A Re-testing Range is Recommended for 13C- and 14C-urea Breath Tests for Helicobacter Pylori Infection in China

Xiangyu Wang
Shenzhen University First Affiliated Hospital: Shenzhen Second People's Hospital
https://orcid.org/0000-0003-2596-8483
Shuzhen Zhang
Shenzhen Kuichong People's Hospital
Eng Guan Chua
University of Western Australia
Yongsheng He
Shenzhen Kuichong People's Hospital
Xiaofeng Li
Shenzhen People's Hospital
Aijun Liu
Shenzhen Kuichong People's Hospital
Haiting Chen
Shenzhen Kuichong People's Hospital
Michael J. Wise
The University of Western Australia
Barry J. Marshall
The University of Western Australia
Dayong Sun
Shenzhen University First Affiliated Hospital: Shenzhen Second People's Hospital
Xuehong Li
Shenzhen Kuichong People's Hospital
Alfred Chin Yen Tay (alfred.tay@uwa.edu.au)
University of Western Australia https://orcid.org/0000-0001-9705-4010

Research

Keywords: Helicobacter pylori, urea breath test, diagnostic performance, indeterminate range

Posted Date: March 31st, 2021

DOI: https://doi.org/10.21203/rs.3.rs-356067/v1

A re-testing range is recommended for ^{13}C- and ^{14}C-urea breath tests for *Helicobacter pylori* infection in China

Xiangyu Wang[1,2]*, Shuzhen Zhang[2]*, Eng Guan Chua[3], Yongsheng He[4], Xiaofeng Li[5], Aijun Liu[4], Haiting Chen[2], Michael J. Wise[3,6], Barry J. Marshall[3], Dayong Sun[1], Xuehong Li[2#], and Chin Yen Tay[3#]

[1] Department of Gastroenterology, Shenzhen Second People's Hospital/ the First Affiliated Hospital of Shenzhen University, Health Science Center, Shenzhen 518035, China.

[2] Department of Clinical Laboratory, Kuichong People's Hospital, Shenzhen 518116, China.

[3] The Marshall Centre for Infectious Diseases Research and Training, University of Western Australia, Perth 6009, Australia.

[4] Department of Gastroenterology, Kuichong People's Hospital, Shenzhen 518116, China.

[5] Department of Nuclear Medicine, Shenzhen People's Hospital, Shenzhen 518001, China.

[6] Department of Computer Science and Software Engineering, University of Western Australia, Perth 6009, Australia.

* Both Xiangyu Wang and Shuzhen Zhang contributed equally to this work.

Corresponding authors:

Dr. Chin Yen Tay[3] (Email: alfred.tay@uwa.edu.au); Dr. Xuehong Li[2] (Email: lihongsz@163.com).

Abstract

Background: The urea breath test (UBT) is widely used for diagnosing *Helicobacter pylori* infection. In our hospital, some UBT findings were contradictory to the histology outcomes, therefore this study aimed to assess and compare the diagnostic performance of both ^{13}C- and ^{14}C-UBT assays.

Methods: We recruited 484 *H. pylori*-treatment naïve patients, among which 217 and 267 were tested by the ^{13}C-UBT or ^{14}C-UBT, respectively. The cutoff value for *H. pylori* positivity based on manufacturer's instruction was 4% DOB for the ^{13}C-UBT, and 100 DPM for the ^{14}C-UBT. Gastric biopsies of the antrum and corpus were obtained during endoscopy for histopathology.

Results: In patients who were tested using the ^{13}C-UBT kit, histopathology was positive in 136 out of 164 UBT-positive patients (82.9% concordance), and negative in 46 out of 53 UBT-negative cases (86.8% concordance). For the ^{14}C-UBT-tested patients, histopathology was positive for *H. pylori* in 186 out of 220 UBT-positive patients (84.5% concordance), and negative in 41 out of 47 UBT-negative cases (87.2% concordance). While the ^{13}C-UBT and ^{14}C-UBT each had a high sensitivity level of 95.1% and 96.9%, respectively, their specificity was low, at 62.2% and 54.7%, respectively. By using new optimal cutoff values and including an indeterminate range (3-10.3% DOB for ^{13}C-UBT and 87-237 DPM for ^{14}C-UBT), the specificity values can be improved to 76.7% and 76.9% for the ^{13}C- and ^{14}C-UBT, respectively.

Conclusions: The establishment of an indeterminate range is recommended to allow for repeated testing to confirm *H. pylori* infection, and thereby avoiding unnecessary antibiotic treatment.

Trial registration: Chinese Clinical Trial Registry, ChiCTR2000041570. Registered 29 December 2020- Retrospectively registered, http://www.chictr.org.cn/edit.aspx?pid=66416&htm=4

(263/350 words)

Key words: *Helicobacter pylori*, urea breath test, diagnostic performance, indeterminate range

Introduction

Helicobacter pylori infection is common in China, with an overall estimated prevalence of 55.8% (1). It is an important gastric pathogen that can lead to several gastroduodenal disorders including chronic gastritis, gastric atrophy and peptic ulcer disease, and less commonly, to gastric adenocarcinoma and mucosa associated lymphoid tissue (MALT) lymphoma (2, 3). *Helicobacter pylori* is able to convert urea into carbon dioxide and ammonia via its urease enzyme, where the ammonia is used to neutralize the acid for its survival in the stomach (4). Based on this feature of *H. pylori*, the urea breath test (UBT), a non-invasive *H. pylori* infection diagnostic method was developed. This requires a patient to swallow a capsule containing a dose of urea labeled with carbon-13 (^{13}C) or carbon-14 (^{14}C) isotope. If the patient is an *H. pylori* carrier, the labeled urea will be hydrolyzed by the bacterial urease enzyme within the stomach, resulting in the release of labeled carbon dioxide which is then absorbed into the bloodstream and expelled from the lungs in a few minutes. Hence the amount of labeled carbon dioxide within a patient's breath sample can be measured to determine current *H. pylori* infection status (5, 6). Due to its accuracy, simplicity and non-invasive nature, the UBT has been the preferred method of many medical professionals for testing *H. pylori* infection in their patients. Both the ^{13}C-UBT and the ^{14}C-UBT are widely used. The former utilizes the stable ^{13}C isotope of carbon while the latter uses the radioactive ^{14}C carbon isotope. Nevertheless, it is important to mention that both are naturally occurring isotopes and the radiation exposure from the ^{14}C-UBT is even lower than that from background radiation (7). In fact, the ^{14}C-UBT has been approved by the Food and

Drug Administration (FDA) of the United States for its usage in everyone, including children and pregnant women (8).

