Validated accuracy of a novel urea breath test for rapid *Helicobacter pylori* detection and in-office analysis

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Background **A novel ^{14}C-urea breath test (UBT) was developed to detect the presence of *Helicobacter pylori* by bench analysis in office, enabling the practitioner to readily reveal *H. pylori* infection.**

Aim **To validate the novel UBT (Heliprobe^{TM}) versus conventional UBT.**

Methods **Pretreatment ($n = 203$) and post-treatment ($n = 147$) detection of *H. pylori*. Additional tests with encapsulated ^{14}C-urea ($n = 37$) were validated. After intake of liquid or encapsulated ^{14}C-urea, exhaled $^{14}CO_2$ in breath was trapped in benzethoniumhydroxide/ethanol, or adsorbed to LiOH-soaked pads on a dry cover surface (Heliprobe BreathCard^{TM}). The amount of adsorbed ^{14}C was detected using a β-scintillator or two Geiger–Müller counters operating in parallel (Heliprobe^{TM} Analyzer).**

Results **For pretreatment detection, we found full concordance between the UBTs, with 100% sensitivity and specificity (CI 95–100% and 97–100%, respectively) and strong agreement ($r = 0.80$, CI 0.75–0.85; $\kappa = 1$, CI 0.86–1.14; $P < 0.0001$). Similarly, for post-treatment follow-up detection, sensitivity and specificity were 100% (CI 85–100% and 97–100%, respectively) with significant agreement ($r = 0.48$, CI 0.34–0.59; $\kappa = 1$, CI 0.84–1.16; $P < 0.0001$). The use of encapsulated ^{14}C-urea did not change agreement between the tests. Sensitivity and specificity were 100% (CI 72–100% and 87–100%, respectively) with strong agreement between the tests ($r = 0.71$, CI 0.50–0.84; $\kappa = 1$, CI 0.68–1.32; $P < 0.0001$).**

Conclusion **The novel Heliprobe UBT, with either liquid or encapsulated ^{14}C-urea, seems equi-efficacious to conventional UBT in fulfilling its role as the non-invasive gold standard for detection of *H. pylori*. *Eur J Gastroenterol Hepatol* 14:1–8 © 2002 Lippincott Williams & Wilkins**

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Introduction

The urea breath test (UBT) was first described by Graham *et al*. [1] in 1987. The idea of developing a ^{14}C-UBT to detect the presence or absence of *Helicobacter pylori* was a result of two publications appearing in letter form in the *Lancet*. The first, from Tytgat and co-workers [2], discussed the finding that *H. pylori* possessed a urease enzyme that was extremely powerful. The second, from Marshall and Langton [3], described how patients infected with *H. pylori* tended to have lower urea and higher ammonia concentrations in gastric juice than did non-infected people. The later development of the UBT in our and others' laboratories has proven the UBT to be a highly standardized, sensitive and specific test [4]. Today, the presence of *H. pylori* in the gastrointestinal tract is most conveniently detected non-invasively using either the ^{13}C- or ^{14}C-UBT [1,5,6]. The UBT detects the metabolically active bacteria, which makes the test suitable for pretreatment detection as well as for monitoring results after eradication treatment against *H. pylori*. The validity of the UBT is generally high, with a reported sensitivity of 90–98% and specificity of 92–100% [5,7–10]. With the aid of an acidified urea cocktail, the diagnostic precision of the method is enhanced [4]. Even if the UBT has become the diagnostic procedure of choice for detection of *H. pylori* infection, the detection procedure using a liquid CO_2-trapping medium and β-scintillation has hampered the further development of UBT to a handy diagnostic tool. Naturally, the ^{13}C-UBT is always an alternative method with high diagnostic precision and accuracy [1,4,6–8], but it seems to complicate the analysis by requiring a centralized, expensive mass spectrometer with a continuous need for maintenance and calibration.

In the search for a UBT for detection of *H. pylori*, fulfilling common demands of easiness, handiness and

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quick in-office analysis, we have developed a simplified ^{14}C-UBT system (Heliprobe^{TM}, Noster System AB, Stockholm, Sweden). The objective of the present work was to evaluate whether this novel UBT, based on a new collection-and-analysis method, has a comparable diagnostic performance and accuracy with the conventional ^{14}C-UBT method as the ^{14}C-UBT previously developed and validated in our laboratory [4]. Furthermore, we aimed to determine the optimal diagnostic cut-off level for the novel UBT in two different situations: (1) for pretreatment of *H. pylori* in antibiotic-naïve patients, and (2) for follow-up detection after eradication treatment. We designed and performed a clinical study in which each patient swallowed an acidified ^{14}C-urea solution and breath samples were collected via both the conventional UBT and the Heliprobe system. In addition, a preliminary evaluation was made replacing the urea solution with an encapsulated form of ^{14}C-urea (PYCap, Tri-Med Specialities Inc., Charlottesville, Virginia, USA), which should further simplify the novel UBT method.

