

RAPID COMMUNICATION

# An optimized $^{13}\text{C}$ -urea breath test for the diagnosis of *H pylori* infection

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## Abstract

**AIM:** To validate an optimized  $^{13}\text{C}$ -urea breath test ( $^{13}\text{C}$ -UBT) protocol for the diagnosis of *H pylori* infection that is cost-efficient and maintains excellent diagnostic accuracy.

**METHODS:** 70 healthy volunteers were tested with two simplified  $^{13}\text{C}$ -UBT protocols, with test meal (Protocol 2) and without test meal (Protocol 1). Breath samples were collected at 10, 20 and 30 min after ingestion of 50 mg  $^{13}\text{C}$ -urea dissolved in 10 mL of water, taken as a single swallow, followed by 200 mL of water (pH 6.0) and a circular motion around the waistline to homogenize the urea solution. Performance of both protocols was analyzed at various cut-off values. Results were validated against the European protocol.

**RESULTS:** According to the reference protocol, 65.7% individuals were positive for *H pylori* infection and 34.3% were negative. There were no significant differences in the ability of both protocols to correctly identify positive and negative *H pylori* individuals. However, only Protocol 1 with no test meal achieved accuracy, sensitivity, specificity, positive and negative predictive values of 100%. The highest values achieved by Protocol 2 were 98.57%, 97.83%, 100%, 100% and 100%, respectively.

**CONCLUSION:** A 10 min, 50 mg  $^{13}\text{C}$ -UBT with no test meal using a cut-off value of 2-2.5 is a highly accurate test for the diagnosis of *H pylori* infection at a reduced cost.

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**Key words:** *H pylori*;  $^{13}\text{C}$ -urea breath test; Diagnosis; Accuracy; Cost

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## INTRODUCTION

*H pylori* infection is present in around 50% of the world population<sup>[1]</sup>, with higher prevalence rates in developing countries where it is the most frequent chronic infection in human kind<sup>[2]</sup>.

*H pylori* infection has been associated with the pathogenesis of gastric disorders such as gastritis, duodenal and gastric ulcer, gastric cancer and MALT lymphoma<sup>[3]</sup>, and a variety of extradigestive disorders including hematologic, such as iron deficiency anemia<sup>[4]</sup>, pernicious anemia<sup>[5]</sup>, autoimmune neutropenia<sup>[6]</sup>, Schönlein-Henoch purpura<sup>[7]</sup>, thrombotic thrombocytopenic purpura<sup>[8]</sup> and idiopathic thrombocytopenic purpura<sup>[9]</sup>. It has also been implicated in the pathogenesis of traditional autoimmune diseases, including rheumatoid arthritis<sup>[10]</sup>, Sjögren syndrome<sup>[11]</sup> and autoimmune thyroiditis<sup>[12]</sup>, dermatologic diseases such as rosacea<sup>[13]</sup> and urticaria<sup>[14]</sup>, and cardiovascular events<sup>[15,16]</sup> among others.

Diagnosis of *H pylori* infection can be established by either invasive or non-invasive techniques. Invasive techniques, by means of endoscopy, are expensive<sup>[17]</sup>, cause patient discomfort and introduce the risk of cross-infection<sup>[18,19]</sup>; moreover, there is morbidity and mortality associated with the procedure<sup>[20]</sup> and is not indicated in all cases where the *H pylori* status must be determined<sup>[21,22]</sup>. Non-invasive methods include serology<sup>[23]</sup>,  $^{13}\text{C}$ -urea or  $^{13}\text{C}$ -urea breath test (UBT)<sup>[24,25]</sup>, stool antigen test<sup>[26]</sup> and blood urea test<sup>[27]</sup>.

The principle of the  $^{13}\text{C}$ -UBT relies upon the ability of the urease, produced by *H pylori* in the gastric mucosa, to hydrolyze the orally administered  $^{13}\text{C}$ -urea. This enzyme breaks down any urea in the stomach to ammonia and carbon dioxide ( $\text{CO}_2$ ), which is absorbed into the blood stream and then released from the lungs. The labelled carbon dioxide ( $^{13}\text{CO}_2$ ) is detected in breath samples<sup>[28]</sup>.

The aim of the present study was to standardize and validate an assay that is cost-effective, while preserving excellent diagnostic accuracy. Two simple protocols were validated against the standard European protocol<sup>[29]</sup>, which included modifications in the dose, formulation and *via* of urea administration, sample collection times and test meal. Appropriate cut-off values for these assays were also established.

## MATERIALS AND METHODS

### Subjects

The study population included 70 volunteers with no gastrointestinal symptoms. The volunteers were informed about the study and the tests, and signed an informed consent in accordance with the Helsinki Declaration<sup>[30]</sup>. The study was classified as a research study with no biological, physiological, psychological or social risks by the Health Ministry of Colombia<sup>[31]</sup>. Because of the nature of the study in healthy volunteers, it was considered non-ethical to perform invasive tests such as biopsy, culture or endoscopy.