While both UBT are useful in detecting *H. pylori* infection, we noticed that several UBT results were contradictory to the outcomes determined via histopathology examination, prompting us to reconsider the diagnostic accuracy of the commercial UBT kits used for screening *H. pylori* infection in our hospital. In this study, we recruited 484 individuals who underwent endoscopic examination at Shenzhen Kuichong People's Hospital, among which 217 and 267 were tested for *H. pylori* infection using the ^{13}C-UBT and ^{14}C-UBT, respectively. By comparing the outcomes to that of histopathology examination of gastric biopsies, which is the gold standard for diagnosing *H. pylori* infection, we assessed the diagnostic performance of both UBT kits. Additionally, as these commercial kits available for use at our hospital provide only a cutoff value for *H. pylori* positivity, we therefore sought to determine a value range that indicates an indeterminate outcome requiring a repeat UBT, below which we can with confidence infer the absence of an infection.

Material and Methods

Overview of entire study

The schematic flow of experimental program was shown in Figure 1.

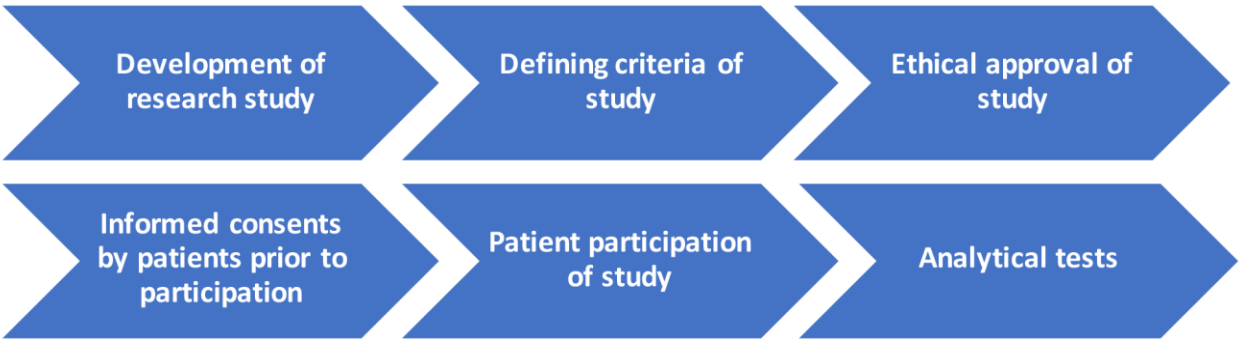

Figure 1. The overview of entire study.

Development of research study

During the clinical practice, we noticed that several UBT results were contradictory to the outcomes determined via histopathology examination, prompting us to reassess the diagnostic performance of both ^{13}C-UBT and ^{14}C-UBT commercial kits used for screening *H. pylori* infection in our hospital.

Defining the criteria of study

The following exclusion criteria on our study subjects were applied: (i) previous treatment of *H. pylori* infection, (ii) received proton pump inhibitor, H_2 receptor antagonist, expectorant, or antibiotic treatment within the last four weeks prior to endoscopy, (iii) history of gastric surgery, (iv) diagnosed with gastrointestinal cancer, (v) had severe heart, lung, liver, kidney or blood system disorder, (vi) aged below 18 or above 70 years old and (vii) pregnancy.

Ethics approval

This study was approved by the research ethics committee of Shenzhen Kuichong People's Hospital (reference no. 201609) and registered at www.chictr.org.cn (reference no. ChiCTR2000041570). Each patient was given a detailed introduction to the purpose and process of the research by a gastroenterologist. Written and informed consents were obtained from all patients prior to their participation in the study.

Study population

From January 2017 to November 2018, following the application of the exclusion criteria, 484 patients (18-70 years of age) who visited Shenzhen Kuichong People's Hospital (26 Kuixin N Road, Dapeng New District, Shenzhen City, China) for endoscopic check-up agreed to participate in this study.

Three gastroenterologists were involved in this study. Prior to the endoscopy session, the patients received either a ^{13}C-UBT or a ^{14}C-UBT at the discretion of a gastroenterologist via the alternating assignment method. The general health information of patients was collected by clinical nurses. During endoscopy, two gastric biopsy specimens (one each from antrum and corpus) were collected for histopathology examination. The different set of people did the examination and analysed the results. The testing was done by the clinical technicians without any implied or actual plan to check the outcome or accuracy of the UBT.

Sample size determination

The samples size required for this study was estimated based on a 95% confidence interval using the following formula (9):

Sample size (n1) based on sensitivity= $\frac{Z^2_{1-\frac{\alpha}{2}}\ \mathrm{Sens}(1-\mathrm{Sens})}{d^2 Prev}$

Sample size (n2) based on specificity= $\frac{Z^2_{1-\frac{\alpha}{2}}\ \mathrm{Spec}(1-\mathrm{Spec})}{d^2(1-Prev)}$

Where Z, the normal distribution value, was set to 1.96 as corresponding with the 95% confidence interval, and *d*, the maximum acceptable width of the 95% confidence interval, was set to 10%. Based on a previous study, the UBT was shown to achieve a sensitivity value of 96% and a specificity value of 93% (10). The prevalence rate (Prev) of *H. pylori* in Shenzhen, China was 35.85% (11). Therefore, this study should recruit at least 41 *H. pylori*-positive patients (n1) and 39 *H. pylori*-negative patients (n2), yielding a total sample size of 80 participants for each UBT test.

