80	90%

**Table V:** Density in comparison with the time of reaction to the LUT.

Time (minutes)	Density			Total	%
	1 few	2 moderate	3 dense		
< 5	5	26	31	62	48.4
5-10	2	20	21	43	33.6
10-15	6	3	2	11	8.6
>15	3	8	1	12	9.4
Total	17	69	58	128	100

## DISCUSSION

The efforts to develop a reliable and faster urease test which could allow the diagnosis of *Helicobacter pylori* at the moment of the upper endoscopy, ahead of pathologic confirmation and, with the possibility of indicating medical treatment in the same day, are completely justified.

Histologic examination of gastric biopsy specimens remains the gold standard method for positive *H. pylori* identification. However, this procedure is expensive and the results may take several days to achieve, therefore using an urease test could be time saving, cost effective and avoid a new visit for appropriate prescription.

The first urease test was described by McNulty in 1985 and used Christensen's 2% urea. Afterward countless tests had been developed using agar medium such as CLOtest®, Pyloritek®, Hpfast®, some liquid test, etc., but these are expensive and, the reading time is considerable long, ranging between 1 to 24 hours; further-

more some of them need incubation at 37° C or to be heated at 40°C to speed up the reaction. (1,12,13)

The LUT described in the present study uses a high concentration of urea providing a rich media that accelerates the reaction, not additional incubation time is required and our results were faster, 82% of positive results were obtained within 1 to 10 minutes and, almost 50% of the total positives sample for LUT, reacted before 5'. If we compare LUT with others liquid tests, the time of reading oscillate between 1-4 hours, which make our test faster indeed. (4,12). Additionally, the price cost of the test is < \$1, therefore it is a extremely affordable device and ideal for large screening studies.

Sensibilities and specificities for others commercial tests or liquid tests are between 80-100%. Our test showed a sensitivity of 91%, specificity of 88.1%, and positive

and negative predictive values of 95% and 80% respectively, an overall accuracy of 90% which is respectable (4, 12).

We confirm that an unique sample of gastric mucosal biopsy specimen is sufficient for a diagnostic urease test as previously reported but, our technique has an additional advantage which is that the same sample could be used for pathology examination, allowing to measure the density of the bacteria by mean of sample staining and visual determination.

The media for LUT was prepared under sterile conditions therefore, the tubes may be stored up to one month before use, at regular refrigeration and suitable for practice (12).

Taking together the cost, fast reaction, high positive predictive value and the possibility of using the same sample for the pathologist analysis, once it is nega-

tively confirmed, we can affirm that our test could be a valuable diagnosis tool in the endoscopic room.

This test can also be used in undeveloped countries where the presence of the *Helicobacter pylori* is elevated and even in rural areas where the pathologist is not available or in epidemiological research, where a rapid test of urease can easily classify the population.

In conclusion our LUT is easy to read and handle, fast and cost effective, with a high positive predictive value.

#### ACKNOWLEDGEMENTS

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### Resumen

El objetivo de este estudio fue desarrollar una prueba en medio líquido utilizando biopsias de antro gástrico para detección de manera indirecta de *Helicobacter pylori*, basado en la alta tasa de actividad de ureasa que posee esta bacteria.

#### Materiales y Métodos:

200 pacientes referidos para la realización de Endoscopia digestiva superior debido a diferentes indicaciones, fueron incluidos en este estudio. Una muestra de antro pilórico fue obtenida durante la endoscopia, la cual se colocó en el medio líquido de urea. La misma muestra fue utilizada para la evaluación histopatológica que se consideró para nuestro trabajo como la prueba diagnóstica de elección para la infección de *Helicobacter pylori*.

#### Resultados:

135 pacientes (67.5%) fueron encontrados positivos and 65 pacientes (32.5%) fueron negativos en nuestra prueba líquida de urea.

128 pacientes (64%) fueron positivos para la presencia de *Helicobacter pylori* en la evaluación histopatológica. Nuestra prueba demostró una sensibilidad de 91%, especificidad de 88.1%, con valores predictivos positivos de 95% y valores predictivos negativos de 80%.

Una alta correlación fue observada entre la densidad de *Helicobacter pylori* y el tiempo de lectura de la prueba líquida de ureasa.

La exactitud de la prueba fue del 90%, lo cual es comparable con las demás pruebas diagnósticas disponibles comercialmente para la detección de *Helicobacter pylori*.

#### Conclusión:

La prueba de ureasa en medio líquido es de bajo costo, rápida, con altos valores predictivos positivos y de fácil preparación.

#### BIBLIOGRAFIA

1. Sato T, Fujino MA, Kitahara F, and Abdullah M, 2001. Recent progress in endoscopy-based diagnosis of *Helicobacter pylori* infection. *Digestive Endoscopy* 13:3-6.
2. Laine L, Lewin D, Naritoku W, Estrada R., Cohen H, 1996. Prospective comparison of commercially available rapid urease tests for the diagnosis of *Helicobacter pylori*. *Gastrointest Endosc* 44:523-6.
3. Anderson JC, Cheng E, Roeske M, Marchildon P, Peacock J, and Shaw R, 1997. Detection of serum antibodies to *helicobacter pylori* by an Immunochromatographic metod. *Am J Gastroenterol* 92:1135-1139.
4. Kent-Man C, Poon R, Tuen HH, Law S, Branicki F, Wong J, 1997. A prospective comparison of locally made rapid urease test and histology for the diagnosis of *Helicobacter pylori* infection. *Gastrointestinal Endosc* 46:503-06.
5. Brown KE, Peura DA, 1993. Diagnosis of *Helicobacter pylori* infection. *Gastroenterol Clin North Am* 22:105-15.

6. Youstfi MM E-ZH, Genta RM, Graham DY, 1996. Evaluation of a new reagent strip rapid urease est for detection of *Helicobacter pylori* infection. *Gastrointest Endosc* 44:519-22.
7. Genta RM, Graham DY, 1994. Comparision of biopsy sites for the histopatologic diagnosis of *Helicobacter pilory*: a topographic study of *H. pylori* density and distribution. *Gastrointest Endosc* 40:342-5.
8. Youstfi M, El-Zimaity H, Cole R, Genta R, Graham D, 1996. Detection of *Helicobacter pylori* by rapid urease tests: is biopsy size a critical variable. *Gastrointest Endosc* 43:222-4.
9. Dixon MF, Genta RM, Yardley JH, Correa P, 1996. Classification and grading of gastritis. *Am J of Surg Pathol* 20:1161-81.
10. Dominguez-Bello MG, Michelangeli F, Romero R, Beker B, Lara D, Morera C, Vezga MA, Spardella V, Guelrud M, Perez ME, Pericchi LR, 1997. Modification of Christensen urease test as an inexpensive tool for detection of *Helicobacter pylori*. *Diagn Microbiol Infect Dis* 28:149-52.
11. Urrestarazu M, Serrano N, Piñero R, Aristimuño L, Beauperthuy Y, Poleo JR, 1989. Prueba de ureasa para el diagnóstico de Infección por *Campilobacter pylori*. *GEN* 43:169-172.
12. Jaup BH, Stenquist B, Brandberg A, 2000. *Helicobacter pylori* Culture from a Positive, Liquid-Based Urease Test for Routine Clinical Use: A cost-Effective Approach. *Helicobacter* 5:22-23.
13. McNulty CAM, Wise R, 1985. Rapid diagnosis of *Campylobacter*-associated gastritis. *Lancet* i:1443-4.

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