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# LOW DOSE CAPSULE BASED $^{13}$C-UREA BREATH TEST COMPARED WITH THE CONVENTIONAL $^{13}$C-UREA BREATH TEST AND INVASIVE TESTS

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**ABSTRACT -** ***Context***- One of the limitations of $^{13}$C-urea breath test for *Helicobacter pylori* infection diagnosis in Brazil is the substrate acquisition in capsule presentation. ***Objectives***- The purpose of this study was to evaluate a capsule-based $^{13}$C-urea, manipulated by the Pharmacy Division, for the clinical practice. ***Methods***- Fifty patients underwent the conventional and the capsule breath test. Samples were collected at the baseline and after 10, 20 and 30 minutes of $^{13}$C-urea ingestion. Urease and histology were used as gold standard in 83 patients. ***Results***- In a total of 50 patients, 17 were positive with the conventional $^{13}$C-urea (75 mg) breath test at 10, 20 and 30 minutes. When these patients repeated breath test with capsule (50 mg), 17 were positive at 20 minutes and 15 at 10 and 30 minutes. The relative sensitivity of $^{13}$C-urea with capsule was 100% at 20 minutes and 88.24% at 10 and at 30 minutes. The relative specificity was 100% at all time intervals. Among 83 patients that underwent capsule breath test and endoscopy the capsule breath test presented 100% of sensitivity and specificity. ***Conclusion***- Capsule based breath test with 50 mg $^{13}$C-urea at twenty minutes was found highly sensitive and specific for the clinical setting.

**HEADINGS-** *Helicobacter pylori*. Breath Test. Urea, analysis.

## INTRODUCTION

*Helicobacter pylori (H. pylori)* infection is the main etiological cause of chronic gastritis, peptic ulcer disease[19] and its recurrence[17]. The eradication of *H. pylori* infection has been considered primary prophylaxis of gastric cancer, and responsible for gastric cancer reduced incidence and mortality[14]; thus, diagnosis of *H. pylori* infection is highly important for the correct management of these disorders[17].

Among the noninvasive tests for *H. pylori* infection diagnosis, urea breath test using urea labeled with carbon-13, non-radioactive isotope, was originally described by Graham et al. in 1987[10], showing excellent correlation with culture and histological analysis. Marshall and Surveyor in the next year[20] reported also a simple breath test with urea labeled with carbon-14, presenting a significant difference between individuals that were *H. pylori* positive in relation to the controls. The principle of urea labeled breath test is the production of urease by *H. pylori* in the acidic environment of the stomach that hydrolyses urea in carbon labeled bicarbonate that is absorbed and, released as carbon dioxide ($^{13}CO_2$ or $^{14}CO_2$) in the exhaled breath[24]. We had previously validated $^{14}$C-urea breath test with 100% sensitivity and specificity[21]; nonetheless, due to its restrictions to pregnant woman and children, the conventional $^{13}$C-urea breath test[30] was further introduced for the clinical practice.

The accuracy of $^{13}$C-urea breath test is very high with sensitivity of 88% to 95% and specificity of 95 to 100%, being an acceptable non-invasive test to diagnose *H. pylori* infection for initial diagnosis and eradication control after treatment[17]. In Brazil, the conventional test with $^{13}$C-urea in powder dissolved in orange juice has been widely performed in children with doses of 50 mg and 75 mg in patients with less than 30 kg and more than 30 kg, respectively[3, 15, 16, 29], and in adults[30]. However, the conventional test with liquid $^{13}$C-urea in orange juice may suffer the influence of urease-producing oral flora, increasing $^{13}CO_2$ excre-

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tion in earlier sample collections with false-positive results, also a higher dose is necessary[7, 25, 27].

Capsule based $^{13}$C-urea breath test has been reported with lower dose than the conventional test with high sensitivity and specificity, avoiding bacterial oral flora interference, and in a shorter time[7, 25, 27, 28]. One of the limitations for $^{13}$C-urea breath test to be incorporated into daily gastroenterology practice in Brazil is the substrate ($^{13}$C-urea) acquisition in capsule presentation[6]. Thus, the purpose of this study was to evaluate the usefulness of a capsule-based $^{13}$C-urea, manipulated by the Pharmacy Division of a Tertiary Care Hospital for the clinical practice.