Urea breath test

^{13}C-UBT

The ^{13}C-UBT (Beijing Boran Pharmaceutical Co. Ltd., China) was performed according to the manufacturer's instructions. Briefly, an initial baseline breath sample was collected from each

patient after fasting for at least four hours prior to ingesting a capsule containing 50 mg ^{13}C isotope labeled urea with 80-100 mL of water. After 30 minutes of sitting, exhaled breath was again collected. The $^{13}CO_2$ content within the initial and 30-min expiratory air bags were analyzed using an HG-IRIS13C infrared spectrometer (Beijing Richen-Force Science & Technology Co., China). Following 30 minutes of administration, a delta over baseline (DOB) value of 4% or above was regarded as a positive indicator of *H. pylori* infection.

^{14}C-UBT

The ^{14}C-UBT (Zhonghe Headway Bio-Sci & Tech Co. Ltd., China) was performed according to the manufacturer's instruction. Briefly, patients who fasted for at least four hours were requested to ingest a gelatin capsule containing 0.75 μCi of ^{14}C isotope with 20 mL of water. After 25 minutes, each patient was then asked to exhale continuously into a bottle until the purple-colored CO_2 capturing liquid within turned colorless. The scintillation fluid was subsequently added, and the homogenized solution was measured for $^{14}CO_2$ quantity. A reading with more than 100 disintegrations per minute (DPM) was classified as *H. pylori* positive.

Histopathology

Two gastric biopsy specimens (one each from antrum and corpus) were sent to Da'an Clinical Laboratory (Guangzhou, China) for histopathology examination. The histopathologists were unaware of the UBT results. The presence of *Helicobacter*-like organism was confirmed with routine hematoxylin and eosin (HE) staining. Giemsa staining was further performed if HE

couldn't confirm the *H. pylori* clearly and in those patients with obvious inflammatory reaction, but no *H. pylori* found in HE staining.

Statistical analysis

The sensitivity and specificity values of each UBT method were reported according to manufacturer's recommended cut-off value. To evaluate the diagnostic capacity of each UBT method, the receiver operating characteristic (ROC) curve was generated by plotting the true-positive rate on the y axis against the true-negative rate on the x axis (12). Our ROC analysis was performed using the R package pROC (version 1.16.2) (13). The area under the curve (AUC) was calculated to quantify the overall accuracy of each UBT method to diagnose *H. pylori* infection outcomes. The optimal cutoff UBT value that generates the highest true positive rate together with the lowest false positive rate, was determined by using maximum Youden index method, where Youden index = sensitivity + specificity − 1 (14). For the comparison of categorical variables, the Fisher's exact test was used. The level of statistical significance was considered at $p<0.05$.

Results

Diagnostic performance of ^{13}C-UBT and ^{14}C-UBT with the manufacturer's recommended cutoff for UBT

The UBT readings, histological findings of *H. pylori* in gastric biopsies and patient demographics including age and sex are available in Table S1. Among the 484 patients recruited in this study, 217 and 267 were tested using the ^{13}C- and ^{14}C-UBT kits, respectively. The numbers of *H. pylori*-positive and -negative patients were 164 (75.6%, 164/217) and 53 (24.4%, 53/217), using the ^{13}C-UBT, and 220 (82.4%, 220/267) and 47 (17.6%, 47/267), as indicated by the ^{14}C-UBT. We next assessed the diagnostic performance of both UBT assays (Table 1). While the ^{13}C-UBT and ^{14}C-UBT each had a high sensitivity of 95.1% (CI:89.8%-97.8%) and 96.9% (CI:93.0%-98.7%), respectively, their specificity was unsatisfactory, at 62.2% (CI:50.1%-73.0%) and 54.7% (CI:42.8%-66.1%), respectively.

Table 1. Diagnostic performance of the ^{13}C-UBT and ^{14}C-UBT.

Method		Histology		Sens (%)	Spec (%)	Acc (%)	FPR (%)	FNR (%)
		+	-	95%CI	95%CI	95%CI	95%CI	95%CI
^{13}C-UBT	+	136	28	95.1	62.2	83.9	37.8	4.9
	-	7	46	89.8-97.8	50.1-73.0	78.4-88.2	27.0-49.9	2.2-10.2
^{14}C-UBT	+	186	34	96.9	54.7	85	45.3	3.1
	-	6	41	93.0-98.7	42.8-66.1	80.2-88.8	33.9-57.2	1.3-7.0

UBT: urea breath test; Sens: sensitivity; Spec: specificity; Acc: accuracy; FPR: false positive rate; FNR: false negative rate; CI: confidence interval.

We also compared the discordance of *H. pylori* infection status as determined by each UBT assay to histopathology in three different age groups (Table 2). Interestingly, in patients aged 18-30 years, there was a significantly higher discordance between the ^{13}C-UBT and histopathology

outcomes as compared to the ^{14}C-UBT counterparts (29.4% versus 8.3%, $P = 0.032$).On the other hand, in patients aged above 50 years, the discordance was significantly greater in the ^{14}C-UBT group than those who were tested by the ^{13}C-UBT (28.4% versus 12.2%, $P = 0.045$). No significant difference was observed in the 31-50 years patient group.

Table 2. Discordance between UBT and histopathology findings among different patient age groups.

