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Comparative study of urease tests for Helicobacter pylori detection in gastric biopsies.

Article

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SUMMARY.

Introduction. A positive urease test is strong evidence of *Helicobacter pylori* (*H. pylori*) infection. This test is widely used as standard procedure for the detection of this bacteria, because it is a simple, reliable and inexpensive test, and provides quick results.

Material and Methods. Ninety-nine biopsies from patients of the Endoscopy Department at San Vicente de Paul Hospital, Heredia, Costa Rica were studied. Diagnosis of *H. pylori* infection was based on histological examination and apposition smear stained with Giemsa of biopsies samples from gastric antrum. A comparative study of urease tests for the diagnosis of *H. pylori* in gastric biopsies was made using O-Cresolphthalein Complexone (OCPC), Phenol Red, Berthelot and Urea Agar methods. The sensitivity and specificity of the urease methods were obtained and compared.

Results. The sensitivities and specificities obtained were for Phenol Red 97% and 100%, for OCPC

99% and 100%, for Berthelot 97% and 93% and Urea Agar 92% and 96% respectively.

Discussion. The OCPC Method presented the best results of the urease tests evaluated, followed by the Phenol Red method. The OCPC method has the advantage of a greater reagent stability and less possibilities of false positive results due to inadequate washing of the glassware. However the Phenol Red test has a significantly faster reaction time, which is an obvious clinical advantage. (*Rev Biomed 1999; 10:145-151*)

Key words: *H. pylori*, urease tests, gastric biopsy, diagnosis.

RESUMEN.

Estudio comparativo de pruebas de ureasa para la detección de *Helicobacter pylori* en biopsias gástricas.

Introducción. Una prueba de ureasa positiva es una

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fuerte evidencia de infección por *Helicobacter pylori* (*H. pylori*). Esta prueba es comúnmente utilizada para la detección de esta bacteria debido a que es un método simple, fácil de realizar y económico, que suministra resultados rápidamente.

Materiales y métodos. Se estudiaron 99 biopsias provenientes de pacientes del Departamento de Endoscopía del Hospital San Vicente de Paul, Heredia, Costa Rica. El diagnóstico de *H. pylori* se basó en el análisis histológico y en el frotis por aposición teñido con Giemsa de biopsias de antro gástrico. Se realizó un estudio comparativo de pruebas de ureasa para el diagnóstico de *H. pylori* en biopsia gástrica utilizando los métodos de O-cresoltaleína complexona (OCPC), rojo fenol, Berthelot y agar urea. La sensibilidad y la especificidad de estos métodos fueron comparadas.

Resultados. Las sensibilidades y especificidades obtenidas para rojo fenol fue de 97% y 100%, para OCPC de 99% y 100%, para Berthelot de 97% y 93% y para agar urea de 92% y 96%, respectivamente.

Discusión. El método de la OCPC presentó los mejores resultados, seguido por el rojo fenol. El método de la OCPC presenta la ventaja de tener un reactivo con mayor estabilidad y una menor posibilidad de presentar falsos positivos debido a la contaminación de la cristalería. Sin embargo el rojo de fenol tiene un tiempo de reacción más corto, siendo una ventaja clínica del método.

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Palabras clave: *H. pylori*, prueba de ureasa, biopsia gástrica, diagnóstico.

INTRODUCTION.

In 1983 Warren and Marshall (1) isolated a new curved Gram negative bacillus from the gastric mucosa of patients with active chronic gastritis, bacteria that was first named *Campylobacter pyloris*, then *Campylobacter pylori* and finally *Helicobacter pylori* (*H. pylori*) (2), establishing an association between the bacteria, gastritis and

peptic ulcer disease.

H. pylori is the most important cause of chronic gastritis (3-6), it is also the most important etiological factor responsible for duodenal ulcer (7-9), gastric ulcer (10-13), and has an important role in the pathogenesis of gastric cancer (14-18). In Costa Rica gastric cancer is the most frequent malignancy, an important reason for developing research in *H. pylori* in our population (19).

The identified virulence factors of *H. pylori* include the flagella used for moving through the mucus, the urease activity used for neutralizing the acid from the stomach and a cytotoxin activity that produces vacuolization of epithelial cells (20-22). It is not well known how the urease acts, but its role in the colonization of the mucosa and the injury that it produces is very clear (23).

Since Marshall and Warren established the association between *H. pylori*, gastritis and peptic ulcer a great number of diagnostic techniques for the identification of this microorganism have been developed (24).