## METHODS

### Subjects

This study was approved by the local Ethics Committee. The inclusion criteria were patients who had not taken antibacterial therapy for at least one month and proton pump inhibitors for less than 15 days prior the urea breath test. Patients that underwent *H. pylori* infection eradication regimen for more than one month and those of initial *H. pylori* infection diagnosis were included in the study. In the first phase of the study fifty patients (mean age of 42.52±11.81 years; thirty-five were women) who underwent $^{13}$C-urea breath test were invited to participate, and gave written informed consent. The purpose of the first phase was to establish the best point time collection of capsule based breath test.

In the second phase of the study after the time point collection was set, $^{13}$C-urea breath test was validated using urease and histology for *H. pylori* status determination. Cases were considered positive when urease and histology were positive. For this analysis 83 patients (mean age of 56.13±12.89 years; 45 were women) that underwent upper gastrointestinal endoscopy and capsule based $^{13}$C-urea breath test entered the study. Cases that had discordant results between histology and urease tests were excluded. One patient with bleeding gastric ulcer that was *H. pylori* negative by urease and histology and *H. pylori* positive by urea breath test (21.3 dob) was excluded.

### Conventional $^{13}$C-urea breath

The procedure was performed according to technique previously described[30]. Briefly, patients had to fast at least 4-hour before the test and not to smoke or drink sparkling water on the day of the test. Breath sample was collected in aluminized bag as the baseline value, followed by ingestion of 75 mg of $^{13}$C-urea (Euriso-top®, France) dissolved in 200 mL of orange juice (pH = 3.0; powdered orange juice in packets prepared with water immediately before use, Dia Brasil Sociedade Ltda., Brazil). Breath samples were collected after 10, 20 and 30 minutes of $^{13}$C-urea ingestion, and analyzed by infrared spectroscopy (IRIS Doc, Wagner Analysen, Germany). A DOB (Delta over baseline-value) ≥4.0‰ was considered positive for *H. pylori* infection according to the manufacturer instruction and previous reports in Brazil[3, 15, 16, 29, 30].

### Capsule based urea breath test

Capsules were prepared by the Pharmacy Division of Hospital das Clínicas da FMUSP. The formulation consisted of 50 mg of $^{13}$C-urea (Euriso-top®, France) and 170 mg of citric acid that were mixed by trituration, reducing all to a fine and uniform powder and filled in hard gelatin capsule size 4, using capsule hand-filling machine (Capsutec, Brazil)[9]. All batches of 50 mg of $^{13}$C-urea capsules were tested for weight variation. Each capsule weight was between 90 per cent and 110 per cent of the average and with the relative standard deviation less than or equal to 5.0%[1]. The capsules were packaged into pouch unit-dose and kept in cardboard boxes with capsules of silica gel[32]. The expiration date of the capsules prepared was 30 days. Not exceeding the period of one week, the patients repeated breath test, ingesting capsules of 50 mg of $^{13}$C-urea with 200 mL of orange juice. Breath samples were collected at basal, and after 10, 20 and 30 minutes of capsule ingestion and analyzed by infrared spectroscopy (IRIS Doc, Wagner Analysen, Germany). A DOB (Delta over baseline-value) ≥4.0‰ was considered positive for *H. pylori* infection.

### Urease test

The biopsy samples taken from the antrum and corpus of the stomach were inserted into the homemade urease test tubes according to the previously described technique. The urease test tube was examined over the next 24 hours[22].

### Histology

Gastric biopsy samples from the antrum and corpus were fixed in 10% formalin and stained with hematoxylin and eosin (H&E) and Giemsa for *H. pylori* identification, as was previously described[23].

### Statistical Analyses

The sample size of 42 patients was calculated through a test between a null hypothesis given by Kappa statistic equal to 0.4 (fair) and an alternative hypothesis with Kappa statistic equal to 0.8 (substantial) for a prevalence of 53% of *H. pylori* in the studied population[18], considering a statistical significance of 5% and a power of 80%.