Age (years)	^{13}C-UBT			^{14}C-UBT			*P*
	# FP	# FN	Discordance [% (n/N)]	# FP	# FN	Discordance [% (n/N)]	
18-30	10	0	29.4 (10/34)	3	0	8.3 (3/36)	0.032
31-50	15	4	14.2 (19/134)	12	4	10.2 (16/157)	0.367
>50	3	3	12.2 (6/49)	19	2	28.4 (21/74)	0.045

FP: false positive; FN: false negative. The distribution of discordant findings between both UBT assays in each age group was tested using the Fisher's exact test, with a *P*-value less than 0.05 considered as statistically significant.

ROC analysis and development of optimal cutoff values

Using a ROC analysis, the optimal cutoff value as a positive indicator for *H. pylori* infection was 10.4% DOB for ^{13}C-UBT, and 238 DPM for ^{14}C-UBT (Figure 2). With these cutoffs, the AUCs for ^{13}C- and ^{14}C-UBT were 86.4% and 87.8%, respectively. While the increase in cutoff value had greatly reduced the number of false positives in each UBT assay, improving the specificity from 62.2% (CI: 50.1%-73.0%) to 81.1% (CI:70.0%-88.9%) for ^{13}C-UBT, and from 54.7% (CI: 42.8%-66.1%) to 84% (CI:73.3%-91.1%) for ^{14}C-UBT, the sensitivities decreased to 83.9% (CI:76.6%-89.3%) and 82.3% (CI:76.0%-87.3%) (Table 3).

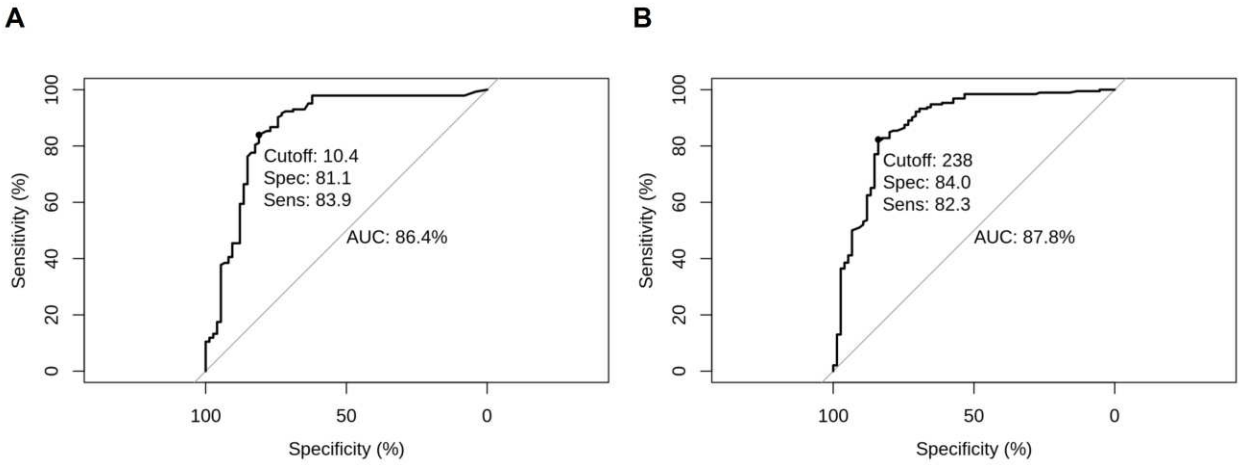

Figure 2. ROC curve of UBT for the diagnosis of *H. pylori* infection.
(A) ROC curve of ^{13}C-UBT. (B) ROC curve of ^{14}C-UBT.

Table 3. Diagnostic performance of the ^{13}C-UBT and ^{14}C-UBT with optimal cutoff values for *H. pylori* positivity.

Method		Histology		Sens (%)	Spec (%)	Acc (%)	FPR (%)	FNR (%)
		+	-	95%CI	95%CI	95%CI	95%CI	95%CI
^{13}C-UBT	+	120	14	83.9	81.1	82.9	18.9	16.1
	-	23	60	76.6-89.3	70.0-88.9	77.4-87.4	11.1-30.0	10.7-23.4
^{14}C-UBT	+	158	12	82.3	84	82.8	16	17.7
	-	34	63	76.0-87.3	73.3-91.1	77.8-86.9	8.9-26.7	12.7-24.0

UBT: urea breath test; Sens: sensitivity; Spec: specificity; Acc: accuracy; FPR: false positive rate; FNR: false negative rate; CI: confidence interval. Based on the maximum Youden index method, the optimal cutoff values for *H. pylori* positivity were 10.4% DOB and 238 DPM for the ^{13}C-UBT and ^{14}C-UBT, respectively.

To improve the sensitivity of each assay, we thought that it was necessary to establish an additional cutoff value as a negative indicator of *H. pylori* infection, following which the values situated between the upper and lower cutoffs would be classified as indeterminate results and therefore require repeated testing. Again, ROC analysis was performed on each UBT assay and

this time, only with values below the optimal cutoff as previously determined. As shown in Figure 3, the new cutoff value was 3% DOB for ^{13}C-UBT, and 87 DPM for ^{14}C-UBT, which in turn implies that UBT values less than 3% DOB or 87 DPM were very likely to be *H. pylori* negative. Taking both upper and lower cutoff values for each assay into consideration, we recommend that for ^{13}C-UBT readings of 3% to 10.3% and ^{14}C-UBT readings of 87 to 237, the *H. pylori* infection status should be considered indeterminate and would therefore require a repeated testing.