Materials and methods

The novel ^{14}C-urea breath test system

The new Heliprobe UBT is a completely 'dry' system consisting of two components, the Heliprobe BreathCard^{TM} and the Heliprobe Analyzer^{TM} (Fig. 1). The Heliprobe BreathCard is a flat, credit-card-sized collection vehicle that adsorbs exhaled CO_2 via chemical bounding to pads soaked in LiOH. The collection process is simple; the patient breathes into a mouthpiece on the card until a pH-sensitive indicator changes colour from orange to yellow as an indication of CO_2 saturation of the pads. The breathing time varies depending on the number of breaths into the card, the average time being approximately 1–2 min. Since the exhaled CO_2 is bound chemically to the pads, the card can be stored for several years without loss or deterioration of its CO_2 content.

Fig. 1

The Heliprobe^{TM} system including BreathCard and Analyzer.

With the Heliprobe Analyzer, the traditionally used liquid β-scintillator has been replaced with an instrument containing two built-in Geiger–Müller counters operating in parallel. This technology swap has made it possible to design a cheap, small (laptop-sized), and fully automatic analyser that can be operated by the nurse or physician in the clinic (Fig. 1).

The Heliprobe BreathCard is simply put into the slot of the Heliprobe Analyzer. By pressing the start button, a fully automatic test sequence is initiated and run for 250 s. The result of the measurement is presented on a liquid-crystal display (LCD) and on a printer. The analysis is based on the number of emitted β-particles that hit the two Geiger–Müller counters during the 250-s measurement cycle and is presented as counts per min (cpm) together with the test result 'negative', 'equivocal' or 'positive'.

The cut-off levels between the different test results are based on the obtained cpm values. The diagnostic cut-off is programmable to different levels by setting lower and upper limits. A cpm value below the lower limit is presented as a negative result, values between the lower and upper limits are presented as equivocal, and values above the upper limit are positive. By setting the lower and upper limits to the same value, equivocal results can be avoided. The Heliprobe Analyzer is continuously compensating for background radioactive variations, thereby eliminating this source of error.

The conventional ^{14}C-urea breath test system

The conventional UBT is based on trapping exhaled CO_2 in a Hyamine solution (1 ml 1.0-mol/l benzethoniumhydroxide in methanol (Hyamine®, Sigma Chem. Co., St Paul, Minnesota, USA) and 1 ml 99.8 % ethanol) kept in a 20-ml scintillation vial. The patient exhales into the scintillation vial through a straw, which is connected to a water-lock to eliminate the possibility of swallowing the solution. Phenolphthalein (Sigma) was used as a colour indicator for saturation of the benzethoniumhydroxide solution with 1 mmol CO_2.

After saturation, indicated by colour change from pink to colourless, 10 ml scintillation liquid Optiphase 'Hi Safe' (Wallac, Fison Chem., Loghborough, Leicestershire, UK) was added, and the sample was analysed in a liquid β-scintillation counter (Beta Rack 1215, Wallac). As a blank, we used 1 ml benzethoniumhydroxide, 1 ml ethanol and 10 ml scintillation liquid. As standard, 0.5 ml ^{14}C-urea cocktail was added to a prepared scintillation vial with benzethoniumhydroxide solution,

ethanol and scintillation liquid. Quench correction was applied by the external standard ratio method to yield sample activities in disintegrations per minute (dpm). The results were presented as CO_2 recovery (% dose recovered/mmol CO_2 trapped multiplied by the weight of patient).

Study design

The study was carried out on consecutive patients scheduled for diagnosis of *H. pylori* infection or post-treatment follow-up at the Department of Gastroenterology and Hepatology, Karolinska University Hospital, Stockholm, Sweden. No attempts were made to select patients based on their diagnosis. Patients could not take proton-pump inhibitors in the week before the test was undertaken. Antacids, H_2-receptor antagonists and sucralfate were stopped 24 h before the test day, and antibiotics and bismuth medications were stopped during the month before the study. Patients were prompted to observe a 6-h fasting period. To minimize exposure to oral microflora, patients were instructed to brush their teeth well in the morning before the UBT, and to swallow the acidified ^{14}C-urea solution quickly.

The inclusion criteria for subjects in the study were age 18–85 years, upper-gastrointestinal discomfort or symptoms, and suspicion of *H. pylori* infection. Exclusion criteria were pregnancy or breastfeeding, previous gastric surgery, drug or alcohol addiction, senile dementia, and a previous diagnostic UBT within 30 days.

The study design was approved by the local Ethics and Radiation Safety Committees of the Karolinska Hospital. Each patient received information about the investigative nature of the study, and informed consent was obtained from each subject.