Kappa Index measure of agreement[31] was performed with SPSS software version 15.0 for Windows (Chicago, Illinois, USA). The diagnostic performance of capsule based breath test was estimated with the software R version 2.15.2 (R Core Team, R Foundation for Statistical Computing Vienna, Austria, http://www.R-project.org). Relative sensitivity and relative specificity were determined based on subjects with positive conventional urea breath test and subjects with negative conventional urea breath test, as the true-positives and the true-negatives, respectively[11]. Breath $^{13}CO_2$ values were compared between patients *H. pylori* positive with those *H. pylori* negative by Mann-Whitney on non-normal distributions, or by Student's *t*-test on normal distribution. The analysis of capsule based breath test in relation to the *H. pylori* status (urease and histology) was performed by Fisher's exact test. Sensitivity, specificity, positive predictive

value and negative predictive value were estimated, using urease and histology as gold standard. A value of $P<0.05$ was considered significant.

## RESULTS

In a total of 50 patients, 17 were positive with the conventional $^{13}$C-urea (75 mg) breath test at 10, 20 and 30 minutes. When these patients repeated the $^{13}$C-urea breath test with 50 mg capsules, 17 were positive at 20 minutes (Kappa Index 1.0 = almost perfect; $P<0.05$) and 15 at 10 and 30 minutes (Kappa Index 0.90 = almost perfect; $P<0.05$) (Figure 1).

The relative sensitivity of $^{13}$C-urea with 50 mg capsules was 100% (95%CI: 77.92%-100%) at 20 minutes and 88.24% (95%CI: 64.16%-97.75%) at 10 and at 30 minutes. The relative specificity was 100% (95%CI: 87.31%-100%) at all time intervals.

Breath $^{13}CO_2$ values were significantly higher ($P<0.05$) in those patients *H. pylori* positive than in those *H. pylori* negative. There was a striking difference of the mean $^{13}CO_2$ excretion between *H. pylori* positive and *H. pylori* negative groups (Table 1), only at 10 (DOB = 2.20‰ and DOB = 2.80‰) and 30 (DOB = 2‰ and DOB = 3.10‰) minutes, four *H. pylori* positive patients presented DOB values lower than

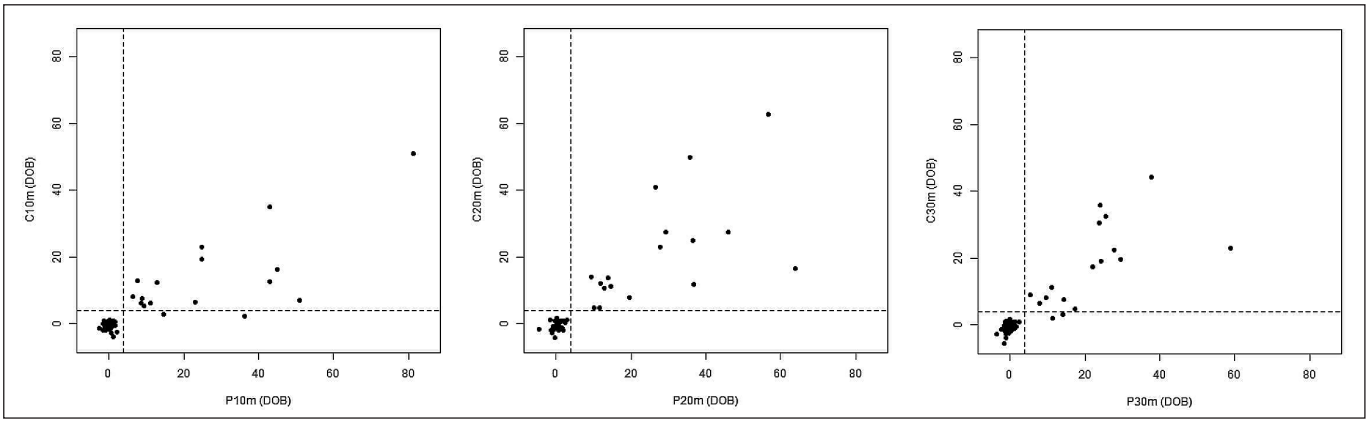


**FIGURE 1.** Comparison of the breath test results obtained with 50 mg $^{13}$C-urea capsules (C) and with the conventional 75 mg $^{13}$C-urea (P) at 10, 20 and 30 minute (m) time point collection

