

ADVANTAGES WITH THE HELIPROBE SYSTEM

The use of ^{14}C -urea breath to detect *Helicobacter pylori* infection is common, but the products used have been developed within the academic world and have not been registered for such use within Europe. There are two ^{13}C -urea breath tests registered within the EU (Pylobactell; *Helicobacter* Test INFAI), but this is the first ^{14}C -urea product. A ^{14}C -urea breath test, called PYTest is marketed in the USA.

The HeliProbe (Urea Breath Test) is a device that quickly, inexpensively and accurately detects whether a patient has *Helicobacter pylori* in his/her gastrointestinal tract. The HeliProbe System is composed of the HeliProbe™ Analyzer a BreathCard™ (credit card size breath collector) and a HeliCap™ (^{14}C Urea Capsule).

The BreathCard™, the shape and size of a credit card, contains two pads soaked in lithium hydroxide. When the patient breathes into the mouthpiece, both pads are exposed to the exhaled air. Exhaled carbon dioxide to form lithium carbonate, fixing the carbon atoms in the pads. A pH sensitive indicator mounted in the card changes colour from orange to yellow to indicate when the pads are saturated with carbon dioxide. Breathing time required varies depending on frequencies of breaths in the card, but averages 1-2 minutes. Once used, the BreathCard is stable and sturdy; it can be shipped to another location for reading, stored, or disposed of without special precautions due to the low radioactivity involved.

The HeliProbe™ analysed contains two shielded Geiger-Müller counters mounted in parallel face-to-face. An opening in the shield allows insertion of the BreathCard between the two counters. When the BreathCard is fully inserted the pads are perfectly lined up with the two counters. Correct position of the BreathCard is verified with an optical sensor and the analysis sequence can only be initiated if the card is properly inserted.

Microprocessor controlled electronics in the analyzer steer the measurements cycle, keeping continuous track of and compensating for variations in background radiation not blocked by the shielding around the Geiger-Müller counters; comparing the result with programmed cut-off levels for determination of disease; and calculating and presenting the result on an LCD display. A set-up interface allows the user to customize the most important performance parameters such as the cut-off level for determination of disease. A thermal printer can be connected to the HeliProbe Analyzer to print the result.

The HeliCap™ is a hard capsule containing 1 μCi ^{14}C -labeled urea and citric acid. The capsule is swallowed intact and its content is released in the stomach thereby assuring that urea will not be exposed to mouth bacteria which poses a risk for false positive results. The purpose of the citric acid is to add acidity in the stomach which firstly eliminates the need for a test meal, and secondly increases the metabolic process where CO_2 is produced thereby the accuracy of the test.

The test procedure is simple: 10 minutes after swallowing the HeliCap, the patient exhales through the BreathCard until it is fully saturated. The BreathCard is inserted into the analysis unit and a one-button operation starts the analysis. The result is presented 250 seconds later as "infected", "not infected", or "borderline", together with a quantitative assessment of the degree of infection.

Other ^{14}C -urea and ^{13}C -urea breath test analysis units are expensive and difficult to run. The urea is taken in liquid form increasing the risk for false positive results if the mouth hygiene is compromised. A test meal or a separate citric acid drink must be administered with the urea. Test sampling and analysis requires laboratory expertise. These properties make the other urea breath tests less suited for small clinics, unless the doctor is prepared to send samples in the mail, wait several days for the test result and recall the patient for a second consultation.

The most important advantages with the HeliProbe system are speed and ease of use. Without external facilities or expertise, the doctor has access to the test result 15-20 minutes after the patient has swallowed the ^{14}C -urea capsule, eliminating the need for a second consultation. Furthermore, the cost of the HeliProbe unit is a fraction of that of a traditional breath test analysis unit. Eliminating the need for a large up-front investment, together with the time the hospital staff saves on the simplified test and analysis procedure, makes this system highly cost effective compared to traditional urea breath tests. The HeliProbe system will make it easier for smaller gastroenter-ological clinics and for general practitioners to adopt the highly accurate urea breath test when diagnosing *H pylori*. The system combines the convenience of serological near-patient test systems with the accuracy of the urea breath test.

NO RADIATION PROTECTION REASONS FOR RESTRICTIONS ON ^{14}C UREA BREATH TESTS IN CHILDREN

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Abstract

Traditional ^{14}C urea breath tests are normally not used for younger children because the radiation exposure is unknown. High sensitivity accelerator mass spectrometry and an ultra-low amount (440 Bq) of ^{14}C urea were therefore used both to diagnose *Helicobacter pylori* (HP) infection in seven children, age 3-6 years, and to make radiation dose estimates. The activity used was 125 times lower than the amount normally used for older children and 250 times lower than that used for adults. Results were compared with previously reported biokinetic and dosimetric data for adults and older children age 7-14 years. ^{14}C activity concentrations in urine and exhaled air per unit administered activity for younger children (3-6 years) correspond well with those for older children (7-14 years). For a child aged 3-6 years who is HP negative, the urinary bladder wall receives the highest absorbed dose, 0.3 mGy MBq⁻¹. The effective dose is 0.1 mSv MBq⁻¹ for the 3-year old child and 0.07 mSv MBq⁻¹ for the 6-year-old child. For two children, the 10 min and 20 min post- ^{14}C administration samples of exhaled air showed a significantly higher amount of ^{14}C activity than for the rest of the children, that is 6% and 19% of administered activity exhaled per hour compared with 0.3-0.9% (mean 0.5%) of administered activity exhaled per hour indicating that these two children that is were HP positive. For a 3-year-old HP positive child, absorbed dose to the urinary bladder wall was 0.3 mGy MBq⁻¹ and effective dose per unit of administered activity was 0.4 mSv MBq⁻¹. Using 55 kBq, which is a normal amount for older children when liquid scintillation counters are used for measurement, the effective dose will be approximately 6 μSv to a 3-year-old HP negative child and 20 μSv to a HP positive child. Thus there is no reason for restrictions on performing a normal ^{14}C urea breath test, even on young children.