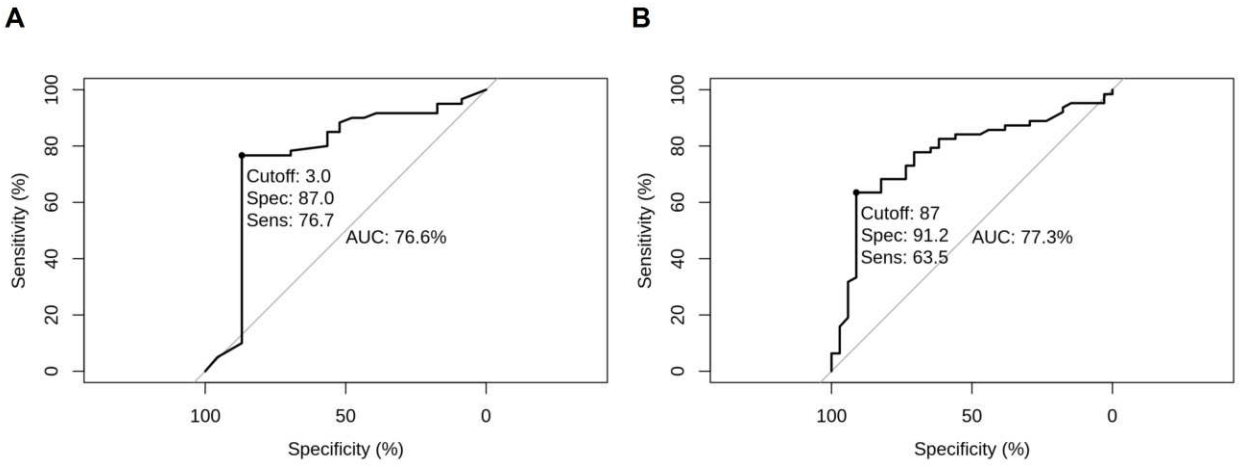

Figure 3. ROC curve of UBT for the diagnosis of H. pylori infection with only UBT readings below the optimal positive cutoff value.
(A) ROC curve of ^{13}C-UBT. (B) ROC curve of ^{14}C-UBT.

More importantly, with the introduction of an indeterminate zone, in which its (indeterminate) readings have been excluded from performance assessment and subjected to other test methods, the sensitivity and specificity can be improved to 96% (CI:90.4%-98.5%) and 76.7% (CI:63.7%-86.2%) for ^{13}C-UBT, and 98.1% (CI:94.2%-99.5%) and 76.9% (CI:62.8%-87.0%) for ^{14}C-UBT

(Table 4). Further, the diagnostic accuracy of the ^{13}C- and ^{14}C-UBT in this population can be boosted from initially 83.9% (CI:78.4%-88.2%) and 85% (CI:80.2%-88.8%), to 89.7% (CI:84.5%-93.4%) and 93% (CI:88.6%-95.8%), respectively.

Table 4. Diagnostic performance of the ^{13}C-UBT and ^{14}C-UBT with optimal *H. pylori*-positive and -negative cutoff values, and the inclusion of an indeterminate range.

Method	Histology			Sens (%)	Spec (%)	Acc (%)	FPR (%)	FNR (%)
		+	-	95%CI	95%CI	95%CI	95%CI	95%CI
^{13}C-UBT	+ (≥10.4%)	120	14	96	76.7	89.7	23.3	4
				90.4-98.5	63.7-86.2	84.5-93.4	13.8-36.3	1.5-9.6
	- (≤2.9%)	5	46					
	IND (3-10.3%)	18	14					
^{14}C-UBT	+ (≥238 DPM)	158	12	98.1	76.9	93	23.1	1.9
				94.2-99.5	62.8-87.0	88.6-95.8	13.0-37.2	0.5-5.8
	- (≤86 DPM)	3	40					
	IND (87-237 DPM)	31	23					

UBT: urea breath test; IND: indeterminate; Sens: sensitivity; Spec: specificity; Acc: accuracy; FPR: false positive rate; FNR: false negative rate; CI: confidence interval.

Discussion

The urea breath test is widely accepted as an accurate non-invasive method for diagnosing *H. pylori* infection. In the present study, we assessed the diagnostic performance of the ^{13}C- and ^{14}C-

UBT commercial kits used in our hospital by comparing each UBT outcome against that of histological examination, which was considered the "gold standard" reference method for determination of *H. pylori* infection. Among the 164 and 53 ^{13}C-UBT-positive and -negative patients, only 136 (82.9%, 136/164) and 46 (86.8%, 46/53) in whom the *H. pylori* infection status was concordant with that of histopathology examination. On the other hand, among the 220 and 47 ^{14}C-UBT-positive and -negative patients, 186 (84.5%, 186/220) and 41 (87.2%, 41/47) were positive and negative for *H. pylori* based on histopathology examination, respectively. Using the original cutoff values of 4% DOB and 100 DPM, both the ^{13}C- and ^{14}C-UBT assays had high sensitivity, at 95.1% and 96.9%, respectively, but inadequate specificity, which was 62.2% for the former and 54.7% for the latter. Given the unexpectedly high number of false positives produced by each test, we investigated and compared the distribution of discordant UBT and histological findings in different age groups. Importantly, based on histology, the ^{14}C-UBT was significantly more accurate than the ^{13}C-UBT in determining *H. pylori* infection status in patients aged 18-30 years, whereas, in patients older than 50 years of age, the ^{13}C-UBT method was more accurate than the ^{14}C-UBT.

The ^{13}C-UBT measures the ratio of labeled CO_2 to human respiratory CO_2 in the breath. Hence its outcome can be affected by one's gender, age, urea hydrolysis rate and CO_2 production rate (15). Therefore, in the event where there were many false-positive ^{13}C-UBT results among the young patients, it is possible that these individuals have a relatively low basal CO_2 production rate and/or a high urea hydrolysis rate, releasing breath with a proportionally higher quantity of