**TABLE 1.** Mean, standard deviation, minimum and maximum values of conventional and capsule based breath test at 10, 20 and 30 minutes of the positive and negative *H. pylori* subjects, and of the 83 patients that underwent breath test with 50 mg capsule at 20 minutes for breath test validation against gold standard

| Test minutes | Mean *Hp+* | SD *Hp+* | Minimum *Hp+* | Maximum *Hp+* | Mean *Hp-* | SD *Hp-* | Minimum *Hp-* | Maximum *Hp-* |
|---|---|---|---|---|---|---|---|---|
| **Cap 10** | 13.80 | 12.67 | 2.20 | 50.9 | -0.69 | 1.16 | -4 | 1.20 |
| **P 10** | 26.61 | 20.64 | 6.50 | 81.20 | 0.08 | 1.12 | -3 | 2.20 |
| **Cap 20** | 21.42 | 16.32 | 4.80 | 62.80 | -0.58 | 1.38 | -4 | 1.90 |
| **P 20** | 27.25 | 16.8 | 9.40 | 64 | 0.29 | 1.44 | -5 | 3.00 |
| **Cap 30** | 17.48 | 12.61 | 2 | 44.20 | -0.66 | 1.61 | -5 | 1.90 |
| **P 30** | 21.49 | 13.06 | 5.50 | 58.90 | -0.27 | 1.22 | -3 | 2.50 |
| **83 pat** | 21.57 | 12.84 | 4.5 | 51.9 | 0.33 | 0.6 | 0.1 | 3.6 |

*Hp: Helicobacter pylori*; SD: standard deviation; Cap: capsule based breath test; P: conventional breath test; pat, patients.

the cutoff of 4‰ with capsule based breath test (Figure 1). For 50 mg $^{13}$C-urea using capsule, cutoff value of DOB≥2‰ gave 100% relative sensitivity and specificity at 10, 20 and 30 minutes. However, at 20 minutes these patients had positive breath test; hence, this time point collection was considered the most reliable for 50 mg capsule based breath test.

When capsule based breath test was validated against a gold standard (urease and histology), 32 patients that were *H. pylori* positive had positive urea breath test and 51 patients that were *H. pylori* negative had negative breath test; thus, the sensitivity (95%CI: 86.96%-100%) and specificity (95%CI: 91.42%-100%) were 100%. The demographic and endoscopic findings of these 83 patients are presented in Table 2, 52 (62.7%) patients had peptic ulcer, 28 (33.7%) had gastritis, 2 (2.4%) had atrophic gastritis and 1 (1.2%) had normal endoscopy. The statistical analysis of the endoscopic findings in relation to the *H. pylori* status, gender and ethnicity was not significant. The mean $^{13}CO_2$ excretion of *H. pylori* positive patients was DOB = 21.57±12.84‰, and of *H. pylori* negative patients was DOB = 0.33±0.6‰ (Table 1), the difference between these two groups was significant ($P$<0.05).

the capsule may help acidifying the stomach milieu, inducing *H. pylori* for the urease synthesis[13]. There is no interference of the oral flora, as the capsule is swallowed and disintegrated directly in the stomach. Capsules prevented the urea to be released before reaching the stomach, requiring a lower dose of $^{13}$C-urea[7, 13, 25, 27].

In this report, we showed that $^{13}$C-urea breath test performed with formulated 50 mg hard gelatin capsule with citric acid and taken with orange juice (pH = 3.0) had almost perfect agreement (Kappa Index measure of agreement = 1; $P$<0.05) with the conventional $^{13}$C-urea breath test in powder dissolved in orange juice. The conventional breath test with 75 mg of $^{13}$C-urea in 200 mL orange juice and considering a cutoff value of DOB≥4‰ was previously validated with 100% sensitivity and specificity[4], and performed in other publications in Brazil[3, 15, 16, 29, 30].