The first rapid and simple test developed for the diagnosis of *H. pylori* infection was the urease test based on the capacity of the organism to produce great quantities of this enzyme (25-27). The urease catalyzes the degradation of urea to ammonia and bicarbonate. This reaction produces an increase in the pH of the medium that can be detected by an acid-base indicator such as phenol red, that changes color from yellow to pink or red (28). The velocity of the change of color depends on the urease concentration according to the number of bacteria present (29).

The great advantage of the urease test in the diagnosis of *H. pylori* is that the results can be obtained before the patient leaves the endoscopy room, making the clinical management easier. The urease tests results are comparable in sensitivity and specificity with the histological and culture techniques, being more economic and faster (24). Nevertheless an endoscopy is always necessary because a gastric biopsy is required to perform the test and also a culture can be required for evaluating

the sensitivity to antibiotics. The urease test should be done jointly with another diagnostic test such as histology or culture. McNulty and Wise (28) were the first ones to use this test for the detection of *H. pylori* infection. From then on, diverse variations for the improvement of the test making it simpler and faster have been proposed (24, 31-34).

The CLO test (*Campylobacter* Like Organism) was the first commercial test available developed by Barry Marshal. This test is done in agar with urea and phenol red, and in the presence of the bacteria the medium changes from yellow to magenta (29,30,35-36).

In the present study two new modifications for the determination of urease in gastric biopsies were developed using o-cresolphthalein complexone (OCPC) indicator and the Berthelot method (37) as alternatives for the *H. pylori* diagnosis in gastric biopsies.

The OCPC method is based on the detection of urease by pH changes, which can be detected by color variation of the indicator from pink to purple. The reagent used in this method includes an ethylenediamine tetracetate solution that avoids calcium interference and pH variations that may be produced by the specimen or by chemical contamination of the test tube. The Berthelot reaction provides evidence of the presence of urease based on the reaction of the ammonia produced with sodium hypochlorite (alkaline solution) and phenol (catalytic agent), producing intense blue colour (indophenol), a procedure that has not yet been used for the detection of *H. pylori*.

A comparative study was done between the histological analysis and apposition smear stained with Giemsa as reference methods and the urease tests with Phenol Red, OCPC, Berthelot and Urea Agar, for determining the best diagnostic sensitivity and specificity.

MATERIALS AND METHODS.

Samples: 99 gastric biopsies from patients

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of the Endoscopy Department of the San Vicente de Paul Hospital, Heredia, Costa Rica were analyzed between April and May 1997. At least five close gastric biopsies were taken from the antrum in each patient for the histological, Phenol Red, OCPC and Urea Agar tests. For the Berthelot test and the apposition smear stained with Giemsa, the same biopsy was used. Patients were informed about the study and those who agreed to continue with it filled a data sheet that included personal information.

Phenol Red rapid urease test (38): A solution of urea 10% and a solution of phenol red 1% were prepared. For the working solution, two drops of phenol red solution were mixed in 1 ml of the urea solution. The reagent is stable for 2 weeks at 4-8°C. Each biopsy was embeded in 0,2 ml of the reagent and incubated at room temperature (24°C) for one minute.

OCPC urease test: A work solution was prepared with EDTA 2Na 2,5 mmol/L, urea 100 mg/dl and OCPC 2,5 mmol/L at pH 7,0. Each biopsy was embeded in 0,5 ml of reagent and incubated at room temperature (25°C). The reagent was stable for at least one year at 4-8 °C.

Berthelot urease test: A gastric biopsy was added to a tube containing 1,0 ml of urea solution and was incubated for 15 minutes at 37 °C. 1,0 ml of phenol solution and 1,0 ml of sodium hipoclorite solution were then added and incubated for another 15 minutes at 37°C. A blank reagent was runned as a control. A blue color indicates the presence of ammonia in the sample (37).

Urea Agar urease test: Urea agar medium (2% urea and 10% phenol red) were inoculated with gastric biopsies and incubated for 24 hours at 37°C. A change in color of the agar from yellow to pink indicates the presence of urease in the sample (38).

The histological study of each biopsy was performed at the Pathology Service of Hospital Mexico, San Jose, Costa Rica, using hematoxilline/eosine stain, for the observation of the presence of rod like *H. pylori* organisms.

RESULTS.

Considering the histological analysis and the apposition smear stained with Giemsa as reference methods, there were 74 patients with true positive results and 25 patients with true negative results.

Table 1 shows the results of the gastric biopsies analyzed by the different urease tests. Table 2 presents the results of sensitivity and specificity of the urease tests studied.

DISCUSSION.