The ^{14}C urea breath test is widely used for detecting *Helicobacter pylori* (HP) infection [1-3] in the stomach. Since it is non-invasive, cheap and easy to perform with standard liquid scintillation counters (LSCs), the test has become very popular. However, owing to the long physical half-life of ^{14}C (5730 years) and uncertainties in the biokinetics, there are generally restrictions on performing the ^{14}C urea breath test on small children and other sensitive groups, such as pregnant or breast-feeding women. We have previously shown that, for older children aged 7-14 years, dose values per unit of administered activity are similar to those for adults [4].

To detect HP infection and to study the biokinetics of ^{14}C urea in younger children, aged 3-6 years, we have used accelerator mass spectrometry (AMS) [5,6] for the breath test instead of LSCs. Use of AMS makes it possible to reduce administered activity to less than 1% of the activity used in connection with measurements with the LSC technique. As an alternative to the ^{14}C urea test, non-radioactive ^{13}C urea has been promoted [7], mainly on the basis of radiation safety aspects. The use of ^{13}C presupposes access to a mass spectrometer, normally not available in a hospital. Moreover, the signal/background ratio for the ^{13}C method is considerably lower than for the ^{14}C method. Thus the ^{14}C alternative is better, both from an analytical and economic point of view. Therefore in case of ^{14}C use, the question of radiation exposure to the patient is critical.

The aims of the present study were two-fold. First, to use the AMS technique and an ultra-low amount of ^{14}C urea to diagnose HP infection, and second, to determine whether there are significant differences between the biokinetics and dosimetry for small children, aged 3-6 years, compared with the previously studied group of adults and children aged 7-14 years.

Material and Methods

Subjects

Seven paediatric patients aged 3-6 years were referred to the Department of Nuclear Medicine at Malmö University Hospital for a ^{14}C urea breath test. As no reliable radiation dose estimates were available for this age group, the investigation was carried out with an ultra-low amount of ^{14}C urea and the exhaled air was analysed with a high sensitivity AMS technique. Following overnight fasting, patients were given 440 Bq ^{14}C urea (Code CFA 41; Amersham Pharmacia Biotech, Uppsala, Sweden) orally in 125 ml water containing 200 mg of non-labeled urea. To reduce possible contamination from urease-producing bacteria in the mouth, subjects brushed their teeth and rinsed their mouths with some help from parents / medical staff before administration. Thus study was approved by the Ethics Committee at Lund University and the Regional Radiation Protection Committee.

Samples of exhaled air

Samples of exhaled air were taken prior to and 10 min, 20 min, 24 h and 120 h after administration of ^{14}C urea. Results from the 10 min and 20 min measurements were used clinically to evaluate whether the patient was HP positive or not. All breath samples were collected in glass vials, containing 1.25 g sodium hydroxide, on a solid support (Ascarite; Thomas Scientific, Swedesboro, NJ). The sample preparation and AMS procedure used at the AMS facility in the Pelletron laboratory in Lund have been described in detail earlier [8].

The amount of ^{14}C exhaled was determined assuming a basal endogenous carbon dioxide (CO_2) production of 20 mmol per kg body weight per hour [9, 10]. The amount of ^{14}C exhaled per hour and unit of administered activity was plotted as a function of time after administration of ^{14}C urea for all patients. A 20 min sample with a normalized ^{14}C activity > 2.2% of administered activity exhaled per hour was considered to indicate that the patient was HP positive. For comparison, results from eight older children from earlier studies [4] were also used. As there was an interest in limiting the number of samples for younger children, no samples were taken in the period between 20 min and 24 h after ^{14}C urea administration. In the analysis the activity as a function of time was compared for small children, with the more complete time-activity curves obtained for older children. Multi-exponential functions were iteratively fitted to each curve using a non-linear least squares regression algorithm. Finally, these curves were analytically integrated to yield the total fraction excreted via exhaled air.

Samples of urine

Urine samples were collected prior to approximately 30 min after and 24 h and 120 h after administration of ^{14}C urea. Urine was collected in plastic bottles and stored at -180°C before analysis with both AMS and LSC techniques. Before the urine was analysed with AMS, the CO_2 was extracted and converted into graphite [11, 12]. For LSC measurements, 1 ml urine was added to 18 ml scintillation liquid (Optiphase Hisafe; Wallac Oy, Turku, Finland) and duplicate samples were measured for 30 min in a LSC (1414 Guardian; Wallac Oy, Turku, Finland). The 24 h post-administration urinary excretion was calculated using a normalized urinary excretion rate of 25ml per kg body weight per day [9].

Kinetic and dosimetric models

The biokinetic model used for dosimetric calculations consists of two parts, a urea model and a CO_2 / bicarbonate model [13]. These models have previously been described in detail [4]. Most administered ^{14}C urea is excreted through the kidneys, most likely as intact ^{14}C urea. The residence times in the kidneys and urinary bladder were calculated according to the International Commission on Radiological Protection (ICRP) [14], with bladder voiding intervals taken from ICRP Publication 56 [15]. A minor quantity of the administered ^{14}C urea is broken down to ammonia and CO_2 and was treated according to the ICRP CO_2 / bicarbonate model [13]. Input parameters in the CO_2 / bicarbonate model, which differ between younger and older children, are bone turnover rate and relationship between the fraction of cortical and trabecular bone in the skeleton. Bone turnover rates were taken from ICRP Publication 70 [16]. 60% of the bone mass was assumed to be cortical bone and 40% trabecular bone for 3-6 year old children [16].

