

VALIDATION OF A RAPID STOOL ANTIGEN TEST FOR DIAGNOSIS OF *Helicobacter pylori* INFECTION

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SUMMARY

The aim of this study was to validate the rapid lateral flow *Helicobacter pylori* stool antigen test (One step *H. pylori* antigen test, ACON laboratories, San Diego, USA; Prime diagnostics, São Paulo), using ^{13}C -Urea Breath Test as the gold standard for *H. pylori* infection diagnosis. A total of 98 consecutive patients, asymptomatic or dyspeptic, entered the study. Sixty-nine were women, with a mean age of 45.76 ± 14.59 years (14 to 79 years). In the *H. pylori*-positive group, the rapid stool antigen test detected *H. pylori* antigen in 44 of the 50 positive patients (sensitivity 88%; 95% CI: 75.7-95.5%), and six false-negative; and in the *H. pylori*-negative group 42 presented negative results (specificity 87.5%; 95% CI: 74.7-95.3%), and six false-positive, showing a substantial agreement (Kappa Index = 0.75; $p < 0.0001$; 95% CI: 0.6-0.9). Forty four of fifty patients that had positive stool antigen were *H. pylori*-positive, the PPV of the stool antigen test was 88% (95% CI: 75.7-95.5%), and 42 patients with negative stool antigen test were *H. pylori*-negative, the NPV of the stool antigen test was 87.5% (95% CI: 74.7-95.3%). We conclude that the lateral flow stool antigen test can be used as an alternative to breath test for *H. pylori* infection diagnosis especially in developing countries.

KEYWORDS: ^{13}C -urea breath test; *H. pylori* stool antigen test; *H. pylori* diagnosis.

INTRODUCTION

Helicobacter pylori (*H. pylori*), spiral or curved gram-negative microaerophilic, flagellate bacilli has been considered the etiological cause of gastritis, peptic ulcer disease^{21,24} and associated with the development of gastric cancer²⁵.

The prevalence of *H. pylori* infection is different worldwide, depending on the socioeconomic status and sanitation conditions¹; in the developed countries being under 40% and more than 80% in developing countries¹⁶, including Brazil where the prevalence varies from 65.6% in the city of Sao Paulo that has a high standard of living³⁴, to 87% in the poor urban areas of Fortaleza, northeastern Brazil²⁹.

The diagnosis of *H. pylori* infection can be achieved by invasive methods such as urease test, which has 97.4% sensitivity and 100% specificity³², widely used and performed in the endoscopy suite as a rapid indirect test to confirm the presence of *H. pylori* in biopsy samples, and histology of the Modified Giemsa stained gastric biopsies (96.2% sensitivity)¹⁸. Non-invasive methods, ^{14}C - urea breath test (^{14}C -UBT)^{22,23} and ^{13}C urea breath test (^{13}C -UBT), are considered gold standard¹⁹. Recently, a noninvasive diagnostic test based on the detection of *H. pylori* stool antigen has been developed. Some *H. pylori* stool antigen tests that use polyclonal antibodies to *H. pylori* have shown heterogeneous results^{2,12,17}. More recently, novel stool antigen tests based on monoclonal

antibodies which could increase the accuracy of this test, have been developed^{2,8,12,14,15,33}.

The non-radioactive isotope ^{13}C -UBT is preferable to ^{14}C -UBT³; however, the acquisition of Infrared has a cost that most places cannot afford, and that makes the stool antigen a cheaper alternative choice for eradication control^{2,15}. It was recommended by the Maastricht III Consensus Report as a non-invasive test¹⁹.

The stool antigen tests based on immunoassay (ELISA) have been tested in several laboratories of the world, including in Brazil^{4,26-28}, and the results have been satisfactory. In Brazil, previous validation of a monoclonal stool antigen test for diagnosis of *H. pylori* was reported in children²⁷; however, the stool antigen test based on lateral flow chromatography has not yet been tested in adults. The major advantage of these tests is the cost, when compared to ^{13}C -UBT and the possibility of being performed in any laboratory^{10,14,15,17}.

The rapid stool antigen test (ACON laboratories, Inc, San Diego, USA) is a rapid 10-min assay based on lateral flow chromatography with polyclonal antibodies that detect *H. pylori* antigens present in human stool. Thus, the aim of this study was to evaluate the rapid *Helicobacter pylori* stool antigen test, using ^{13}C -UBT as the "gold standard" method for *H. pylori* infection diagnosis.

MATERIAL AND METHODS

Patients: This study was approved by the local Ethics Committee (CAPPesq 0311/2009). A total of 98 consecutive patients, asymptomatic or dyspeptic, entered the study after providing written informed consent. Sixty-nine were women with a mean age of 45.76 ± 14.59 years (14 to 79 years). None had taken antibiotics at least one month before the test or acid secretor inhibitors in the previous ten days. The interval between the tests for *H. pylori* infection diagnosis did not exceed 14 days. The stools were fresh and processed immediately. Patients were instructed to store the stools at 4-16 °C until arrival at the laboratory.