295 labeled CO_2 and thus, generating a false-positive DOB value. In the situation where ^{14}C-UBT
296 generated substantially more false-positive results than ^{13}C-UBT in older patients, some of these
297 patients might have hypochlorhydria, a condition where there is a low-level production of gastric
298 acid and which is commonly associated with aging, leading to the growth of urease-producing
299 non-*H. pylori* bacteria originating either from the oral cavity or the intestine and thus, a UBT-
300 positive outcome (16-18).
301 Also, it is also possible that the four-hour fasting time (prior to ingesting a capsule containing 50
302 mg ^{13}C isotope-labeled urea) of this current study is insufficient to empty the stomach in some of
303 these individuals. This situation is most likely to generate a less-acidic gastric environment,
304 which would be rather permissive for the growth of other bacteria with urease activity that could
305 eventually induce a false-positive UBT reaction. Therefore, a longer fasting period, potentially
306 overnight when possible, should be considered the preferred option before testing.
307 Additionally, attention to detail when performing the tests could improve the accuracy. As an
308 example, cleaning teeth and mouth immediately prior to the test might decrease gastric
309 contamination from swallowed oropharyngeal (urease positive) bacteria. Ensuring the patient was
310 sitting quietly prior to the test would lower the amount of endogenous CO_2 resulting in a slightly
311 higher breath enrichment of the isotope.
312 Depending on populations and the doses of ^{13}C-urea or ^{14}C-urea, no one-size-fits-all UBT cutoff
313 value can be used to define whether an individual is *H. pylori*-positive or -negative (19-22). In
314 our study, to overcome the low specificity of each UBT kit, two optimal cutoff points, indicating

315 UBT-positive and -negative, respectively, along with an indeterminate zone to address UBT
316 readings that are inconclusive, were established. The intermediate zone, defined as ranging from
317 3% to 10.3% DOB for ^{13}C-UBT, and from 87-237 DPM for ^{14}C-UBT, contained at least half of
318 the false-positive test results in this study. By using new optimal cutoff values and including an
319 indeterminate range, the false positive rates can be greatly reduced. More importantly, we suggest
320 that for any patient who had an indeterminate UBT result, a repeated UBT or other diagnostic test
321 such as stool antigen or serum antibody test should be performed to confirm *H. pylori* infection,
322 avoiding misdiagnosis and unnecessary antibiotic treatment.

323 ## Limitations of study

324 We concede that there are limitations in this study which was performed in a busy clinical setting
325 rather than in a formal research environment. Despite with the new cutoffs and the establishment
326 of an indeterminate range, the specificity of each UBT kit was only improved to approximately
327 77%, which is still considerably lower than reported (10). The lower specificity of each UBT kit
328 used in this study was probably due to the misdiagnosis of *H. pylori* infection when histology
329 alone was used as the reference method, as its accuracy depends on the skills of the operator, the
330 size and number of biopsies taken and whether or not the biopsy site contained *H. pylori* or
331 missed it by chance.
332 To address this issue, a larger cohort study with a more even distribution of different age
333 categories, as well as the ability to test every study participant using both ^{13}C- and ^{14}C-UBT, and
334 the concordant use of two or three methods as references should be further performed. For

example, rapid urease test, bacterial culture, histology and even PCR, would create a truer "gold standard" and allow for better comparison of the diagnostic accuracy of both tests and the validation of our suggested indeterminate zone. At the same time, observation of how the tests were administered in a busy clinical setting could increase the value of the test even further.

Conclusions

Via comparing the UBT outcomes to that of histopathology examination, we demonstrated that both ^{13}C- and ^{14}C-UBT kits used in this study have high sensitivity but low specificity. Based on ROC analysis and the maximum Youden index method, new optimal cutoff values were identified and used to establish an indeterminate range (3-10.3% DOB for ^{13}C-UBT and 87-237 DPM for ^{14}C-UBT), improving the specificity from 62.2% to 76.7% and 54.7% to 76.9% for the ^{13}C- and ^{14}C-UBT, respectively. We strongly suggest that for any patient who had an indeterminate UBT result, a repeated UBT or other diagnostic test should be performed to confirm *H. pylori* infection, avoiding misdiagnosis and unnecessary antibiotic treatment. For future studies, a larger cohort study with two or three methods as references should be further performed to validate our suggested indeterminate zone.

Declarations

Ethics approval and consent to participate

This study was approved by the research ethics committee of Shenzhen Kuichong People's Hospital (reference no. 201609) and registered at www.chictr.org.cn (reference no. ChiCTR2000041570). Informed consents were obtained from all participants.

Consent for publication

Not applicable.

Availability of data and materials

All data generated or analysed during this study are included in this published article (and its supplementary information files).

Competing interests

The authors declare that they have no competing interests.

Funding

This work was funded by the National Natural Science Foundation of China (No. 81902022) and the Sanming Project of Medicine in Shenzhen (No. SZSM201510050).

Authors' contributions

Preparation of draft manuscript: XY and SZ. Manuscript revision: EGC, MJW and CYT. Data analysis: EGC, MJW and CYT. Endoscopy: YH, AL, DS, The urea breath test and histopathology test: SZ, XL, HC and DS. Study design and conceptualisation: CYT, BJM and XL. Funding and resources: XY, YH. All authors read and approved the final manuscript.

Acknowledgements

Not applicable.

Authors' information

[1] Department of Gastroenterology, Shenzhen Second People's Hospital/ the First Affiliated Hospital of Shenzhen University, Health Science Center, Shenzhen 518035, China.

[2] Department of Clinical Laboratory, Kuichong People's Hospital, Shenzhen 518116, China.

[3] The Marshall Centre for Infectious Diseases Research and Training, University of Western Australia, Perth 6009, Australia.

[4] Department of Gastroenterology, Kuichong People's Hospital, Shenzhen 518116, China.

[5] Department of Nuclear Medicine, Shenzhen People's Hospital, Shenzhen 518001, China.

[6] Department of Computer Science and Software Engineering, University of Western Australia, Perth 6009, Australia.

References

1. Hooi JKY, Lai WY, Ng WK, Suen MMY, Underwood FE, Tanyingoh D, et al. Global Prevalence of Helicobacter pylori Infection: Systematic Review and Meta-Analysis. Gastroenterology. 2017;153(2):420-9.

2. Marshall BJ, Warren JR. Unidentified curved bacilli in the stomach of patients with gastritis and peptic ulceration. Lancet. 1984;1(8390):1311-5.