### Protocols

**Reference protocol:** *H pylori* infection status of individuals was determined by the <sup>13</sup>C-UBT, according to the European protocol described before<sup>[29]</sup> and using commercial kits (TAU-KIT, Isomed SL, Madrid, Spain) that provide both a sensitivity and specificity close to 100%<sup>[25]</sup>. This protocol was standardized and was validated for our region, with over 15000 assays performed, and used as the gold standard. The <sup>13</sup>C-UBT was analyzed by means of continuous flow-isotope ratio mass spectrometry (ABCA, SerCon, Cheshire, UK) at the Laboratorio Clínico Hematológico® in Medellín, Colombia.

The reference protocol was performed as follows: After fasting for at least 8 h, individuals were given 4.2 g of citric acid dissolved in 200 mL of water. Ten minutes later, a duplicate basal breath sample was collected. Immediately after, individuals were given 100 mg of <sup>13</sup>C-urea dissolved in 125 mL of water. After 30 min, a duplicate post-urea breath sample was collected. Results over 2.5 delta-over-baseline (DOB) were considered positive for *H pylori* infection.

**Protocol 1:** After fasting for at least 8 h, a first basal breath sample was collected. Individuals were given 50 mg of <sup>13</sup>C-urea (99%, Isotec, Miamisburg, Ohio, USA) dissolved in 10 mL of water, taken as a single swallow. Immediately after, individuals were given 200 mL of water (pH 6.0). Volunteers, with a final volume of 210 mL, were asked to make a circular motion around the waistline for a few times to homogenize the aqueous solution and allow contact of the <sup>13</sup>C-urea with the entire gastric mucosa. Additional breath samples were collected afterwards at 10, 20 and 30 min.

**Protocol 2:** Same as Protocol 1, except that 4.2 g of dehydrated citric acid were added to the 200 mL of water.

The performance of both protocols was analyzed at various cut-off values from 0.5 to 5.5, at the different time intervals (10, 20 and 30 min).

### Statistical analysis

The  $\chi^2$  test was used to analyze associations between qualitative variables. For quantitative variables, the Wilcoxon's signed rank sum tests and Student's *t*-test were applied. Normality of the distribution of the data was assessed with the Wilk-Shapiro test. Sensitivity, specificity, positive predictive value, negative predictive value, accuracy,

Youden index, likelihood ratios for a positive (LR+ve) or negative (LR-ve) test were calculated against the defined gold standard. The effectiveness of each protocol was evaluated by ROC analysis. Processing and analysis of data were done with the SPSS (Statistical Product for Service Solutions) version 12.0 and EPIDATE Version 3.0. A value of *P* < 0.05 was considered statistically significant.

## RESULTS

This study included 70 individuals, 24 (34.3%) males and 46 (65.7%) females, with an average age of 39.63 (SD  $\pm$  12.58) years for males and 34.33 (SD  $\pm$  10.17) years for females. There were no significant differences between the mean age for males and females (*P* = 0.061). According to the reference protocol, 46 (65.7%) individuals were positive for *H pylori* infection and 24 (34.3%) were negative. When assessed by gender, 17 (70.8%) males and 29 (63%) females were positive for *H pylori*; this association was not statistically significant (*P* = 0.515).

Table 1 shows the performance of the protocols in terms of sensitivity, specificity, accuracy, positive and negative predictive values, Youden index and likelihood ratios for a positive (LR+ve) or (LR-ve) test with the different DOB cut-off values at 10, 20 and 30 min. Only Protocol 1 (with no test meal) achieved accuracy, sensitivity, specificity, positive and negative predictive values of 100%. The highest values achieved by Protocol 2 were 98.57%, 97.83%, 100%, 100% and 100%, respectively.

There were no significant differences in the ability of both protocols to correctly identify positive and negative *H pylori* individuals at 10 (*P* = 0.32), 20 (*P* = 0.32) and 30 min (*P* = 0.32). These results were confirmed by ROC analysis (Figure 1). The areas under the ROC curves for both protocols were as follows: for Protocol 1, 1.0 at 10, 20 and 30 min; for Protocol 2, 0.9837 at 10 and 30 min, and 0.9873 at 20 min. Although these results were not statistically different, Protocol 1 shows the maximum optimal values for an assay.

Table 2 shows the distribution of the DOB values at 10, 20, 30 min for *H pylori* positive and negative individuals for Protocols 1 and 2. For Protocol 1, the median DOB for *H pylori* infected individuals at 10 min was 13.64, while for Protocol 2 was 12.02. There was no statistically significant difference between these 2 values (Wilcoxon, *P* = 0.121). In contrast, median DOB values at 20 and 30 min for both protocols showed significant differences (*P* = 0.006 and *P* = 0.001, respectively). In addition, for non-infected individuals there were no statistically significant differences in the median DOB values at 10, 20 and 30 min (*P* = 0.710, *P* = 0.440 and *P* = 0.346, respectively) between both protocols.