Ten minutes after ingestion of the solution, breath samples were collected via both the conventional UBT and the Heliprobe system. At 20 min, a second breath sample was obtained for the conventional UBT. Since our aim was to develop a simplified test procedure, we did not repeat this second test with the Heliprobe system.

Preparation of the acidified urea solution

Liquid ^{14}C-urea was obtained commercially from the Swedish National Pharmacy (Apoteksbolaget AB, Stockholm, Sweden) in 25-μCi vials at a concentration of 5 μCi/ml and stored in refrigerator. Immediately before the test, aliquots of 1 μCi (0.2 ml) were pipetted into a plastic cup and 50 mL 0.05-mol/l citric acid water solution was added.

In a separate study group, a 1-μCi encapsulated form of ^{14}C-urea (PYCap, Tri-Med Specialities Inc.) was used instead of the regular urea solution.

Determination of Helicobacter pylori-positive and -negative cases

The conventional UBT was used to determine *H. pylori*-infected patients. Optimal cut-off criteria were determined in a previous validation study of the conventional method [4]. A 10-min test value above 0.80 % mmol CO_2^{-1} kg, or a 20-min test value above 0.50 % mmol CO_2^{-1} kg, was classified as positive. Patients with values equal to or below the above-mentioned values were classified as negative.

Analysis and statistics

Examinations for pretreatment detection and post-treatment detection after eradication treatment were analysed separately. All evaluations were done per protocol, but the effects of protocol violations were also explored.

The results of the Heliprobe tests were divided into two classes, *H. pylori*-positive and *H. pylori*-negative, based on the outcome of the conventional UBT used for reference.

Normality was assessed by the Wilk–Shapiro W test. Descriptive data are presented as mean ± SD for normally distributed data, and as the median and range for non-normally distributed data. The Mann–Whitney U test was used for comparisons between groups. Sensitivity and specificity together with 95% exact (Clopper–Pearson) confidence limits for the proportion were determined for all possible cut-off points with AccuROC ver. 2.3 (Accumetric Corporation, Montreal, Canada). Association between the Heliprobe system and the conventional UBT was assessed by correlation analysis (Spearman rank method) and explored further by inter-rater agreement and Cohen's unweighted κ statistics. A *P*-value of less than 0.05 was considered significant. All statistics, except the sensitivity and specificity analyses, were carried out using StatsDirect (CamCode, Ashwell, Hertfordshire, UK).

Results

Pretreatment detection of *Helicobacter pylori*

The characteristics of the 192 evaluated patients in the pretreatment population are summarized in Table 1. Eleven additional patients did not meet the enrolment criteria (8 taking proton-pump inhibitors in the week before UBT; 1 on proton-pump inhibitor and antibiotics; 2 did not fast for 6 h) and were excluded from the main analysis.

Figure 2 shows the spread of the Heliprobe cpm values in the *H. pylori*-positive and -negative groups. The *H. pylori*-negative group consisted of 119 patients, and the *H. pylori*-positive group consisted of 73 patients, revealing an *H. pylori* infection prevalence of 38%. The minimum, median and maximum Heliprobe values

Table 1 Summary of the study population for pretreatment detection of *Helicobacter pylori*

	Patients for analysis		
Patient characteristic	*H. pylori*-positive	*H. pylori*-negative	All
Number of subjects (*N*)	73	119	192
Male/female (*N*/*N*)	46/27	46/73	93/99
Age (years; mean ± SD)	52 ± 17	46 ± 16	48 ± 16
Weight (kg; mean ± SD)	72 ± 14	70 ± 14	71 ± 14

were 41, 400 and 1291 cpm, respectively, in the *H. pylori*-positive group, and 0, 1 and 25 cpm, respectively, in the *H. pylori*-negative group. The Shapiro–Wilk W test indicated non-normality, and the Mann–Whitney U statistical test revealed a highly significant difference (median difference 391 cpm, CI 323–429 cpm; $P < 0.0001$).

Sensitivity and specificity of the Heliprobe system

Choosing a Heliprobe cut-off level in the gap between the highest *H. pylori*-negative (25 cpm) and the lowest *H. pylori*-positive (41 cpm) gave 73 true positive, 0 false positive, 119 true negative, and 0 false negative measurements. This created 100% sensitivity and specificity (CI 95–100% and 97–100%, respectively) of the Heliprobe system versus the conventional UBT.