Considering a cutoff value of DOB≥4‰ at 20 minute time collection or a cutoff value of DOB≥2‰ at 10, 20 and 30 minutes, the capsule (50 mg) urea breath test had 100% relative sensitivity and relative specificity. A cutoff value of DOB≥4‰ at 20 minute time collection was chosen for the

**TABLE 2.** Demographic and endoscopic findings of the 83 patients that performed $^{13}$C-urea breath test with 50 mg capsule at 20 min time point collection (%)

| Endoscopy | Normal | Gastritis | Peptic ulcer | Atrophic gastritis | Total |
|---|---|---|---|---|---|
| ***H. pylori* neg.** | 1 (2) | 13 (25.5) | 35 (68.6) | 2 (3.9) | 51 (61.4) |
| ***H. pylori* pos.** | | 15 (46.9) | 17 (53.1) | | 32 (38.6) |
| **White** | 1 (1.4) | 25 (36.2) | 41 (59.4) | 2 (2.9) | 69 (83.1) |
| **Brown** | | 1 (12.5) | 7 (87.5) | | 8 (9.6) |
| **Black** | | 1 (25) | 3 (75) | | 4 (4.8) |
| **Japanese-Brazilian** | | 1 (50) | 1 (50) | | 2 (2.4) |
| **Men** | | 13 (34.2) | 25 (65.8) | | 38 (45.8) |
| **Women** | 1 (2.2) | 15 (33.3) | 27 (60) | 2 (4.4) | 45 (54.2) |

Four patients that were *H. pylori* positive and were taking omeprazole until the day of the urea breath test gave positive results as follows: DOB = 7.6‰, 8.1‰, 24.1‰ and 37.7‰.

## DISCUSSION

According to the Maastricht IV Consensus report[17] urea breath test and stool antigen testing are acceptable non-invasive tests for *H. pylori* infection diagnosis with 88%-95% sensitivity and 95%-100% specificity. In Brazil, commercial capsule of $^{13}$C-urea is not available, hindering the incorporation of this test to the daily gastroenterology practice[6]. The Pharmacy Division of our Institution may formulate capsule of $^{13}$C-urea, and provide for the patients of our Hospital to undergo urea breath test.

The comparison of capsule based breath test with the conventional test showed that citric acid used as excipient of clinical setting, as was previously demonstrated that a change in the cutoff value within 2 and 5 would have little effect on the clinical accuracy of the test, as positive and negative results tend to group outside this range[12].

In this study powdered orange juice (pH = 3.0) was taken with the capsule to increase sensitivity of the test, although citric acid as excipient of the capsule formulation may provide an acidic microenvironment. The activity of bacterial urease and the $^{13}CO_2$ excretion were significantly higher with apple juice (pH = 3.0) or with acidified meal (pH = 3.0) than with neutral meal, and independent of gastric emptying[26]. Other authors[7, 13, 27] that tested 50 and or 100 mg capsule of $^{13}$C-urea with water, showed sensitivity of 95%-100% and specificity of 97.6%-100%, suggesting that acid test meal may be omitted, lowering the cost of the exam. Nonetheless, powdered orange juice had low cost, long expiration date, and stability. Citric acid solution instead of juice showed high sensitivity

(89.1%-100%) and specificity (100%) with low concentrations of 10 to 25 mg $^{13}$C-urea solution[8]. Conversely, exam performed with 25 mg $^{13}$C-urea solution in orange juice had lower sensitivity and specificity, and was not recommended for the clinical setting in our country[5].