***H. pylori* status:** The *H. pylori* status was based on the results of ¹³C-Urea breath test, and ¹³C-Urea breath test was considered the gold standard to ascertain the *H. pylori*-positive and the *H. pylori*-negative groups.

¹³C-Urea breath (UBT): Patients had to avoid smoking and sparkling water or soft drinks on the day of the test and observe a 4-hour fast. ¹³C-Urea breath was performed using acid meal, composed of 75 mg of ¹³C-labeled urea dissolved in 200 mL of orange juice. Breath samples were collected in aluminized plastic bag to determine the baseline value before ingestion of acid meal and at 30 minutes after ingestion of the juice. Breath samples were analyzed by infrared spectroscopy (IRIS DOC, Wagner Analysen - Technik, Bremen, Germany). A DOB (Delta over baseline-value) $\geq 4.0\%$ was considered positive for *H. pylori* infection, as previously described¹⁷.

***H. pylori* stool antigen test by Lateral Flow Immunoassay:** Specimens were tested using the stool antigen test (One step *H. pylori* antigen test device, IHP-602, ACON laboratories, Inc, San Diego, USA; Prime diagnostics, São Paulo, Brazil) according to the manufacturer's instructions (94.9%-100% sensitivity and 95.1-100% specificity, according to the manufacturer). Briefly, small samples of stool specimens collected from three different parts of the stool sample were transferred to a vial with diluent, vigorously agitated and after two minutes of resting the tube, dropping around two to three drops into the round window of the test cassette. Reading was made after 10 minutes of incubation at room temperature, and based on the appearance of colored lines across the central window of the cassette, two lines, C (control) and T (test), indicated positive test, only one line in C indicated negative result. A pale colored line in T was also considered positive.

Statistical analysis: The statistical analysis was performed by Kappa index measure of agreement of diagnostic tests, and Chi-square using SPSS. Confidence intervals were calculated by Exact Binomial Test with program R 2.10.1.

RESULTS

Fifty of ninety-eight patients were positive according to UBT, being defined as *H. pylori*-positive, and 48 patients were negative according to

UBT and considered *H. pylori*-negative. In the *H. pylori*-positive group, the rapid stool antigen test detected *H. pylori* antigen in 44 of the 50 positive patients (sensitivity 88%; 95% confidence interval (CI): 75.7 - 95.5%), and six false-negative; and in the *H. pylori*-negative group, 42 presented negative results (specificity 87.5%; 95% CI: 74.7 - 95.3%), and six false-positives, showing a substantial agreement (Kappa Index = 0.75; $p < 0.0001$; CI: 0.6 - 0.9). Considering that 44 patients of 50 that had positive stool antigen were *H. pylori*-positive according to UBT, the positive predictive value of the stool antigen test was 88% (95% CI: 75.7 - 95.5%), and that 42 patients with negative stool antigen test were *H. pylori*-negative, the negative predictive value of the stool antigen test was 87.5% (95% CI: 74.7 - 95.3%), as shown in **Table 1**. Among six patients that had positive stool antigen test and negative UBT, four of them were eradication control.

DISCUSSION

H. pylori colonizes the human stomach during childhood²⁶ and survives in the human stomach, the only niche known to date, for the lifetime of the carrier⁵. In most of the individuals *H. pylori* infection may be asymptomatic, causing chronic gastritis. Around 20% to 30% of the infected individuals may develop peptic ulcer disease²⁰, and less than 2% gastric cancer¹⁶. Gastrointestinal endoscopy has been widely performed for gastrointestinal disorders and *H. pylori* infection diagnosis²⁰. Nonetheless, the Maastricht III consensus report recommended in primary care, a test and treat strategy using a noninvasive test in adult patients under the age of 45 with persistent dyspepsia. The urea breath test, stool antigen tests, and serological kits with a high accuracy are non-invasive exams which should be used for the diagnosis of *H. pylori* infection¹⁹.

For eradication control, ideally after three months of treatment, the breath test rapidly confirms the disappearance of *H. pylori*³¹, unlike the serological techniques, which need a prolonged period of time to confirm the eradication effect. Thus, because antibody titers can take up to six months to fall after successful treatment, serological tests cannot readily be used to assess the efficacy of *H. pylori* eradication regimens shortly after treatment⁸.

Even though the radiation exposure from ¹⁴C-urea breath test is less than 1% of that received from an upper gastrointestinal series²², the cost of a β counter is low, and the test has 100% specificity and sensitivity²³, the long half life (5.730 years)³ of ¹⁴C restricts its use due to the environmental protection policy. That means ¹³C-urea breath test and the stool antigen test are the only choices of non-invasive methods for *H. pylori*-infection eradication control.