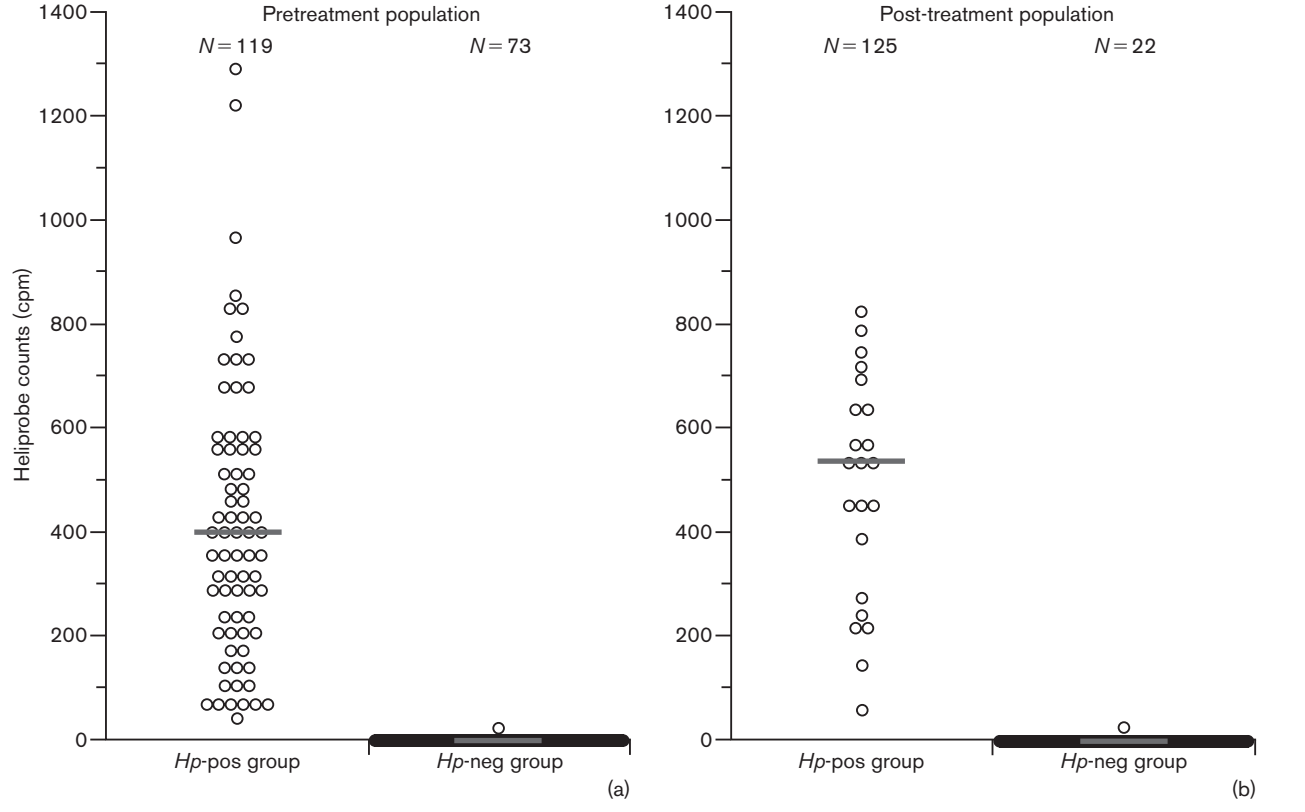
Association between the Heliprobe system and the conventional urea breath test

As shown in Figure 3, there was a significant correlation between the Heliprobe cpm values and the conventional UBT dpm values at 10 min, with a Spearman rank correlation coefficient of 0.80 (CI 0.75–0.85; $P < 0.0001$). An alternative association analysis, the κ statistics, also revealed a significant concordance between the two methods ($\kappa = 1$, CI 0.86–1.14; $P < 0.0001$).

Post-treatment detection of *Helicobacter pylori*

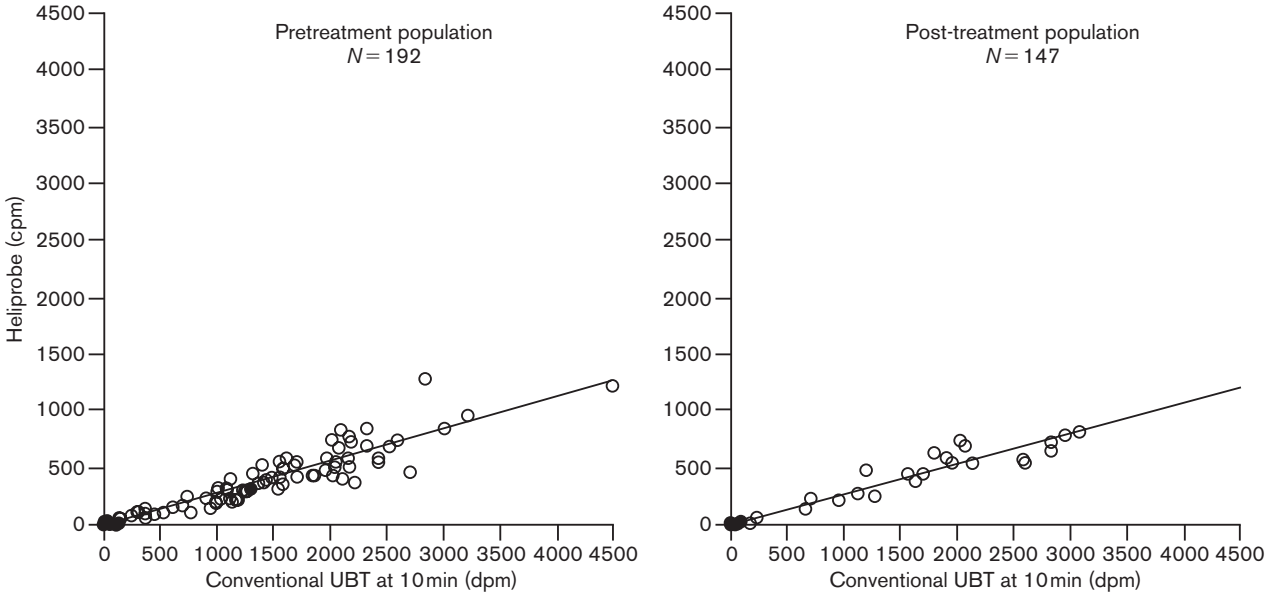
The main characteristics of the 147 patients evaluated with UBTs after eradication of *H. pylori* are summarized in Table 2. In this population, 31 patients were post-treatment verifications from the pretreatment