## DISCUSSION

The <sup>13</sup>C-UBT has become the gold standard of the non-invasive tests for diagnosing *H pylori* infection, before and after eradication treatment. Recently, The Maastricht III Consensus Report has recommended the <sup>13</sup>C-UBT as the best option to establish the diagnosis of *H pylori* infection, especially in patients in whom endoscopy is not indicated<sup>[22]</sup>.

Table 1 Performance of protocols (P1 and P2) in terms of sensitivity, specificity, accuracy, positive and negative predictive values, Youden index and likelihood ratios for a positive (LR+ve) or (LR-ve) test with the different DOB cut-off values at 10, 20 and 30 min

Time (min)	DOB	Sensitivity		Specificity		Accuracy		Positive predictive value		Negative predictive value		Youden index		LR +ve		LR-ve	
		P1	P2	P1	P2	P1	P2	P1	P2	P1	P2	P1	P2	P1	P2	P1	P2
10	0.5	100	97.83	45.83	62.5	81.43	85.71	77.97	83.33	100	93.75	0.46	0.6	1.85	2.61	<sup>1</sup>	0.03
	1.0	100	97.83	83.33	79.17	94.29	91.43	92	90.0	100	95	0.83	0.77	6.00	4.7	<sup>1</sup>	0.03
	1.5	100	97.83	95.83	95.83	98.57	97.14	97.87	97.83	100	95.83	0.96	0.94	24.00	23.48	<sup>1</sup>	0.02
	2.0	100	97.83	100	100	100	98.57	100	100	100	96	1.00	0.98	<sup>2</sup>	<sup>2</sup>	<sup>1</sup>	0.02
	2.5	100	95.65	100	100	100	97.14	100	100	100	92.31	1.00	0.96	<sup>2</sup>	<sup>2</sup>	<sup>1</sup>	0.04
	3.0	97.83	95.65	100	100	98.57	97.14	100	100	96.0	92.31	0.98	0.96	<sup>2</sup>	<sup>2</sup>	0.02	0.04
	3.5	97.83	95.65	100	100	98.57	97.14	100	100	96.0	92.31	0.98	0.96	<sup>2</sup>	<sup>2</sup>	0.02	0.04
	4.0	95.65	95.65	100	100	97.14	97.14	100	100	92.31	92.31	0.96	0.96	<sup>2</sup>	<sup>2</sup>	0.04	0.04
	4.5	93.48	95.65	100	100	95.71	97.14	100	100	88.89	92.31	0.93	0.96	<sup>2</sup>	<sup>2</sup>	0.07	0.04
	5.0	86.96	95.65	100	100	91.43	97.14	100	100	80	92.31	0.87	0.96	<sup>2</sup>	<sup>2</sup>	0.13	0.04
	5.5	84.78	95.65	100	100	90	97.14	100	100	77.42	92.31	0.85	0.96	<sup>2</sup>	<sup>2</sup>	0.15	0.04
	20	0.5	100	97.83	66.67	88.57	85.71	85.19	83.33	100	93.75	0.67	0.6	3.00	2.61	<sup>1</sup>	0.03
	1.0	100	97.83	95.83	75	98.57	90	97.87	88.24	100	94.74	0.96	0.73	24.00	3.91	<sup>1</sup>	0.03
	1.5	100	97.83	100	83.33	100	92.86	100	91.84	100	95.24	1.00	0.81	<sup>2</sup>	5.87	<sup>1</sup>	0.03
	2.0	100	97.83	100	100	100	98.57	100	100	100	96	1.00	0.98	<sup>2</sup>	<sup>2</sup>	<sup>1</sup>	0.02
	2.5	100	95.65	100	100	100	97.14	100	100	100	92.31	1.00	0.96	<sup>2</sup>	<sup>2</sup>	<sup>1</sup>	0.04
20	3.0	97.83	95.65	100	100	98.57	97.14	100	100	96	92.31	0.98	0.96	<sup>2</sup>	<sup>2</sup>	0.02	0.04
	3.5	95.65	95.65	100	100	97.14	97.14	100	100	92.31	92.31	0.96	0.96	<sup>2</sup>	<sup>2</sup>	0.04	0.04
	4.0	89.13	95.65	100	100	92.86	97.14	100	100	82.76	92.31	0.89	0.96	<sup>2</sup>	<sup>2</sup>	0.11	0.04
	4.5	84.78	95.65	100	100	90	97.14	100	100	77.42	92.31	0.85	0.96	<sup>2</sup>	<sup>2</sup>	0.15	0.04
	5.0	82.61	95.65	100	100	88.57	97.14	100	100	75	92.31	0.83	0.96	<sup>2</sup>	<sup>2</sup>	0.17	0.04
	5.5	82.61	93.48	100	100	88.57	95.71	100	100	75	88.89	0.83	0.93	<sup>2</sup>	<sup>2</sup>	0.17	0.07
	30	0.5	100	97.83	54.17	84.29	80	80.7	77.59	100	91.67	0.54	0.44	2.18	1.81	<sup>1</sup>	0.05
	1.0	100	97.83	91.67	70.83	97.14	88.57	95.83	86.54	100	94.44	0.92	0.69	12.00	3.35	<sup>1</sup>	0.03
	1.5	100	97.83	100	83.33	100	92.86	100	91.84	100	95.24	1.00	0.81	<sup>2</sup>	5.87	<sup>1</sup>	0.03
	2.0	95.65	97.83	100	91.67	97.14	95.71	100	95.74	92.31	95.65	0.96	0.89	<sup>2</sup>	11.74	0.04	0.02
	2.5	91.3	97.83	100	100	94.29	98.57	100	100	85.71	96	0.91	0.98	<sup>2</sup>	<sup>2</sup>	0.09	0.02
	3.0	84.78	95.65	100	100	90	97.14	100	100	77.42	92.31	0.85	0.96	<sup>2</sup>	<sup>2</sup>	0.15	0.04
	3.5	82.61	95.65	100	100	88.57	97.14	100	100	75	92.31	0.83	0.96	<sup>2</sup>	<sup>2</sup>	0.17	0.04
	4.0	78.26	95.65	100	100	85.71	97.14	100	100	70.59	92.31	0.78	0.96	<sup>2</sup>	<sup>2</sup>	0.22	0.04
	4.5	78.26	95.65	100	100	85.71	97.14	100	100	70.59	92.31	0.78	0.96	<sup>2</sup>	<sup>2</sup>	0.22	0.04
	5.0	76.09	95.65	100	100	84.29	97.14	100	100	68.57	92.31	0.76	0.96	<sup>2</sup>	<sup>2</sup>	0.24	0.04
	5.5	71.74	93.48	100	100	81.43	95.71	100	100	64.86	88.89	0.72	0.93	<sup>2</sup>	<sup>2</sup>	0.28	0.07