The development of non-dispersive isotope selective infrared spectrometers opened up a lower-priced analytical alternative for high-resolution mass spectrometers with sensitivity and specificity of 95% in multiple studies and a meta-analysis including more than 3500 patients³. However, most of the Health Centers in Brazil cannot afford to pay for infrared spectrometers in foreign currency, even though the cost of

Table 1
Performance of the stool antigen test using UBT to define *H. pylori*-negative and *H. pylori*-positive groups

| Group | Method | True positive | True negative | False positive | False negative | Sensitivity | Specificity | PPV | NPV |
|-------|--------------------|---------------|---------------|----------------|----------------|-------------|-------------|-----|-------|
| 98 | Stool antigen test | 44 | 42 | 6 | 6 | 88% | 87.5% | 88% | 87.5% |

¹³C-urea is lower than the ¹⁴C-urea. Thus, the stool antigen test may be the only option of a non-invasive method of *H. pylori* eradication control in these Health Centers.

The stool antigen test may be performed by conventional immunoassay (EIA) using polyclonal antibodies^{4,6,7,10,13,17}, showing sensitivity of 88.9% to 98.3% and specificity of 77.8% to 98.4%, and by rapid lateral flow immunoassay using monoclonal antibodies^{10,12,14,15,33}, presenting 52.5-94.6% sensitivity, 55.5-98.4% specificity, 94.6% positive predictive value and 98.4% of negative predictive value^{2,10,12,33}. The lateral flow immunoassay is useful for small laboratories that do not have equipment for performing the EIA and that work with few samples. The lateral flow immunoassay is faster than the conventional EIA that takes more than two hours to be performed; in addition, the cut-off value is still a matter of debate^{2,33}.

We tested rapid lateral flow immunoassay stool antigen (a commercial immunoassay kit of ACON), using the ¹³C-urea breath test as the gold standard method, and detected a sensitivity of 88% and specificity of 87.5%, presenting good agreement with the breath test ($\kappa = 0.755$), data consistent with those previously reported.

As the patients guaranteed that they had followed the instruction of avoiding proton pump inhibitors for at least ten days before the test, temporary inhibition of the growth would not be the cause of false-negative results. Nonetheless, low colonization of bacteria in the stomach, and consequently, low concentration of antigens of *H. pylori* in the feces could not be enough to react in the test¹⁵, causing the false-negative tests. Coccoid form of *H. pylori* may explain the false-positive results that is the morphologic manifestation of bacterial cell death and does not mean an infection risk²; conversely, three patients with false-positive result were not eradication control; however, two of them had *H. pylori*-positive relatives sharing the same bathroom. Even though patients were instructed to collect the stools in sterile vials supplied by the laboratory, we cannot exclude the possibility of external contamination. Another possibility would be cross-reaction among the polyclonal antibodies of the test with antigens of bacteria from the intestinal flora.

KRAUSSE *et al.* (2008)¹⁴ evaluated lateral flow rapid fecal antigen tests and found that the incubation time was an important factor for the reading of the result: readings at 30 min (76.9%) and 60 min (78.6%) had higher sensitivity than after 20 min (59.1), suggesting a new reading strategy: first interpretation at 15-20 min, and a long incubation of 30 min when the sample is negative for up to 20 min. We also observed in some samples that turned up positive after 20 minutes of incubation (data not shown), that this strategy may increase sensitivity.

We conclude that the lateral flow stool antigen test can be used as an alternative to breath test for diagnosis of primary infection of *H. pylori*, especially in developing countries.

RESUMO

Validação de teste rápido de antígeno fecal para diagnóstico de infecção por *Helicobacter pylori*

O objetivo desse trabalho foi avaliar o teste rápido de antígeno de *H. pylori* nas fezes (One step *H. pylori* antigen test, ACON laboratories, San

Diego, USA; Prime diagnostics, São Paulo), usando teste respiratório com uréia marcada com ¹³C (TRU-¹³C), como padrão ouro. Noventa e oito pacientes assintomáticos ou com dispesia participaram do estudo. Sessenta e nove eram mulheres; a média de idade dos pacientes foi de 45.76 ± 14.59 (14 a 79 anos). No grupo *H. pylori* positivo, o teste rápido detectou antígenos de *H. pylori* nas fezes em 44 dos 50 pacientes positivos (sensibilidade de 88%; 95% IC: 75.7-95.5%), com seis falso-negativos; e no grupo *H. pylori* negativo, 42 apresentaram resultados negativos (especificidade de 87,5%; 95% IC: 74.7-95.3%), com seis falso-positivos, mostrando concordância substancial (índice Kappa = 0.75; $p < 0.0001$; 95% IC: 0.6-0.9). Quarenta e quatro dos 50 que tiveram teste de antígeno fecal positivo eram *H. pylori* positivos, sendo o VPP do teste 88% (95% IC: 75.7-95.5%), e 42 pacientes com teste de antígeno fecal negativo eram *H. pylori* negativos, com VPN de 87,5% (95% IC: 74.7-95.3%). Concluímos que o teste de antígeno fecal imunocromatográfico pode ser usado como alternativa ao teste respiratório para diagnóstico de infecção pelo *H. pylori*, principalmente em países em desenvolvimento.

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