Fig. 2



Spread of Heliprobe test results for the *Helicobacter pylori*-positive group (*Hp*-pos) and the *H. pylori*-negative group (*Hp*-neg), as categorized by the conventional ^{14}C-urea breath test (UBT) method. The individual test results in counts per minute (cpm) are shown together with each group's median value. (a) Spread for the pretreatment population; (b) spread for the post-treatment population. Not all points are visualized adequately in the graphs due to superposition.

Fig. 3



Scatter plot between the Heliprobe counts per minute (cpm) values (Heliprobe) and the conventional ^{14}C-urea breath test (UBT) disintegrations per minute (dpm) values at 10 min (conventional UBT at 10 min). (a) Scatter for the pretreatment population; (b) scatter for the post-treatment population.

Table 2 Summary of the study population for post-treatment detection of *Helicobacter pylori*

	Per protocol		
Patient characteristic	*H. pylori*-positive	*H. pylori*-negative	All
Number of subjects (*N*)	22	125	147
Male/female (*N*/*N*)	10/12	62/63	74/73
Age (years; mean ± SD)	51 ± 17	53 ± 16	53 ± 16
Weight (kg; mean ± SD)	72 ± 11	72 ± 14	72 ± 14
Months after treatment (median, range)	2 (1–24)	2 (1–24)	2 (1–24)

population at our lab. The remaining 116 patients had been diagnosed and treated for *H. pylori* at other hospitals, and were referred for follow-up examination more than 1 month after finishing their *H. pylori*-eradication treatment. Four patients did not meet all enrolment criteria (1 on proton-pump inhibitor during the week before UBT; 2 on antibiotics; 1 did not fast for 6 h) and were excluded from the main analysis.

The *H. pylori*-eradication success rate was 85% after the first treatment (125/147 patients). The success rate following the second and third treatments was not assessed in this study. The spread of the Heliprobe cpm values in the *H. pylori*-positive and -negative groups are shown in Figure 2. Again, the difference was highly significant (median difference 532 cpm, CI 452–569 cpm; $P < 0.0001$). The minimum, median and maximum Heliprobe cpm values were 58, 537 and 824 cpm, respectively, for the *H. pylori*-positive group, and 0, 1 and 25 cpm, respectively, for the *H. pylori*-negative group.

Sensitivity and specificity of the Heliprobe system

Since there was complete separation between the two groups in Figure 2, the Heliprobe follow-up sensitivity and specificity were 100% when choosing a cut-off level in the gap between 25 and 58 cpm (CI 85–100% and 97–100%, respectively).

Association between the Heliprobe system and the conventional urea breath test

Figure 3 shows the correlation analysis for the post-treatment population. As for the pretreatment detection population, the correlation was highly significant ($P < 0.0001$), but due to the high number of *H. pylori*-negative patients, the correlation coefficient of 0.48 (CI 0.34–0.59) was not as distinct as for the pretreatment population. The κ statistics did, however, reveal an equally strong association at follow-up as that seen for the pretreatment population ($\kappa = 1$, CI 0.84–1.16).

Influence of protocol violations

Including the four patients violating the enrolment criteria in the follow-up population did not change the

range of the Heliprobe cpm values. One patient was classified as positive by the conventional UBT (Heliprobe value 439 cpm) and the remaining three as negative (Heliprobe values 0, 4 and 11 cpm). These values were within the per-protocol distribution, and the sensitivity and specificity remained at 100%.