3. Marshall BJ, Windsor HM. The relation of Helicobacter pylori to gastric adenocarcinoma and lymphoma: pathophysiology, epidemiology, screening, clinical presentation, treatment, and prevention. Med Clin North Am. 2005;89(2):313-44, viii.

4. Bauerfeind P, Garner R, Dunn BE, Mobley HL. Synthesis and activity of Helicobacter pylori urease and

catalase at low pH. Gut. 1997;40(1):25-30.

5. Savarino V, Vigneri S, Celle G. The 13C urea breath test in the diagnosis of Helicobacter pylori infection. Gut. 1999;45 Suppl 1:I18-22.

6. Hamlet AK, Erlandsson KI, Olbe L, Svennerholm AM, Backman VE, Pettersson AB. A simple, rapid, and highly reliable capsule-based 14C urea breath test for diagnosis of Helicobacter pylori infection. Scand J Gastroenterol. 1995;30(11):1058-63.

7. Stubbs JB, Marshall BJ. Radiation dose estimates for the carbon-14-labeled urea breath test. J Nucl Med. 1993;34(5):821-5.

8. Bentur Y, Matsui D, Koren G. Safety of 14C-UBT for diagnosis of Helicobacter pylori infection in pregnancy. Canadian family physician Medecin de famille canadien. 2009;55(5):479-80.

9. Negida A, Fahim NK, Negida Y. Sample Size Calculation Guide - Part 4: How to Calculate the Sample Size for a Diagnostic Test Accuracy Study based on Sensitivity, Specificity, and the Area Under the ROC Curve. Advanced journal of emergency medicine. 2019;3(3):e33.

10. Ferwana M, Abdulmajeed I, Alhajiahmed A, Madani W, Firwana B, Hasan R, et al. Accuracy of urea breath test in Helicobacter pylori infection: meta-analysis. World journal of gastroenterology. 2015;21(4):1305-14.

11. Yang B, Chen H, Li S. Analysis of helicobacter pylori infection in Longgang distric of Shenzhen. Proceeding of Clinical Medicine. 2016;25(11):816-7+74.

12. Zweig MH, Campbell G. Receiver-operating characteristic (ROC) plots: a fundamental evaluation tool in clinical medicine. Clinical chemistry. 1993;39(4):561-77.

13. Robin X, Turck N, Hainard A, Tiberti N, Lisacek F, Sanchez JC, et al. pROC: an open-source package for R and S+ to analyze and compare ROC curves. BMC Bioinformatics. 2011;12:77.

14. Youden WJ. Index for rating diagnostic tests. Cancer. 1950;3(1):32-5.

15. Klein PD, Malaty HM, Czinn SJ, Emmons SC, Martin RF, Graham DY. Normalizing results of 13C-urea breath testing for CO2 production rates in children. J Pediatr Gastroenterol Nutr. 1999;29(3):297-301.

16. Osaki T, Mabe K, Hanawa T, Kamiya S. Urease-positive bacteria in the stomach induce a false-positive reaction in a urea breath test for diagnosis of Helicobacter pylori infection. J Med Microbiol. 2008;57(Pt 7):814-9.

17. Michaud L, Gottrand F, Ganga-Zandzou PS, Wizla-Derambure N, Turck D, Vincent P. Gastric bacterial overgrowth is a cause of false positive diagnosis of Helicobacter pylori infection using 13C urea breath test. Gut. 1998;42(4):594-5.

18. Brandi G, Biavati B, Calabrese C, Granata M, Nannetti A, Mattarelli P, et al. Urease-positive bacteria other than Helicobacter pylori in human gastric juice and mucosa. Am J Gastroenterol. 2006;101(8):1756-61.

19. Zhou Q, Li L, Ai Y, Pan Z, Guo M, Han J. Diagnostic accuracy of the (14)C-urea breath test in Helicobacter pylori infections: a meta-analysis. Wien Klin Wochenschr. 2017;129(1-2):38-45.

20. Kato C, Sugiyama T, Sato K, Saito S, Kudara N, Abiko Y, et al. Appropriate cut-off value of 13C-urea breath test after eradication of Helicobacter pylori infection in Japan. J Gastroenterol Hepatol. 2003;18(12):1379-83.

21. Mauro M, Radovic V, Zhou P, Wolfe M, Kamath M, Bercik P, et al. 13C urea breath test for (Helicobacter pylori): determination of the optimal cut-off point in a Canadian community population. Can J

433 Gastroenterol. 2006;20(12):770-4.

434 22. Campuzano-Maya G. An optimized 13C-urea breath test for the diagnosis of H pylori infection. World

435 J Gastroenterol. 2007;13(41):5454-64.

436

Figures

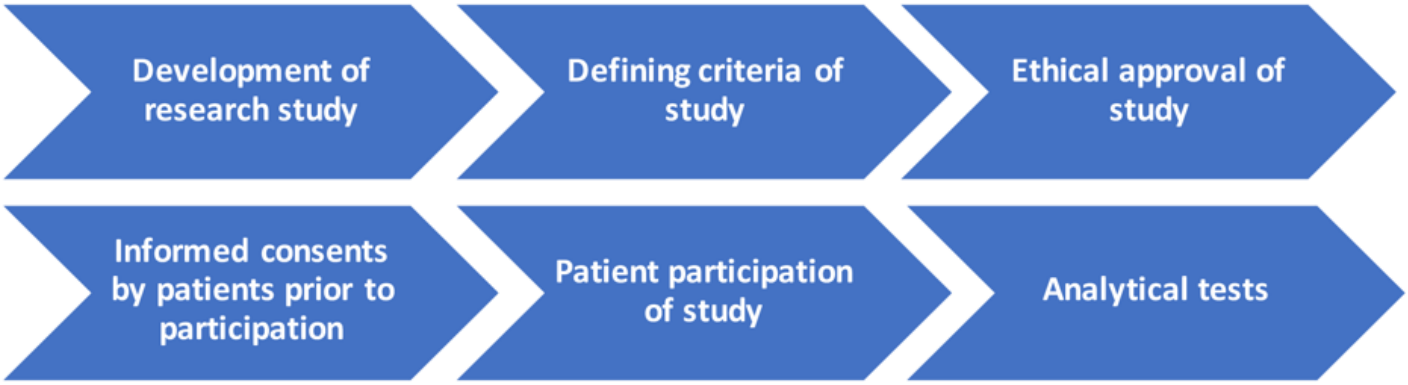

Figure 1

The overview of entire study.