Previously was shown that even after 7-day treatment with omeprazole, no patients had false negative results with the tablet based breath test (two tablets of 50 mg $^{13}$C-urea and 463 mg citric acid)[13]. In this report four patients that did not stop taking omeprazole also had positive breath test. This is an important finding, as some patients with continuous proton pump inhibitor therapy may not discontinue medication with risk of gastrointestinal bleeding[2]. Although the recommendation is to avoid all proton pump inhibitors and $H_2$-receptor antagonists, among omeprazole, lansoprazole and pantoprazole, the latter did not interfere in the accuracy of $^{13}$C-urea breath test. In addition, $H_2$-receptor antagonists, that increase intragastric pH, have no inhibitory effect on *H. pylori* density, different from proton pump inhibitors that decreases the density of *H. pylori* on the stomach surface[12]. Thus, the false negative effect of $H_2$-receptor antagonists and proton pump inhibitors on capsule $^{13}$C-urea breath test and the real necessity of discontinuing these medications should be further evaluated. Although the effects of increasing gastric pH by $H_2$-receptor antagonists and sodium bicarbonate solution and of density of *H. pylori* were already explored during $^{13}$C-urea breath test in solution by Graham et al.[12] that concluded $H_2$-receptor antagonists differ from proton pump inhibitors as high intragastric pH may cause a reduction in urease activity, unrelated to a reduced bacterial load and reversed by citric acid.

When capsule based breath test was validated against a gold standard (urease and histology), the sensitivity and the specificity were high and comparable to previous reports with $^{13}$C-urea in capsule presentation that showed sensitivity of 95%-100% and specificity of 92-100%[7, 13, 25, 27, 28].

In conclusion, capsule based breath test with 50 mg $^{13}$C-urea at 20 minutes was found suitable for the clinical setting with 100% sensitivity and specificity.

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Mattar R, Villares CA, Marostegam PFF, Chaves CE, Pinto VB, Carrilho FJ. Teste respiratório com $^{13}$C-uréia em cápsula de baixa dose em comparação com o teste respiratório com $^{13}$C-uréia convencional e testes invasivos. Arq Gastroenterol. 2014,51(2):133-8.

**RESUMO –** ***Contexto*** - Uma das limitações para o teste respiratório com $^{13}$C-uréia ser incorporado na prática clínica no Brasil para diagnóstico de infecção pelo *Helicobacter pylori* (*H. pylori*) é a aquisição do substrato em apresentação de cápsula. ***Objetivos*** - O objetivo deste estudo foi avaliar a utilidade de $^{13}$C-uréia em cápsula, manipulada pela Divisão de Farmácia de um Hospital terciário para a prática clínica. ***Métodos*** - Cinquenta pacientes foram submetidos ao teste respiratório convencional com 75mg de $^{13}$C-uréia e ao teste com cápsula de 50 mg de $^{13}$C-uréia. Amostras de ar expirado foram coletadas no basal e após 10, 20 e 30 minutos da ingestão de $^{13}$C-uréia para definição do melhor tempo de coleta. A urease e a histologia foram usadas como padrão ouro em 83 pacientes que se submeteram ao teste respiratório com único ponto de coleta. ***Resultados*** - Num total de 50 pacientes, 17 foram positivos com teste respiratório convencional com 75 mg de $^{13}$C-uréia aos 10, 20 e 30 minutos. Quando esses pacientes repetiram o teste respiratório com $^{13}$C-uréia em cápsulas de 50 mg, 17 foram positivos aos 20 minutos (Índice Kappa 1,0; $P$<0,05) e 15 aos 10 e 30 minutos (Índice Kappa 0,90; $P$<0,05). A sensibilidade relativa da $^{13}$C-uréia em cápsulas de 50 mg foi 100% aos 20 minutos e 88,24% aos 10 e 30 minutos. A especificidade relativa foi de 100% em todos os intervalos de tempo. Entre 83 pacientes que se submeteram ao teste respiratório com cápsula e endoscopia, aqueles que eram *H. pylori* positivos tiveram teste respiratório positivo e aqueles *H. pylori* negativo tiveram teste respiratório negativo, mostrando sensibilidade e especificidade de 100%. ***Conclusão*** - O teste respiratório com cápsula de 50 mg de $^{13}$C-uréia aos 20 minutos foi considerado altamente sensível e específico para a prática clínica.

**DESCRITORES** - *Helicobacter pylori*. Testes respiratórios. Ureia, análise.

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