DOB: Delta-over-baseline; <sup>1</sup>: ≈ 0; <sup>2</sup>: Φ +.

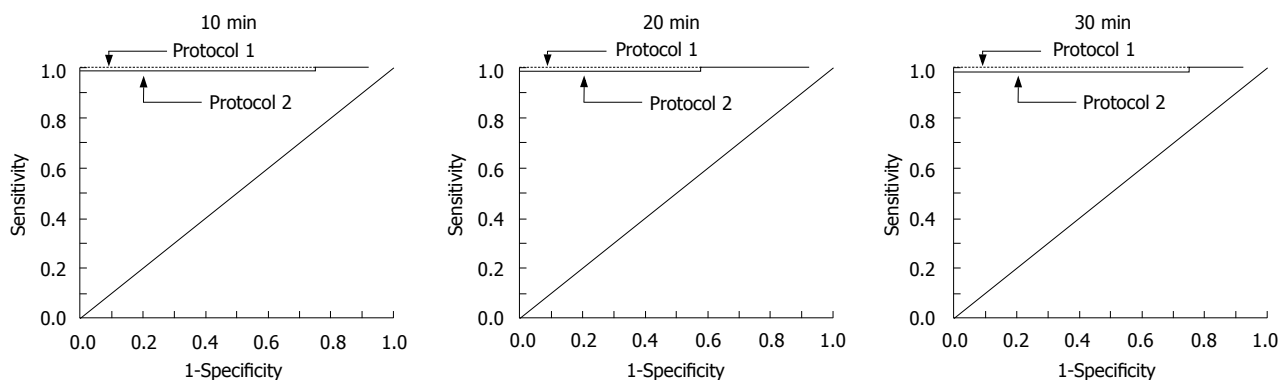


Figure 1 ROC curves for protocols 1 and 2 at 10, 20 and 30 min to establish the diagnosis of *H. pylori* infection.

Table 3 shows a selection of 40 studies from the literature where relevant variations to the original <sup>13</sup>C-UBT protocol<sup>[28]</sup> have been implemented. From each study, the protocol with the best diagnostic performance was selected<sup>[32-71]</sup>. Of these, 12 (30%) yielded sensitivities and specificities of 100%<sup>[33,37,43,45,47,56,61,63,65,67-69]</sup>.

Based on the results after reviewing the literature, the present study introduced several variations to simplify even further the technique and make it more cost-efficient,

without compromising the high standards of sensitivity, specificity, positive and negative predictive values of the test. Below is a brief review of the evolution of the assay, since its first description, which led to the designing of the protocols evaluated in the present study.

#### Urea dose

Originally, the <sup>13</sup>C-UBT was described with a dose of <sup>13</sup>C-urea of 5 mg/kg of bodyweight<sup>[28]</sup>. Later on, doses of

**Table 2** Distribution of DOB values in *H pylori* positive and negative individuals for both protocols at 10, 20 and 30 min

<i>H pylori</i> -positive individuals						
	10 min		20 min		30 min	
	Protocol 1	Protocol 2	Protocol 1	Protocol 2	Protocol 1	Protocol 2
Mean	17.38	17.69	18.25	22.32	15.44	22.08
Median	13.64	12.02	12.63	17.07	10.98	17.50
SD	14.47	12.68	22.80	15.01	17.75	13.00
<i>H pylori</i> -negative individuals						
	10 min		20 min		30 min	
	Protocol 1	Protocol 2	Protocol 1	Protocol 2	Protocol 1	Protocol 2
Mean	0.32	0.33	0.11	0.45	0.21	0.62
Median	0.51	0.32	0.28	0.34	0.38	0.59
SD	0.91	0.8	0.77	0.86	0.84	0.91

DOB: Delta-over-baseline.