Voiding time used in the calculation of cumulated activity in the urinary bladder also differs between younger and older children [14].

In domestic model, source organs were the stomach, urinary bladder, cortical bone, trabecular bone and remaining tissues. Residence times obtained from the compartment model were used to estimate absorbed doses with the Medical Internal Radiation Dose (MIRD) technique using the MIRDose 3.1 software package (Oak Ridge Associate Universities, Oak Ridge, TN) [17]. Organ doses and effective dose were calculated according to ICRP Publications 60 and 67 [18, 19]. Residence times, absorbed dose and effective dose were calculated for a 3 year-old HP negative child (body weight approximately 15 kg) and for a 6-year-old HP negative child (body weight approximately 20kg) using biological half-time and fractions obtained for older children [4].

Results and Discussion

^{14}C activity in the samples of exhaled air taken 20 min after administration of ^{14}C urea are given in Figure 1. This shows that two patients exhaled significantly more ^{14}C than the others, and these two were considered to be HP positive. The rest of the younger children were considered HP negative. All the older children were considered HP negative according to a standard breath test performed with 55 kBq ^{14}C and liquid scintillation counting [4]. The ^{14}C concentrations in urine and exhaled air, normalized to the activity administered to the patient, are shown in Figures 2 and 3. AMS results show that for younger children, no ^{14}C could be detected in exhaled air taken 5 days post administration. This agrees well with results for older children. This was also the case for the ^{14}C urea found in urine and measured with the LSC. However, for three of the younger children, small amounts of ^{14}C above normal background level of 0.258 ± 0.008 (standard deviation) Bq g $^{-1}$ carbon could still be detected in the urine with AMS 5 days post administration (0.041, 0.066 and 0.083 Bq g $^{-1}$ C). Values for younger children are almost the same as those for older children. Therefore, we consider it reasonable to use biological half-times and fractions obtained for older children when calculating absorbed doses to younger children. For a HP positive 3-year-old child, absorbed doses were estimated assuming the fraction excreted in exhaled air to be 65% [13].

The urinary bladder wall received the highest absorbed dose, 0.3mGy MBq $^{-1}$ for both a 3-year-old and 6-year-old HP negative child (Table 1). For the 3-year-old HP positive child, absorbed dose to the urinary bladder wall was also 0.3 mGy MBq $^{-1}$. Effective dose was 0.1mSv MBq $^{-1}$ for a 3-year-old HP negative child and 0.07 mSv MBq $^{-1}$ for a 6-year-old HP negative child. For the 3-year-old HP positive child, effective dose was 0.4mSv MBq $^{-1}$. In addition to the uncertainties in the assumption that biokinetic data are the same for younger children as for older children, there are uncertainties in the determination of the fraction of ^{14}C excreted in urine and via exhaled air owing to uncertainties in estimating 24-h urinary production rate and endogenous CO_2 production. This has previously been discussed by Leide-Svegborn et al [4]. Absorbed dose to the urinary bladder wall is dependent on urinary bladder volume and voiding time. The absorbed dose given in Table 1 is based on a voiding period depending on the age of the child, e.g. 2.0 h for a 3-year-old child. Absorbed dose and effective dose are higher for a HP positive patient than for a HP negative patient owing to the fact that there is a larger fraction entering the

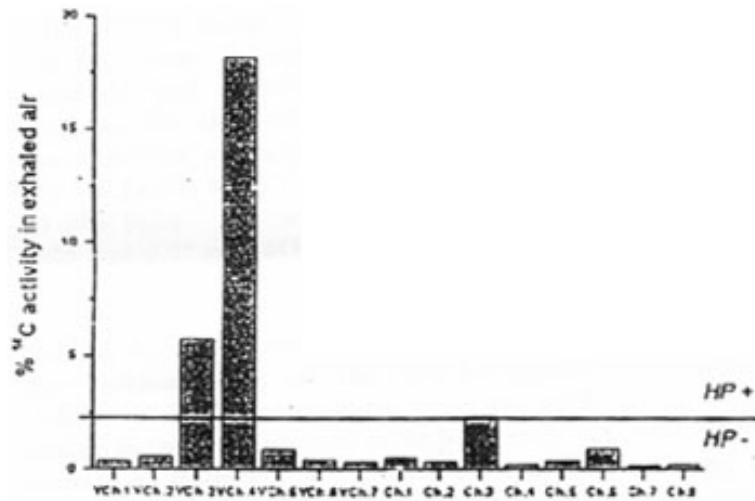


Figure 1. ^{14}C activity in exhaled air, given as percentage of administered ^{14}C urea activity per hour, in samples taken 20 min after administration and measured with accelerator mass spectrometry. A normalized ^{14}C activity $>2.2\%$ of administered activity per hour indicates that the patient is *Helicobacter pylori* (HP) positive. YCh, younger children (3-6 years); Ch, older children (>7 years).