One of the 11 protocol violations (1 patient on proton-pump inhibitor) in the detection population raised the maximum Heliprobe value in the *H. pylori*-negative group from 25 to 43 cpm. The remaining ten patients did not impose any changes on the ranges in either group. Since the minimum Heliprobe value in the *H. pylori*-positive group remained at 41 cpm, this meant that one patient had to be misclassified, either false positive or false negative, depending on how the cut-off was set. The optimal cut-off range that allowed only one to two erroneous patient classifications was set between 26 and 47 cpm.

Optimal cut-off value for the Heliprobe system

Ideally, the same Heliprobe cut-off level would be used for both the pre- and post-treatment populations. That is possible if the optimal cut-off ranges for the two groups overlap. As shown in Table 3, a cut-off value between 25 and 41 cpm fulfils this criterion, and any value within this range would be appropriate to use. Another sensible alternative is to set a range where the result is presented as equivocal. A practical range for equivocal result would be between 25 and 50 cpm, which is the default setting of the Heliprobe system.

The Heliprobe system with encapsulated ^{14}C-urea

The characteristics of 37 patients undergoing UBT with an encapsulated form of ^{14}C-urea in combination with the Heliprobe system are summarized in Table 4. In this group, 21 patients were tested for detection of *H. pylori* before, and 16 for detection after, eradication treatment. Due to the small sample size, both populations were analysed together.

The *H. pylori* eradication success rate was 88% (14/16 patients) after treatment. The minimum, median and maximum cpm values in the *H. pylori*-positive group were 47, 131, 618 cpm, respectively; in the *H. pylori*-negative group, the corresponding values were 0, 1 and 28 cpm. The difference was highly significant (median difference 130 cpm, CI 76–356 cpm; $P < 0.0001$).

Table 3 **Summary of cut-off values for pre- and post-treatment detection of *Helicobacter pylori* infection**

	Pre-treatment		Post-treatment	
Patient category	Per protocol	All patients	Per protocol	All patients
Maximum value of *H. pylori*-negative group (cpm)	25	25	25	25
Minimum value of *H. pylori*-positive group (cpm)	41	47	58	58
Misclassification	0	1	0	0

Table 4 **Summary of the study group for detection of *Helicobacter pylori* using encapsulated urea**

	Per protocol		
Patient characteristic	*H. pylori*-positive	*H. pylori*-negative	All
Number of subjects (*N*)	11	26	37
Male/female (*N*/*N*)	6/5	13/13	19/18
Age (years; mean ± SD)	54 ± 15	54 ± 15	54 ± 15
Weight (kg; mean ± SD)	73 ± 15	70 ± 15	71 ± 15

As the maximal value in the *H. pylori*-negative group was 28 cpm and the minimal value in the *H. pylori*-positive group was 47 cpm, any value between these two values might be used as a cut-off between negative and positive responses.

Eleven true positives and 26 true negatives were found, while no false positives or false negatives were found. In comparison with the conventional UBT, the sensitivity using encapsulated urea was calculated to be 100% (CI 72–100%) and the specificity 100% (CI 87–100%).

As for the liquid urea group, the correlation was highly significant, with a correlation coefficient of 0.71 (CI 0.50–0.84; $P < 0.0001$). The κ statistics revealed a strong agreement between the conventional UBT and Heliprobe system using encapsulated urea ($\kappa = 1$, CI 0.68–1.32; $P < 0.0001$).

Discussion

All the biological test results obtained with the Heliprobe system were confined to a 95% confidence interval. The test results with the Heliprobe system were indistinguishable from those of our conventional UBT. In terms of both sensitivity and specificity, no significant differences between the two tests were detectable, with data approaching equality. Thus, full concordance between the two tests seems to prevail. This outcome permits us to draw the conclusion that the two systems are equi-efficacious in diagnosing *H. pylori* status. The advantages of the Heliprobe system are speed and simplicity. With no aid required from external facilities or expertise, the diagnostician will, through the use of this novel system, have access to the test result within 15–20 min after the patient has swallowed the urea. This increases the options for when and where to perform the analysis. The small size and handiness of the BreathCard also make handling very easy. In cases where mailing might be needed, a

regular envelope can be used for mailing the test to the analyser. Due to the stability of the chemical binding of CO_2 to the LiOH in the BreathCard, later reanalysis of a specific sample is possible, even years after the test was first carried out. The swap in technology has also made the Heliprobe Analyzer comparably cheaper to produce, with an estimated cost of about one-tenth of that of a β-scintillator.