125<sup>[35,72]</sup> and 100 mg<sup>[36,40,47,48,51,54,57,60,62,65,73-83]</sup> were validated by several American, European and Asian groups, and more recently 75 mg<sup>[32,37,43-45,49,50,55,72,84-93]</sup>, 50 mg<sup>[52,56,58,94,95]</sup>, 38 mg<sup>[96]</sup>, 25 mg and even 10 mg<sup>[69]</sup> of <sup>13</sup>C-urea have proved to be sufficient.

### Test meal

From the beginning, there has been a belief that a delay in gastric emptying is necessary for optimal performance of the test, to allow enough time for the *H pylori*-urease to react in the gastric mucosa, if present. Initially individuals were given a meal consisting of one can of "Sustacal" pudding or 120 mL of 25% glucose polymer, followed 10 min later by a polycose solution containing the <sup>13</sup>C-urea<sup>[28]</sup>. Through the years there have been numerous modifications to the test meal, including the use of citric acid alone before administering the urea<sup>[37,62,67,79,84,85,97]</sup>, or mixed with the <sup>13</sup>C-urea at the time of administration<sup>[43-45,88,91,98]</sup>, or as a presentation in combination with the <sup>13</sup>C-urea<sup>[42,61,72,94,99]</sup>. Several alternatives to citric acid have also been tested, including orange juice<sup>[43,49,67,90,95]</sup> and apple juice<sup>[100]</sup>, as well as other types of food such as milk<sup>[48,58,101,102]</sup> and a pudding test meal<sup>[34,46]</sup>, and even water<sup>[41,103]</sup>. As shown in Table 3, the majority of the test meals have provided reliable results. Even the complete absence of a test meal has shown little, if any, variation in the diagnostic performance of the assay<sup>[35,40,47,53,56,59,60,102,104]</sup>.

### Via of administration and formulation of <sup>13</sup>C-urea

Another issue that has been addressed by different groups is the interference of other urease-producing bacteria in the oral cavity and oropharynx<sup>[105,106]</sup>, leading to an increase in false positive values. As a result, there have been different approaches in the formulation and way of administration of the labeled urea, including the development of <sup>13</sup>C-urea tablets<sup>[42,61,63,64,95]</sup>, capsules<sup>[68,96,99,107]</sup>, and even the intragastric instillation of the urea through the endoscope<sup>[80,108,109]</sup>. Some have also suggested mouth rinsing before and after urea administration<sup>[39-41,48,51,52,54,58,60,68,110]</sup>.

### Sample collection times

The <sup>13</sup>C-UBT was originally described with a basal sample and 18 post-urea samples taken during the following

180 min<sup>[28]</sup>. Rapidly the assay was modified and currently only 2 samples are obtained: pre and post-urea. Sampling times, although shorter than initially, have differed among protocols.

Ways of reducing the cost of the <sup>13</sup>C-UBT could include decreasing the amount of <sup>13</sup>C-urea used, reducing the duration of the test, and improving the ease with which the test can be administered and tolerated. The conventional European <sup>13</sup>C-UTB protocol used in our region is sensitive and specific enough (values close to 100%), but it takes 40-45 min to complete and is performed using 100 mg of <sup>13</sup>C-urea. For the present study we decided to use 50 mg of <sup>13</sup>C-urea to reduce the cost of the assays by half, a dose that has proved to be as accurate as higher doses<sup>[52,56,58,61,63,68]</sup>. The <sup>13</sup>C-urea was administered diluted in 10 mL of water and taken as a single swallow, to try to avoid cross-contamination with urease-producing oropharyngeal bacteria. Immediately after 200 mL of water (pH 6.0) with 4.2 g of citric acid (Protocol 2) and without citric acid (Protocol 1) were administered, and volunteers were asked to make a circular motion around the waistline for a few times to homogenize the aqueous solution and allow contact of the <sup>13</sup>C-urea with the entire gastric mucosa. It has been shown that *H pylori* urease operates in a pH range from 3.1 to 10, with an optimal activity at pH 6.0<sup>[111,112]</sup>. By utilizing water at pH 6.0, activity of the *H pylori* urease was optimized for Protocol 1, where no citric acid was used. Acid solutions have been used by many to delay gastric emptying and to provide a higher acidic environment to induce *H pylori*-urease activity<sup>[43,98]</sup>, although it has been demonstrated by Pantoflickova *et al*<sup>[100]</sup> that the emptying is determined by the caloric density of the test meal rather than by its pH. Finally, in order to reduce the duration of the test, both protocols were tested at different sampling times: 10, 20 and 30 min.