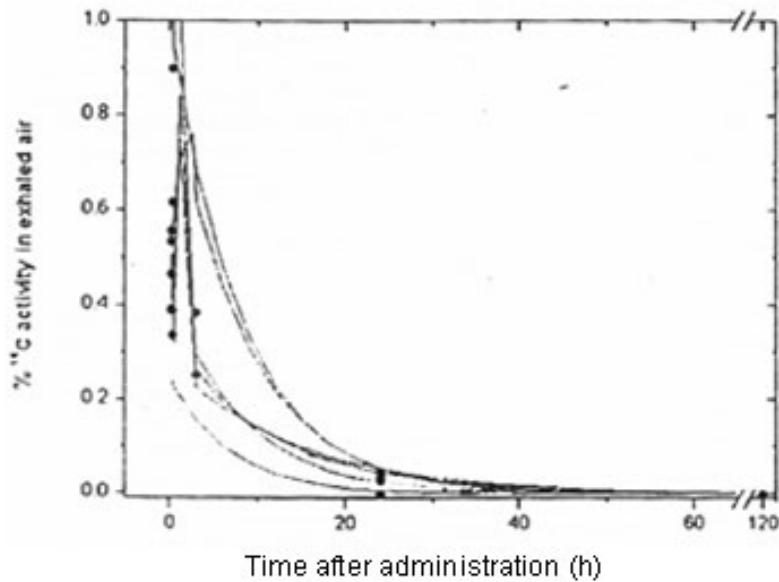


Figure 2. Fractional excretion of ^{14}C in exhaled air as a function of time after administration of ^{14}C urea. Lines correspond to the curves fitted to the data for older children (>7 years) and symbols to those of younger children (3-6 years).

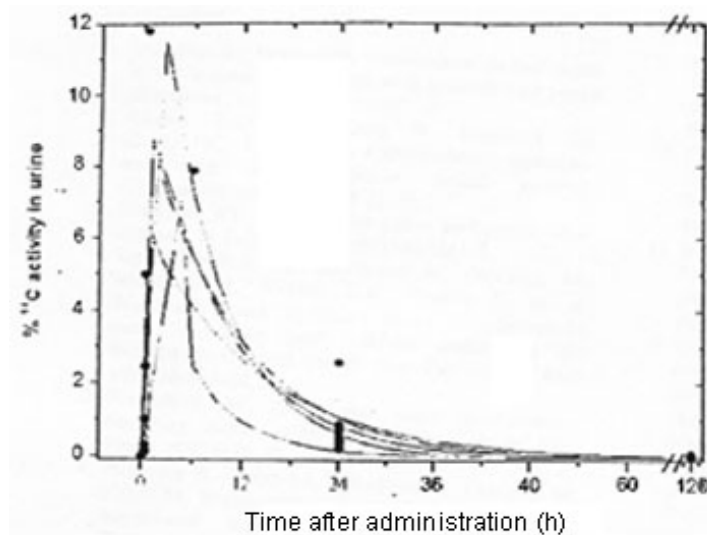


Figure 3. Fractional excretion of ^{14}C in urine as a function of time after administration of ^{14}C urea. Lines correspond to the curves fitted to the data for older children (>7 years) and symbols to those of younger children (3-6 years).

CO₂/bicarbonate pool in the first case. Accordingly, remaining tissues get a higher cumulated activity and absorbed dose will be higher for all organs.

Table 1. Mean absorbed doses (mGy MBq⁻¹) to various organs and tissues and effective dose (mSv MBq⁻¹) from ¹⁴C urea to *Helicobacter pylori* (HP) negative children aged 3 years and 6 years and HP positive children aged 3 years.

	HP negative		HP positive
	3-year-olds (~15 kg)	6-year-olds (~20kg)	3-year-olds (~15 kg)
Mean absorbed dose			
Urinary bladder	0.34	0.26	0.32
Stomach	0.08	0.05	0.24
Bone surfaces	0.09	0.07	0.45
Other organs ^a	0.09	0.07	0.38
Effective dose	0.10	0.07	0.38

Other organs include adrenals, brain, breasts, gall bladder, small intestine, colon, heart, kidneys, liver, lungs, muscles, oesophagus, ovaries, pancreas, red marrow, skin, spleen, testes, thymus, thyroid and uterus.

Conclusion

The highly sensitive AMS technique has provided the possibility of carrying out ¹⁴C urea breath tests on young children, investigations that were not previously considered acceptable, and has also enabled investigation of the biokinetics and dosimetry of ¹⁴C in these children. This study has shown that radiation exposure to children is low. Using 55 kBq ¹⁴C urea, which is a usual amount for older children when measurements of ¹⁴CO₂ are performed with liquid scintillation counting, the effective dose to a 3-year-old HP positive child will be approximately 20 μSv, and 6 μSv in the case of a HP negative child, which is of the same magnitude as a few days of natural background radiation. Thus there are no radiation protection reasons for restrictions on performing a normal ¹⁴C urea breath test on young, 3-6-year-old children.