The diagnostic sensitivity represents the percentage of patients with positive results that really are infected with *H. pylori*. The diagnostic specificity represents the percentage of patients with negative results that are not infected with *H. pylori*.

Of all the urease methods studied, the best results were obtained with the OCPC method, with a sensitivity of 99% and a specificity of 100%, followed by Phenol Red method with a sensitivity of 97% and a specificity of 100%. These results indicate that OCPC and Phenol Red methods have no false positives and the OCPC method only one false negative and the Phenol Red method two false

negative results.

The Urea Agar and Berthelot methods have lower sensitivities and specificities, the first showed 6 false negatives and 1 false positive and the second 2 false negatives and 2 false positives results.

Some authors have reported urease tests with specificities between 98 and 100% and sensitivities between 64 and 98%, speed being the great difference between the methods of analysis (24). Arvid (38) found in the rapid urease test of Phenol Red a sensitivity of 91% and specificity of 100%, also Vaira (39), Thillainayagam (34) and Malfertheiner (32) found specificities of 100% and sensitivities of 94%, 89% and 90% respectively. Hernández (26) reports a sensitivity of 72% and a specificity of 83%. For the Christensen's urea broth Arvid (39), McNulty (28), Morris (36) and Westblom (27) report specificities of 86%, 98%, 92% and 100% and sensitivities of 84%, 92%, 100%, 88%, respectively. For the CLO-test Morris (36), Vaira (40) and Malfertheiner (32) report specificities of 100% and sensitivities of 96%, 58% and 88% respectively.

The presence of false negative and false positive results in the different methods tested may be explained by the patchy distribution that *H. pylori* presents in gastric mucosa, especially in the

Table 1
Results of gastric biopses studied by different urease tests

Test	<u>N° of specimens</u>		<u>N° of false results</u>	
	positive	Negative	positives	negatives
Phenol Red	72	27	0	2
O-cre-Solphthalein complexone	73	26	0	1
Urea agar	69	30	1	6
Berthelot	70	23	2	2

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Table 2
Clinical sensitivity and specificity of the different urease tests used.

<u>Test</u>	<u>Diagnostic sensitivity*</u>	<u>Diagnostic specificity</u>
Phenol Red	97%	100%
o-cresolphthalein complexone	99%	100%
Urea Agar	92%	96%
Berthelot	97%	93%

* Diagnostic sensitivity: $\frac{\text{True positive}}{\text{True positive} + \text{false negatives}} \times 100$

Diagnostic specificity: $\frac{\text{True negatives}}{\text{True negatives} + \text{false positives}} \times 100$

body and fundus of the stomach, so the microorganism can be present in one biopsy and absent in another from the same patient (30-41). This situation is overcome by the use of several specimens of gastric biopsies from the same patient (42,43) to minimize the specimen error caused by the non-homogeneous distribution of the microorganism in the stomach.

If only the histological analysis is used as reference method, the sensitivity and specificity of the apposition smear stained with Giemsa will be 95% and 100% respectively. Because we used the same gastric biopsy for the Giemsa stain and for the Berthelot urease test we can state that 2 of the 4 false negatives that were present in the Giemsa stain were caused by the patchy distribution of the bacteria. These two specimens were negative with Berthelot method and Giemsa stain but were positive with the other urease methods studied confirming this condition. Only the other two negative results are really false negatives, because they were negative only with Giemsa stain and positive with the other tests. If we considerate this

situation the sensitivity of the test is better.

If only the apposition smear stained with Giemsa is considered as reference method, the histological analysis will have a sensitivity of 97% and a specificity of 100% with 2 false negative results, probably due to the patchy distribution of *H. pylori*.

The advantage that we found in the OCPC test over the Phenol Red test was the larger stability of the reagent. The working Phenol Red reagent was stable only for two weeks while the OCPC reagent was stable at least for one year at 4-8°C. Also OCPC test has lower possibility of false positives due to the inadequate washing of the glassware.

We consider the OCPC test adequate for the diagnostic of *H. pylori* in gastric biopsy specimens, but those samples with low urease activity due to a small number of microorganisms should have longer incubation periods to obtain positive results, an advantage of the Phenol Red test is its faster reaction period in this conditions. It is recommended that those specimens that do not

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react immediately with OCPC shall be incubated at room temperature (24°C) for at least 12 hours.

The Berthelot and Urea Agar tests have the disadvantage of requiring longer reaction periods and incubation at 37°C, however they represent another alternative test for the diagnosis of *H. pylori* in gastric biopsy specimens.

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