This study included 70 individuals, 34.3% males and 65.7% females, with an average age of 39.63 ± 12.58 years for males and 34.33 ± 10.17 years for females. According to the reference protocol, 46 (65.7%) individuals were positive for *H pylori* infection. No statistically significant association was found between gender and presence of *H pylori* infection ( $P = 0.515$ ).

There were no significant differences in the ability of both protocols to correctly identify positive and negative *H pylori* individuals at the various sampling times. However, only Protocol 1, with no test meal, yielded a test with sensitivity, specificity, positive and negative predictive values, and accuracy of 100% when compared to the gold standard, when using a DOB cut-off value between 2 and 2.5 at 10 and 20 min, and a DOB cut-off value of 1.5 at 20 and 30 min. For Protocol 2, with citric acid, the highest accuracy (98.57%) was achieved at 10 min using a DOB cut-off value of 2.0, at 20 min a DOB cut-off value of 2.0, and at 30 min with a DOB of 2.5.

Median DOB for *H pylori* infected individuals at 10 min was 13.64, while for Protocol 2 was 12.02. There was no statistically significant difference between these 2 values (Wilcoxon,  $P = 0.121$ ). However, median DOB values at 20 and 30 min for both protocols showed significant differences ( $P = 0.006$  and  $P = 0.001$ , respectively). These results are in accordance with those by Atherton *et al*<sup>[113]</sup>



**Table 3**  $^{13}\text{C}$ -UBT protocol with best diagnostic performance from each study with samples obtained within 30 min of  $^{13}\text{C}$ -urea administration: Review of literature

First author (reference)	Year	Measuring equipment	Gold standard	n	Pre-analytical	$^{13}\text{C}$ -urea dose (mg)	$^{13}\text{C}$ -urea formulation and via of administration	Test meal	Additional information related to $^{13}\text{C}$ -urea administration	t	Cut-off point (DOB)	Sens. (%)	Spec. (%)	PPV (%)	NPV (%)	Acc. (%)
Braden <sup>[32]</sup>	1994	IRMS	$^{13}\text{C}$ -UBT	217	Overnight fasting	75	NA	None		20	5	99	100			
Koletzko <sup>[33]</sup>	1995	IRMS, NDIRS	$^{13}\text{C}$ -UBT	51	Overnight fasting	75	Powder in 150 mL 0.033 mol/L citric acid solution	Taken with $^{13}\text{C}$ -urea		15	5	100	100			
Klein <sup>[34]</sup>	1996	IRMS	H	465	NA	125	Powder in 90 mL sterile water (Meretek kit)	Ensure		30	2.4	95.4	87.9			94.8
Malaty <sup>[35]</sup>	1996	IRMS	H, RUT, C	66	Overnight fasting	125	Powder in 100 mL water	None		20	2.4	96	100			
Taniguchi <sup>[36]</sup>	1996	NDIRS	H	153	Overnight fasting	100	Powder in 30 mL water	None		15	1	97.8	74			
Domínguez-Muñoz <sup>[37]</sup>	1997	IRMS	H, RUT, C	80	Overnight fasting	80	Powder in 50 mL water	200 mL 0.1 mol/L citric acid solution		30	4	100	100			
Eppe <sup>[38]</sup>	1997	IRMS	H	77	Overnight fasting	75	NA	Citric acid		30	1.3	96	100			
Kato <sup>[39]</sup>	1998	IRMS	H, C, RUT	133	Overnight fasting	100	Powder in 100 mL of water	None	Mouth rinsing after $^{13}\text{C}$ -urea	10	3.5	99	100			
Miwa <sup>[40]</sup>	1998	IRMS	H	409	8 h fasting	100	Powder	None	Mouth rinsing before and after $^{13}\text{C}$ -urea	20	5	97	97			
Ohara <sup>[41]</sup>	1998	IRMS	H, RUT, C	248	Overnight fasting	100	Powder in 100 mL tap water	None	Mouth rinsing after $^{13}\text{C}$ -urea	20	2.5	98	98			98
Hamlet <sup>[42]</sup>	1999	IRMS	$^{13}\text{C}$ -UBT, H, RUT, C	134	Overnight fasting	100	Two tablets (Diabact UBT) with 50 mg of $^{13}\text{C}$ -urea + 456 mg of anhydrous citric acid swallowed with 200 mL of water	Taken with $^{13}\text{C}$ -urea		10	1.8	95	100			
Leodolter <sup>[43]</sup>	1999	IRMS	H, RUT, C	50	NA	75	Powder in 200 mL 0.1 mol/L citric acid solution	Taken with $^{13}\text{C}$ -urea		30	4	100	100			
Leodolter <sup>[44]</sup>	1999	IRMS	H, RUT, C	233	NA	75	Powder in 200 mL citric acid solution	Taken with $^{13}\text{C}$ -urea		30	4	95	98			97
Savarino <sup>[45]</sup>	1999	IRMS	H, RUT	134	Overnight fasting	75	Powder in 150 mL 0.033 mol/L citric acid solution	Taken with $^{13}\text{C}$ -urea		30	5	100	100			
Van der Hulst <sup>[46]</sup>	1999	LARA	H, C	544	NA	100	Powder in 50 mL sterile water	Ensure		30	6.3-7.5	93-95	94-96	95-98	86-94	
Gisbert <sup>[47]</sup>	2000	IRMS	$^{13}\text{C}$ -UBT, H	53	Overnight fasting	100	Powder in 50 mL water (TAU-KIT)	None		30	3.3-3.9	100	100			
Peng <sup>[48]</sup>	2000	IRMS	H, RUT, C	136	6 h fasting	100	Powder in 50 mL sterile water	100 mL milk	Mouth rinsing after $^{13}\text{C}$ -urea and laid on their sides, changing sides every 5 min	15	4.8	94	89			
Riepl <sup>[49]</sup>	2000	NDIRS	H, C	100	Overnight fasting	75	Powder in 200 mL orange juice	Taken with $^{13}\text{C}$ -urea		15	6.5	92	94	89	94	