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References

1. Marshal BJ, Warren JR. Unidentified curved bacilli in the stomach of patients with gastritis and peptic ulceration, *Lancet* 1984;1:1311-5
2. Rauws EAJ, Langenberg W, Houthoff HJ, Zanen HC, Tytgat GNJ. *Campylobacter pyloridis*-associated chronic active antral gastritis. *Gastroenterology* 1988;94:33-40.
3. Peterson WL. *Helicobacter pylori* and peptic ulcer disease. *N Engl J Med* 1991;324:1043-8.
4. Leide-Svegborn S, Stenström K, Olofsson M, Mattsson S, Nilsson L-E, Nosslin B, et al. Biokinetics and radiation doses for carbon-14 urea in adults and children undergoing the *Helicobacter pylori* breath test. *Eur J Nucl Med* 1999;26:573-80.
5. Kutschera W. Accelerator mass spectrometry: counting atoms rather than decays. *Nucl Phys News* 1993;3:15-21.
6. Hellborg R, Curtis LJ, Erlandsson B, Faarinen M, Kiisk M, Magnusson C-E, et al. Development of accelerator mass spectrometry at the Lund Pelletron. *Physica Scripta* 2000;61:530.
7. Graham DY, Klien PD, Evans DJ Jr, Evans DG, Alpert LC, Opekun AR, et al. *Campylobacter pylori* detected noninvasively by the ¹³C urea breath test. *Lancet* 1987;1:1174-7.
8. Stenström K, Leide-Svegborn S, Erlandsson B, Hellborg R, Skog G, Mattsson S, et al. A programmed for long-term retention studies of ¹⁴C-labelled compounds in man using Lund AMS facility. *Nucl Instr Meth* 1997;123:245-8.
9. Lentner C, editor. *Geigy Scientific Tables Vol. 1. Units of Measurement, Body Fluids, Composition of the Body, Nutrition* (8th edn.). Basle, Switzerland: CIBA-GEIGY Ltd., 1981:55-56.
10. Klien PD. Normalising results of ¹³C urea breath testing for CO₂ production rates in children. *J Pediatr Gastroenterol Nutr* 1999;29:297-301.
11. Persson J. Development of a sample preparation procedure for the production of elemental carbon from urine for AMS analysis. Results from long-term studies after a ¹⁴C urea breath test. Report 02/97, LUNFD6/(NFFR-5010)/1-40(1997).
12. Pau K. Preparation system for the production of elemental carbon from urine for AMS analysis. Master Thesis. Lund, Sweden: Lund University, 1999.
13. International Commission on Radiological Protection. Radiation dose to patients from radio-pharmaceuticals (Addendum to ICRP 53), ICRP Publication 80. *Annals of the ICRP* 1998;28(3).
14. International Commission on Radiological Protection. Radiation dose to patients from radio-Pharmaceuticals, ICRP Publication 53, *Annals of the ICRP* 1987;18 (1-4).
15. International Commission on Radiological Protection. Age-dependent doses to members of the public from intake of radionuclides: Part J, ICRP Publication 56. *Annals of the ICRP* 1992;20(2).
16. International Commission on Radiological Protection. Basic anatomical and physiological data for use in radiological protection: the skeleton, ICRP Publication 70. *Annals of the ICRP* 1995;25(2).

17. Stabin MG. MIRDOSE: personal computer software for internal dose assessment in nuclear medicine. *J Nucl Med* 1996;37:538-46.
18. International Commission on Radiological Protection. 1990 Recommendations of the International Commission on Radiological Protection, ICRP Publication 60. *Annals of the ICRP* 1991;21(1-3).
19. International Commission on Radiological Protection. Age-dependent doses to members of the public from intake of radionuclides: Part 2 Ingestion dose coefficients, ICRP Publication 67. *Annals of the ICRP* 1994;23(3/4).

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 (Helicobacter™) 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 $^{14}\text{CO}_2$ in breath was trapped in benzethoniumhydroxide/ethanol, or absorbed to LiOH-soaked pads on a dry cover surface (Heliprobe BreathCard™). The amount of absorbed ^{14}C was detected using a β -scintillator or two Geiger-Müller counters operating in parallel (Heliprobe™ 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 Lipponcott 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 other's 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 -tapping 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 quick in-office analysis, we have developed a simplified ^{14}C -UBT system (Heliprobe™, Noster System AB, Stockholm, Sweden). The objective of the present 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 Specialties 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 Breath-Card™ and the Heliprobe Analyzer™ (Fig.1). The Heliprobe BreathCard is a flat, credit-card-sized collection vehicle that absorbs exhaled CO_2 via chemical bonding to pads soaked in LiOH . The collection process is simple; the patient breathes into a mouth-piece 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.

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 analyzer 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, 10ml, scintillation liquid Optiphase 'Hi Safe' (Wallac, Fison Chem., Loughborough, 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 be 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 diagnostic 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 $\mu\text{Ci}/\text{ml}$ 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- μ Ci encapsulated form of 14 C-urea (PYCap, Tri-Med Specialties 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 CO₂-1 kg, or a 20-min test value above 0.50% mmol CO₂-1 kg, was classified as positive. Patients with values equal to or below the above-mentioned values were classified as negative.

Analysis and statistics

Examination for pretreatment detection and post-treatment detection after eradication treatment were analyzed 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 \pm 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 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).