The present investigation showed excellent concordance between the conventional ^{14}C-UBT and the novel Heliprobe UBT. In order to simplify the test and interpretation process, the expression of results in recovery standard units (% dose mmol CO_2^{-1} kg) has been abandoned in the Heliprobe system in favour of simply basing cut-off levels directly on measured cpm values. As reviewed elsewhere, several groups have argued that it is illogical to make allowance for endogenous CO_2 production by incorporating a 'fudge factor' involving the patient's body weight. Indeed, most groups no longer express their results as a recovery of administered dose adjusted for weight, preferring instead to use radioactive cpm or dpm, since the correlation between the two measures is excellent [11]. This is also what is to be expected due to the apparent dependency of the two correlation factors evaluated. Hence, dpm from our conventional UBT could be used and further correlated to the cpm as given by the Heliprobe Analyzer for validation of this system.

With the use of a urea cocktail for administration of ^{14}C and then detection of $^{14}CO_2$ by simultaneously using the conventional UBT and Heliprobe systems, we found a few restrictions that have to be taken in consideration when performing the UBT. First, careful tooth brushing seems important for obtaining conditions representative for the *H. pylori* status in the stomach, not being blurred by the patient's oral microflora and microbial conditions. As a further development of this method, we are aiming to produce an encapsulated form of the urea/citric acid composition needed to achieve a standardized and reliable test with stable outcomes. Second, acid suppression was detrimental for the outcome of the test. We therefore decided to withhold potent acid-inhibitory drugs, such as proton-pump inhibitors, for 7 days before the test was carried out, while less potent acid inhibitors, such as H_2-receptor antagonists, were stopped 24 h before the test was carried out. Preliminary reports indicate that the addition of citric acid to the urea solution/capsule diminishes the effect of acid-inhibitory drugs on the accuracy of the test [12]. Further verification is needed, however, before we can recommend continued drug use with proton-pump inhibitors or H_2-receptor blockers in conjunction with UBT. Third, drugs known to retain binding capacity to different substances, such as antacids and sucralfate, were withheld for 24 h before the UBT. Fourth, antibiotics or bismuth treatment were not allowed during the month preceding the UBT. By keeping a tight hand over these rules, we were able to optimize the diagnostic procedure with a minimum of variation and with the lowest amount of radioactivity (1 μCi, 37 kBq) known to be effective in order to achieve accurate test results [4,11]. Thus, both the conventional UBT as well as the Heliprobe UBT were carried out with 1-μCi ^{14}C dose per test.

Concern with ^{14}C usually arises because of its long half-life, but this is less important for organic compounds such as CO_2 and urea, which are excreted rapidly. In the ^{14}C-UBT, urea either undergoes hydrolysis, being exhaled as $^{14}CO_2$, or is eliminated unchanged in urine. Because the biological half-life of urea is short, the cumulated radiation dose from each breath test is small and far below variations in natural radiation. According to data reported by Munster *et al.* [13], approximately 90% of the ^{14}C from a UBT is eliminated as CO_2 in breath or as urea in urine. This would mean that after 3 days, the amount of isotope retained in the body is negligible. The cumulative lifetime radiation exposure from this test has been calculated to be not more than 0.3 mrem/μCi [14], considered to equal the background radiation a person is exposed to in 1 day [15]. Due to the very low level of radioactive exposure, the 1-μCi ^{14}C dose has been permitted for general use in UBTs in the USA (Nuclear Radioactive Committee, USA, 10CFR § 30.21 Radioactive drug: Capsules containing carbon-14 urea for diagnostic use in humans). We therefore consider the radioactive bioburden on each person to be very limited, even not precluding repeated tests in the same person. Some reports even conclude that there is no restriction on repeated investigations in whole families, including children [16]. We do, however, fully accept that in children and pregnant women, it is preferable to use the ^{13}C-UBT.

For the evaluation of the conventional UBT with the Heliprobe system, we used duplicate samples for the conventional test, whereas single samples were taken for the Heliprobe test. A detailed discussion has appeared previously of the relative merits of multiple as opposed to single samples for the UBT [11]. In accordance with our findings showing high concordance between results using either the conventional or Heliprobe UBTs, we consider one sample at a single time interval after administration of the labelled urea to be acceptable for most non-research purposes in the clinic. Using the ^{14}C-UBT, even a baseline sample seems unnecessary, as there should be no detectable ^{14}C in breath under basal conditions. Furthermore, recommendations have been given to roll the patient over and on the sides in an attempt to get the tracer distributed

evenly over the gastric lining. There is, however, no evidence that moving about should increase the sensitivity or specificity of the test, therefore this recommendation should be abandoned.

The cut-off for *H. pylori* positivity was chosen to not overlook any cases of true *H. pylori* infection. Rather, false positives were considered acceptable, as this would lead only to another antibiotic treatment in a few cases with suspected infection. This approach to the diagnostic performance of the UBT would not leave any patient without treatment for a potentially ulcerogenic infection.