Savarino <sup>[50]</sup>	2000	IRMS	H, RUT	117	Overnight fasting	75	Powder in 150 mL 0.033 mol/L citric acid solution	Taken with <sup>13</sup> C-urea	30	5	98	97	98	97	98	
Sheu <sup>[51]</sup>	2000	IRMS	H, C	441	Overnight fasting	100	NA	100 mL of fatty test meal	Mouth rinsing before and after <sup>13</sup> C-urea	15	4	98	97			
Sheu <sup>[52]</sup>	2000	IRMS, NDIRS	H, C	177	Overnight fasting	50	NA	100 mL citric acid solution	Mouth rinsing after <sup>13</sup> C-urea	15	3.5	96	99	99	97	
Wong <sup>[53]</sup>	2000	IRMS	H, RUT	202	Overnight fasting	75	Powder in 50 mL distilled water	2.4 g of citric acid		30	5	96	98	98	96	97
Yoshida <sup>[54]</sup>	2000	LARA	H, C, PCR	104	Overnight fasting	100	Powder in 50 mL distilled water	None	Mouth rinsing after <sup>13</sup> C-urea	20	2.7	98	100			99
Mana <sup>[55]</sup>	2001	NDIRS	H	223	Overnight fasting	75	Powder	20 mL 0.1 mol/L citric acid solution		10		100	95	94	100	
Wong <sup>[56]</sup>	2001	IRMS	H, RUT	101	Overnight fasting	75	Powder in 50 mL water	None		30	3.5-4.5	100	100			100
Chua <sup>[57]</sup>	2002	IRMS	H, RUT, S	100	NA	100	Powder in solution containing citric acid and <sup>13</sup> C-urea		Pacients laid on their left side for 30 min	30	3.5	94	100	100	89	
Liao <sup>[58]</sup>	2002	IRMS	H, RUT	152	Overnight fasting	50	Powder in 50 mL sterile water	200 mL full-cream cow's milk	Patients gargled with water 3 times after <sup>13</sup> C-urea and laid on their sides, changing sides every 3 min	15	2.5-3.0	99	97	99	97	99
Ng <sup>[59]</sup>	2002	IRMS	H, RUT	123	Regular meal within 2 h of the <sup>13</sup> C-urea	75	Powder in 50 mL water	2.4 g citric acid in 200 mL solution		30	5.5	93	97	100	97	
Chen <sup>[60]</sup>	2003	NDIRS	H, RUT, C, SAT	586	Overnight fasting	100	Powder in 100 mL of water	None	Patients gargled with water 3 times after <sup>13</sup> C-urea and laid down on the left side for 5 min	20	3.5	98	97			98
Gatta <sup>[61]</sup>	2003	IRMS	H, RUT, C	200	Overnight fasting	50	Tablet (Diabact UBT) with 50 mg of <sup>13</sup> C-urea and 456 mg of anhydrous citric acid swallowed with 200 mL of water	Citric acid		10	1.65-3.15	100	100			
Gisbert <sup>[62]</sup>	2003	IRMS	H, RUT	36	Overnight fasting	100	Powder in 50 mL water (TAU-KIT)	200 mL solution with 4.2 g citric acid		30	5	96	100	100	91	
Wong <sup>[63]</sup>	2003	IRMS	H, RUT	150	Overnight fasting	50	Tablet (Diabact UBT) with 50 mg of <sup>13</sup> C-urea and 456 mg of anhydrous citric acid swallowed with 200 mL of water	Citric acid		20	2.1	100	100			
Ohara <sup>[64]</sup>	2004	IRMS	<sup>13</sup> C-UBT, H, C, RUT	254	Overnight fasting	100	Film-coated tablet swallowed with 100 mL of water	None		20	2.5	98	98			98