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

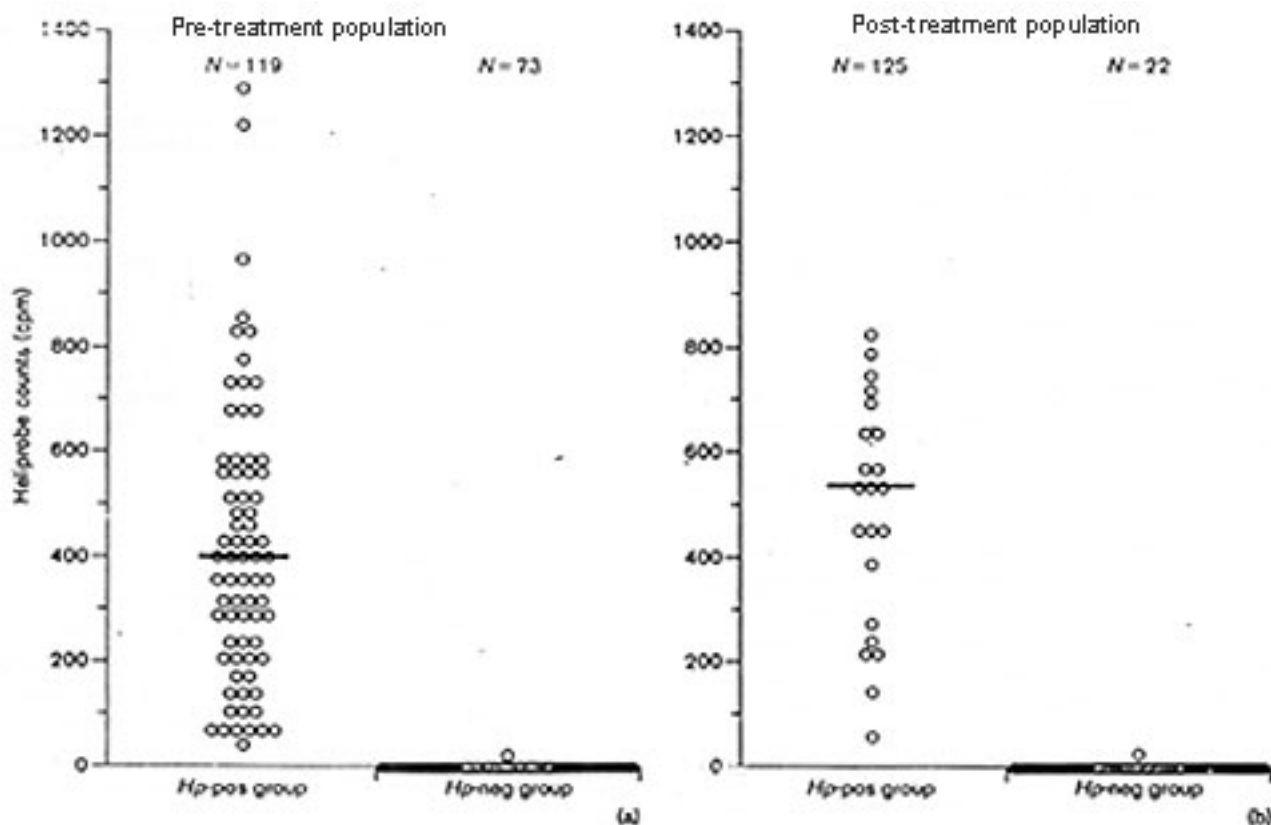
Patient characteristic	Patients for analysis		
	H. pylori-positive	H. pylori-negative	All
Number of subjects (N)	73	119	192
Male/Female (N/N)	46/27	46/73	93/99
Age (years; mean \pm SD)	52 \pm 17	46 \pm 16	48 \pm 16
Weight (kg; mean \pm SD)	72 \pm 14	70 \pm 14	71 \pm 14

Sensitivity and specificity of the Heliprobe system

Choosing a Heliprobe cut-off level in the gap between the highest H. pylori-negative (25cpm) 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).

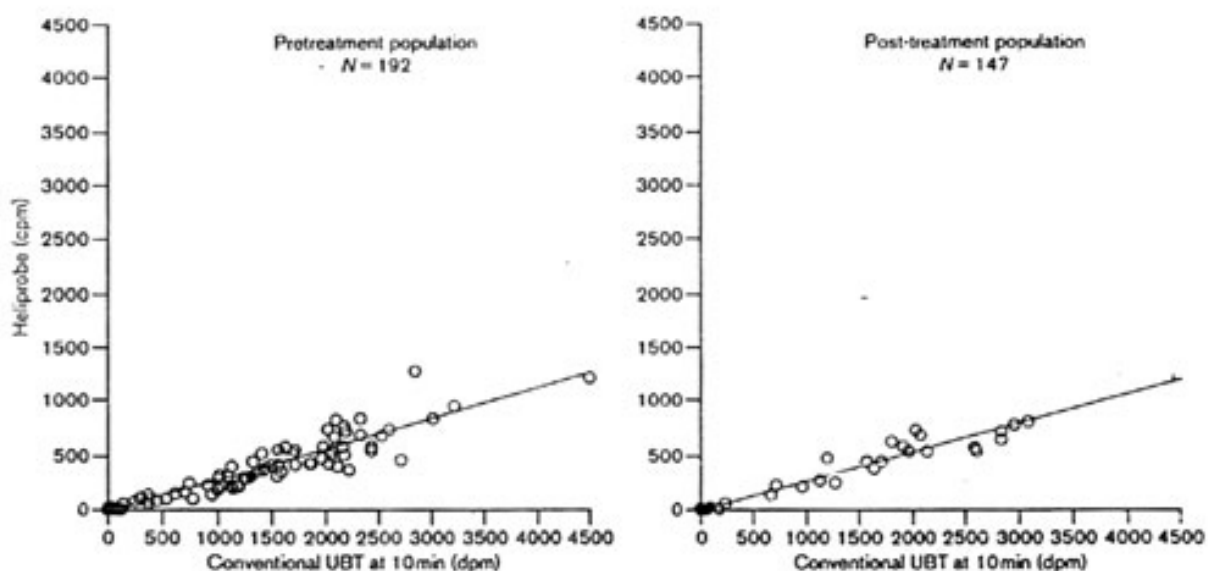


Spread of Heliprobe test results for the *Helicobacter pylori*-positive group (Hp-pos) and the *H. pylori*-negative group (Hp-neg), as categorized by the conventional ^{14}C urea breath test (UBT) method. The individual test results in counts per minute (cpm) are shown together with each group's media 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

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 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.



Scatter plot between the Heliprobe counts per minute (cpm) values (Heliprobe) and the conventional ¹⁴C 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*.