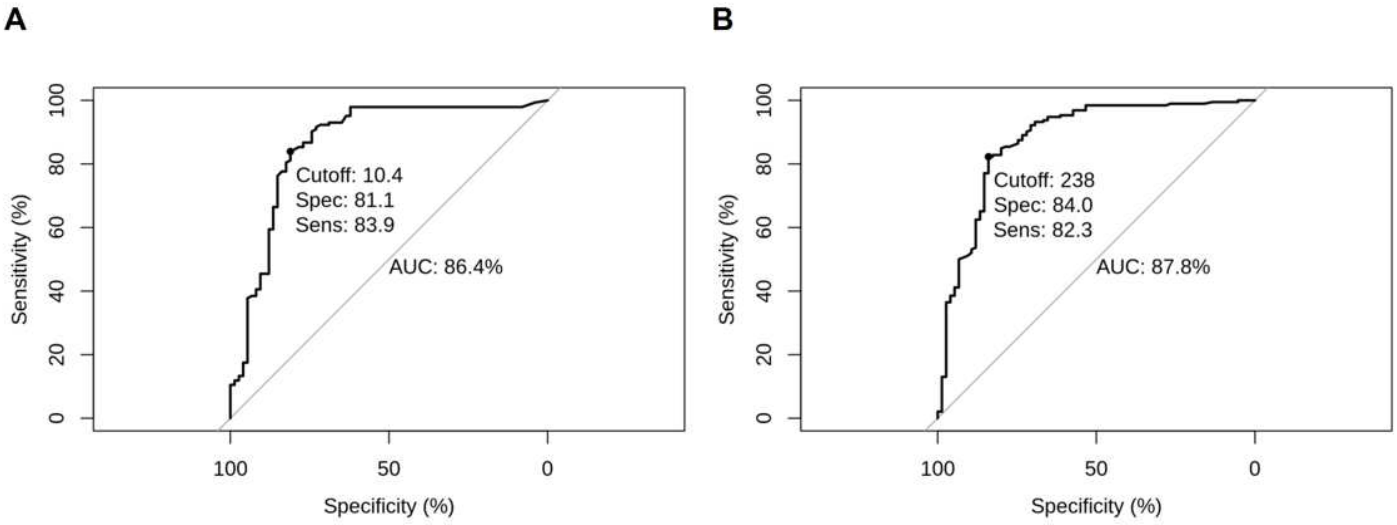

Figure 2

ROC curve of UBT for the diagnosis of H. pylori infection. (A) ROC curve of 13C-UBT. (B) ROC curve of 14C-UBT.

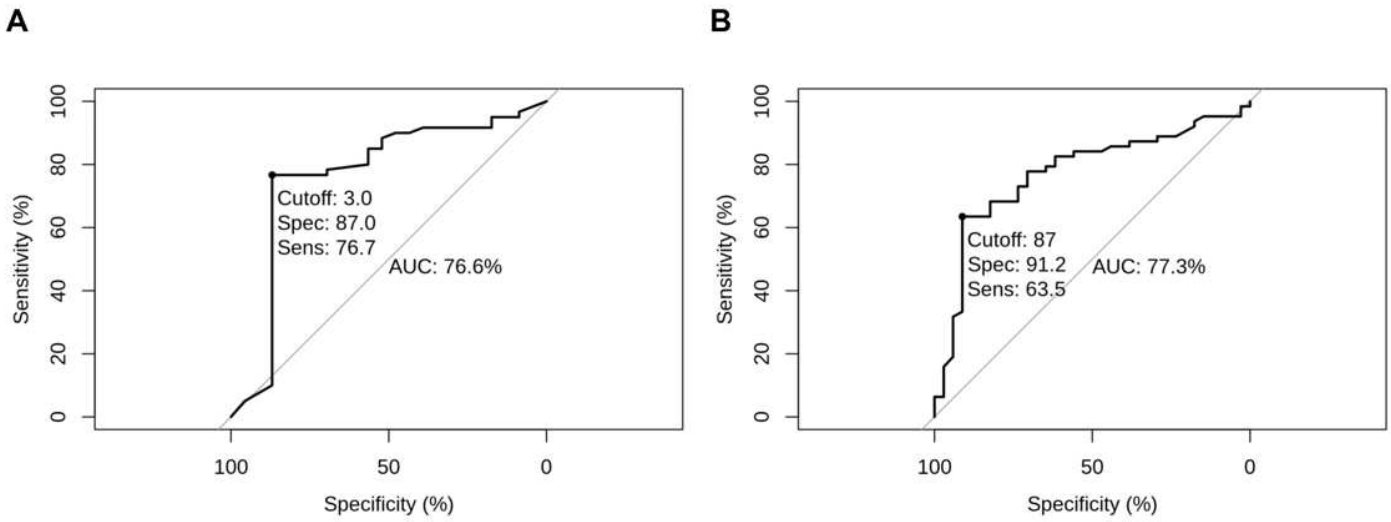

Figure 3

ROC curve of UBT for the diagnosis of H. pylori infection with only UBT readings below the optimal positive cutoff value. (A) ROC curve of 13C-UBT. (B) ROC curve of 14C-UBT.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- TableS1.xlsx