Urita <sup>[65]</sup>	2004	IRMS	H, S	129 Overnight fasting	100	Powder in 100 mL tap water	None	Sample taken through nostril	20	2.5	100	100			100
Beiki <sup>[66]</sup>	2005	NDIRS	<sup>14</sup> C-UBT, H, RUT	76 Overnight fasting	75	Powder in 200 mL orange juice			30	3.5	100	97	98	100	99
Kopacova <sup>[67]</sup>	2005	IRMS	<sup>13</sup> C-UBT	27 Overnight fasting	100	Powder in 50 mL distilled water with 1 g citric acid	150 mL distilled water with 3 g citric acid, orange juice or distilled water		10	3.5	100	100			100
Peng <sup>[68]</sup>	2005	IRMS	H, RUT, C	50 6 h fasting	100	Capsule with water	None	Mouth rinsing before and after <sup>13</sup> C-urea and laid on their sides, changing sides every 5 min	15	4-5	100	100			100
Gatta <sup>[69]</sup>	2006	IRMS	H, RUT	100 Overnight fasting	25	Dissolved in water	Citric acid (1 g)		30	4.4-6.26	100	100			
Mauro <sup>[70]</sup>	2006	IRMS	H, C	176 Overnight fasting	75	Powder in 100 mL citric acid solution	Taken with <sup>13</sup> C-urea		30	3	100	99	95-98	100	
Mauro <sup>[71]</sup>	2006	IRMS	H, C	67 Overnight fasting	75	Powder in 100 mL citric acid solution	Taken with <sup>13</sup> C-urea		10	3	100	96	95-98	99-100	
Present study	2007	IRMS	<sup>13</sup> C-UBT	70 Overnight fasting	50	Powder in 10 mL sterile water immediately followed by 200 mL sterile water	None	Patients made a circular motion around the waistline for a few times	10	2.0-2.5	100	100	100	100	100

n: Participating individuals; t: Sampling time; PPV: Positive predictive value; NPV: Negative predictive value; Acc: Accuracy; UBT: Urea breath test; H: histology; C: Culture; RUT: Rapid urease test; S: Serology; NA: Not available; IRMS: Isotope ratio mass spectrometry; NDIRS: Non-dispersive infrared spectrometry; LARA: Laser assisted ratio analyser; DOB: Delta-over-baseline.

and Gisbert *et al*<sup>[47]</sup>, who showed that the test meal did not affect <sup>13</sup>C-UBT results at 10 min, but increased the values thereafter.

In conclusion, an optimal laboratory test should be non-invasive, easy to perform, highly reproducible, cost-efficient and with a sensitivity and specificity close to 100%. When compared to other protocols published in the literature, the present conditions of Protocol 1 have further optimized the <sup>13</sup>C-UBT assay, as this is the only protocol with a sampling time of 10 min, a <sup>13</sup>C-urea dose of 50 mg and no test meal that can yield a test with 100% accuracy for the diagnosis of *H pylori* infection. These variations provide a protocol that can reduce the cost of the <sup>13</sup>C-UBT assay, is innocuous, well tolerated, has no restrictions and could be implemented for all patients in whom endoscopy is not an indication<sup>[21,22]</sup> and as a screening test for *H pylori* epidemiological studies. Further studies are underway to try to decrease the <sup>13</sup>C-urea to an even lower dose, using biopsy as the gold standard.

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## COMMENTS

### Background

*H pylori* infection is present in around 50% of the world population and has been associated with the pathogenesis of gastric disorders such as gastritis, gastric ulcer and MALT lymphoma, and a variety of extradigestive diseases, including idiopathic thrombocytopenic purpura, iron deficiency anemia and autoimmune thyroiditis, among others. Diagnosis of *H pylori* infection can be established by either invasive techniques, by means of endoscopy, or non-invasive techniques such as the <sup>13</sup>C-urea breath test.

### Research frontiers

The <sup>13</sup>C-urea breath test relies upon the ability of an enzyme (urease), produced by *H pylori* in the stomach, to break down the administered urea. Patients swallow the urea labelled with a non-radioactive isotope (<sup>13</sup>C). After a few minutes, the isotope-labelled carbon dioxide (<sup>13</sup>CO<sub>2</sub>) is exhaled in the breath if there is presence of *H pylori* urease in the stomach. The difference in the <sup>13</sup>CO<sub>2</sub> values before and after ingestion of the labelled urea will determine the presence of infection.

### Innovations and breakthroughs

Many have attempted to lower the high cost of the <sup>13</sup>C-urea breath test, while preserving excellent diagnostic accuracy. For this purpose, modifications in the

dose, formulation and way of administration, sample collection times and test meals have been evaluated.

## Applications

A low cost <sup>13</sup>C-urea breath test for the detection of *H pylori* infection before and after eradication treatment will make this non-invasive assay more accessible for patients, especially in developing countries.

## Terminology

<sup>13</sup>C-UBT: Breath test that includes urea labelled with <sup>13</sup>C, a non-radioactive isotope. DOB: Delta over base line, units used to express the amount of <sup>13</sup>CO<sub>2</sub> contained in the breath sample.

## Peer review

This is a well written and comprehensively referenced article. The methods section is adequately described and the results clearly presented. The conclusions are a fair interpretation of the results.

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