Patient characteristic	Per protocol		
	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)

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 cutoff 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 the 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 ¹⁴C-urea

The characteristics of 37 patients undergoing UBT with an encapsulated form of ¹⁴C-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

Patient category	Pre-treatment		Post-treatment	
	Per protocol	All patients	Per protocol	All patients
Maximum value of <i>H. pylori</i> -negative group (cpm)	25	25	25	25
Minimum value of <i>H. pylori</i> -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

Patient characteristic	Per protocol		
	<i>H. pylori</i> -positive	<i>H. pylori</i> -negative	All
Number of subjects (N)	11	26	37
Male/Female (N/N)	6/5	13/13	19/18
Age (years; mean \pm SD)	54 \pm 15	54 \pm 15	54 \pm 15
Weight (kg; mean \pm SD)	73 \pm 15	70 \pm 15	71 \pm 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 Breath Card also make handling very easy. In cases where mailing might be needed, a regular envelope can be used for mailing the test to the analyzer. Due to the stability of the chemical binding of CO₂ 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 ¹⁴C-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 CO₂-1 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 CO₂ 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 ¹⁴C and then detection of ¹⁴CO₂ by simultaneously using the conventional UBT and Heliprobe systems, we found a few restrictions that have to be taken on 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 H₂-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 H₂-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 radioactivity (1 μ Ci, 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- μ Ci ¹⁴C dose per test.

Concern with ¹⁴C usually arises because of its long half-life, but this is less important for organic compounds such as CO₂ and urea, which are excreted rapidly. In the ¹⁴C-UBT, urea either undergoes hydrolysis, being exhaled as ¹⁴CO₂, 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 ¹⁴C from a UBT is eliminated as CO₂ 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/ μ Ci [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 UA (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 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 labeled 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 be 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 antibiotics-naïve 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, Alpart LC, Opekun AP et al. Campylobacter pylori detected noninvasively by the ^{13}C -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; 1:12???
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, Granstrom 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* 19???: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 ^{13}C 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 ^{13}C -urea breath test modifications still meet the criteria for valid diagnosis of Helicobacter pylori infection. *Z Gastroenterol* 1994;32:198-202.
9. Rollan 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 helical rapid blood test and ^{14}C -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, Heatly RV, editors. *Helicobacter pylori and Gastrointestinal 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 ^{13}C -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 ^{14}C -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;34:821-825.
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, Heatly RV, editors. *Helicobacter pylori and gastrointestinal disease*. Oxford: Blackwell Scientific Publications; 1992. pp. 88-106.

A NEW, PRACTICAL, LOW-DOSE ¹⁴C-UREA BREATH TEST FOR THE DIAGNOSIS OF HELICOBACTER PYLORI INFECTION: CLINICAL VALIDATION AND COMPARISON WITH THE STANDARD METHOD

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Abstract

The carbon-14 urea breath test (UBT) is a reliable and non-invasive technique for the diagnosis of *Helicobacter pylori* (HP) infection. In this study we evaluated the diagnostic performance of a new, practical and low-dose ¹⁴C-UBT system for the diagnosis of HP and compared the results with those obtained using the standard method. Seventy-five patients (56 female, 19 male) with dyspepsia underwent ¹⁴C-UBT and endoscopy with antral biopsies for histological analysis. The rapid urease test (CLO test) was applied to 50 of these patients. After a 6-h fasting period, a 37-kBq ¹⁴C-urea capsule was swallowed for UBT. Breath samples were collected and counted using two different methods, the Heliprobe method and the standard method. In the Heliprobe method, patients exhaled into a special dry cartridge system (Heliprobe BreathCard) at 10 min. The activities of the cartridges were counted using a designated small GM counter system (Heliprobe analyzer). Results were expressed both as counts per minute (HCPM) and as grade (0, not infected; 1, equivocal; 2, infected) according to the counts. In the standard method, breath samples were collected by trapping in a liquid CO₂ absorber. Radioactivity was counted as disintegrations per minute (SDPM) using a liquid scintillation counter after addition of a liquid scintillation cocktail.

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Histological examination was used as a gold standard. Two patients were excluded from the study because of inadequate biopsy sampling. Forty-eight patients (65%) were found to be HP positive on histology. The Heliprobe method correctly classified 48 of 48 HP-positive patients and 19 of 25 HP-negative patients (sensitivity 100%, specificity 76%, PPV 88%, NPV 100%, accuracy 91%). The standard method correctly classified 48 of 48 HP-positive and 20 of 25 HP-negative patients (sensitivity 100%, specificity 80%, PPV 90%, NPV 100%, accuracy 93%). On the other hand, the CLO test identified 26 of 32 HP-positive and 12 of 16 HP-negative patients (sensitivity 81%, specificity 75%, PPV 86%, NPV 66%, accuracy 79%). With the Heliprobe method, all of the positive results were grade 2, and all of the negative results were grade 0. No patients were defined as having grade 1 results. Counts allowed clear discrimination of HP-positive and negative patients with both methods, the difference being statistically significant in each case ($P < 0.001$). A significant correlation was found between HCPM and SDPM ($r = 0.863$, $P < 0.001$). According to the ROC analysis, the area under the curve was nearly the same with HCPM (AUC, 0.888; 95% CI, 0.785-0.992) and SDPM (AUC, 0.898; 95% CI, 0.802-0.994). In conclusion, the new ¹⁴C-UBT system is a highly accurate method for the diagnosis of HP infection. It is rapid and practical, and therefore suitable for clinical and office practice.

Keywords: *Helicobacter pylori* Carbon -14 urea breath test- Peptic ulcer disease.

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Introduction

Helicobacter pylori (HP) is a spiral, gram-negative bacterium that has been found to be associated with gastritis, peptic ulcer disease, gastric adenocarcinoma and MALT lymphoma [1,2,3]. There is consequently increasing demand for treatment and a great need for simple and accurate methods for the diagnosis of HP infection.