With the introduction of the Heliprobe system, broader applications of the UBT are at hand without compromising accuracy, as is the case for serology-based tests. With the portability of the equipment, it may well be used for epidemiological studies, especially in elderly people in whom serology tests may not be reliable. The UBT also has the advantage over serology in testing the current infection status, as it is known that serology for *H. pylori* may remain positive for several years in a significant percentage of patients whose infections have been eradicated [17]. In addition, with our current evaluation of the Heliprobe system, we have the possibility of not only detecting *H. pylori* in antibiotic-naive subjects but also of retesting in post-treatment patients followed up at least 1 month after termination of anti-*H. pylori* treatment.

We conclude that the Heliprobe system is a rapid and reliable UBT for pre- and post-treatment follow-up detection of *H. pylori*. The Heliprobe system offers the first on-site fully accurate diagnostic system for detection of *H. pylori* directly in the doctor's surgery within minutes after oral intake of the urea tracer.

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References

1 Graham DY, Klein PD, Evans DJJ, Evans DG, Alpert LC, Opekun AR, *et al*. *Campylobacter pylori* detected noninvasively by the 13C-urea breath test. *Lancet* 1987; **1**:1174–1177.
2 Langenberg ML, Tytgat GN, Schnipper MEI. *Campylobacter*-like organisms in the stomach of patients and healthy individuals. *Lancet* 1984; **1**:1348.
3 Marshall BJ, Langton SR. Urea hydrolysis in patients with *Campylobacter pyloridis* infection. *Lancet* 1986; **1**:965–966.
4 Rehnberg AS, Bengtsson C, Befrits R, Granström M, Hellström PM. Refinement of the ^{14}C-urea breath test for detection of *Helicobacter pylori*. *Scand J Gastroenterol* 2001; **36**:822–6.
5 Lee A. The nature of *Helicobacter pylori*. *Scand J Gastroenterol* 1996; **214**(Suppl):5–8.
6 Atherton JC. Non-endoscopic tests in the diagnosis of *Helicobacter pylori* infection. *Aliment Pharmacol Ther* 1997; **11**(Suppl 1):11–20.
7 Logan RP, Polson RJ, Misiewicz JJ, Rao G, Karim NQ, Newell D, *et al*. Simplified single sample 13Carbon urea breath test for *Helicobacter pylori*: comparison with histology, culture, and ELISA serology. *Gut* 1991; **32**:1461–1464.
8 Braden B, Duan LP, Caspary WF, Lembcke B. More convenient 13C-urea breath test modifications still meet the criteria for valid diagnosis of Helicobacter pylori infection. *Z Gastroenterol* 1994; **32**:198–202.
9 Rollàn A, Giancaspero R, Arrese M, Figueroa C, Vollrath V, Schultz M, *et al*. Accuracy of invasive and noninvasive tests to diagnose *Helicobacter pylori* infection after antibiotic treatment. *Am J Gastroenterol* 1997; **92**:1268–1274.
10 Mowat C, Murray L, Hilditch TE, Kelman A, Oien K, McColl KE. Comparison of helisal rapid blood test and 14C-urea breath test in determining *Helicobacter pylori* status and predicting ulcer disease in dyspeptic patients. *Am J Gastroenterol* 1998; **93**:20–25.
11 Bell GD, Weil J. Detection of *Helicobacter pylori* by the ^{14}C-urea breath test. In: Rathbone BJ, Heatley RV, editors. Helicobacter pylori *and Gastroduodenal Disease*. Oxford: Blackwell Scientific Publications; 1992. pp. 74–87.
12 Hamlet A, Stage L, Lönroth H, Cahlin C, Nyström C, Pettersson A. A novel tablet-based 13C urea breath test for *Helicobacter pylori* with enhanced performance during acid suppression. *Scand J Gastroenterol* 1999; **34**:367–374.
13 Munster DJ, Chapman BA, Burt MJ, Dobbs BR, Allardyce RA, Bagshaw PF, *et al*. The fate of ingested 14C-urea in the urea breath test for *Helicobacter pylori* infection. *Scand J Gastroenterol* 1993; **28**:661–666.
14 Stubbs JB, Marshall BJ. Radiation dose estimates for the carbon-14-labeled urea breath test. *J Nucl Med* 1993; **34**:821–825.
15 Goddard AF, Logan RP. Urea breath tests for detecting *Helicobacter pylori*. *Aliment Pharmacol Ther* 1997; **11**:641–649.
16 Leide-Svegborn S, Stenström K, Olofsson M, Mattsson S, Nilsson LE, Nosslin B, *et al*. Biokinetics and radiation doses for carbon-14 urea in adults and children undergoing *Helicobacter pylori* breath test. *Eur J Nucl Med* 1999; **26**:573–580.
17 Logan RPH. Detection of *Helicobacter pylori* by the ^{13}C-urea breath test. In: Rathbone BJ, Heatley RV, editors. Helicobacter pylori *and gastroduodenal disease*. Oxford: Blackwell Scientific Publications; 1992. pp. 88–106.