Invasive diagnostic methods require mucosal biopsy during endoscopy, with the specimens being subjected to culture, rapid urease test, polymerase chain reaction or histological analysis. Non-invasive methods include antibody detection (serology), stool antigen test and urea breath test (UBT). While serology (ELISA) is simple and easy to perform, it is not a reliable test for the diagnosis of HP infection in elderly people or for determination of eradication of HP, since it remains positive for a long

period despite adequate treatment [4, 5].

The production of high amounts of urease by HP has been used in the development of UBTs. An oral dose of urea is rapidly broken down by HP in the gastric mucosa to ammonia and carbon dioxide. Labeled carbon dioxide derived from labeled urea can be detected in the breath as a marker of infection. UBTs with either carbon-13 or carbon-14 urea are non-invasive methods, sample the whole stomach and reflect the actual status of infection. Both tests are highly accurate, with reported sensitivities of 97-100% and specificities of 95-100% for both diagnosis and proof of eradication of HP infection after therapy [1, 2, 6, 7, 8, 9]. While the two isotopes seem to offer similar diagnostic accuracy, ¹³C-UBT has the inconvenience of requiring (a) more complex and expensive equipment on site or else analysis off-site by an external laboratory and (b) administration of a test meal and cold urea to the patient. These are not necessary with ¹⁴C, and the test is thus simpler, faster and cheaper.

The routine test protocol of ¹⁴C-UBT requires ingestion of ¹⁴C-urea, collection of breath samples at frequent intervals using a liquid CO₂, trapping medium, addition of a liquid scintillation cocktail and counting with a βscintillation counter. Several different methodological approaches have been suggested to simplify the UBT. Recently a new, practical dry breath collection cartridge (Heliprobe BreathCard) and counting system (Heliprobe analyzer) have been developed for this purpose.

In this prospective study we evaluated the diagnostic performance and accuracy of this new ¹⁴C-UBT system and compared the results with those of the rapid urease test (CLO test) and the standard (liquid CO₂ absorber and liquid scintillation counting) method.

Materials and Methods

Patients. Seventy-five patients (56 female, 19 male; mean age 41±14 years) with dyspepsia were included in the study. Informed consent was obtained from each patient. All patients underwent upper gastrointestinal endoscopy as well as ¹⁴C-UBT within 1 week. CLO test was applied to 50 of these patients.

Rapid urease test (CLO) test and histological examination. During upper gastrointestinal endoscopy, three biopsy specimens were taken from antral mucosa, one for CLO test and two for histological analysis. A home made kit was used for the CLO test. One biopsy specimen was placed in a test tube and the colour reaction was read after 6 h. Histology samples were fixed in formalin, embedded in paraffin, sectioned in routine fashion and stained with Giemsa.

¹⁴C-urea breath test. Antacids were stopped at least 24 h before the test, sucralfate and H₂ receptor antagonists were discontinued for 1 week before the test, and proton pump inhibitors, bismuth compounds and antibiotics were stopped for 1 month beforehand. After overnight fasting, patients swallowed 37 kBq (1 μCi) of an encapsulated form of ¹⁴C-urea/citric acid composition (Helicap, Noster System AB Stockholm, Sweden) with 25 ml water. Breath samples were collected and counted using two different methods.

1. Heliprobe method: Breath samples of patients were collected with a special dry cartridge system (Heliprobe BreathCard, Noster System AB Stockholm, Sweden) at 10 min. Patients exhaled gently into the cartridge mouthpiece until the indicator membrane changed colour from orange to yellow. The breath-card was inserted into a special small desktop Geiger-Müller counter (Heliprobe-analyzer, Noster System AB Stockholm, Sweden) and activity counted for 250 s. Results were expressed both as counts per minute (HCPM) and as grade (0: not infected, CPM <25; 1: equivocal, CPM 25-50; 2: infected, CPM >50), as suggested by the producer according to the counts obtained from the cartridges.

2. Standard method: After completion of the breath sample collection in method 1, patients were asked to blow through a drinking straw into a 20-ml glass scintillation vial containing 0.1 ml CO₂ absorber solution (Carba Sorb E, Packard) as well as a trace of the pH indicator thymolphthalein. Sampling was considered complete when the colour of the solution changed from blue to colourless. After addition of 10 ml of liquid scintillation cocktail (Pico-flour 40, Packard), radioactivity was counted for 10 min in a liquid scintillation counter (tri-carb 2500 TR, Packard). Counts were corrected to disintegrations per minute (SDPM) using an external standard method. The cut-off value was chosen as 100 DPM for the standard method in accordance with the results of our previous biopsy-controlled study.

Data analysis and statistics. Both ¹⁴C-UBT methods and the CLO test were validated against histological examination, and their sensitivity, specificity, positive/negative predictive values (PPV, NPV) and accuracy were determined.

The HCPM and SDPM counts of HP-positive and negative patients were compared using the Mann-Whitney U test. Spearman's correlation and ROC curve analysis were performed for comparison of HCPM and SDPM counts.

All statistical analyses, except for the diagnostic performance values, were performed with Systat (ver.10) statistical package (SPSS, Chicago, IL)

Table 1. Comparative results of histology, the CLO test, the Heliprobe method and the standard method

Histology	CLO test		Heliprobe UBT		Standard UBT	
	(+)	(-)	(+)	(-)	(+)	(-)
HP (+)	26	6	48	-	48	-
HP(-)	4	12	6	19	5	20

Table2. Diagnostic performance of the tests

	CLO test	Heliprobe UBT	Standard UBT
Sensitivity (%)	81	100	100
Specificity (%)	75	76	80
Positive Predictive value (%)	86	88	90
Negative predictive value (%)	66	100	100
Accuracy (%)	79	91	93

Table3. The HCPM and SDPM values of Helicobacter pylori positive and negative patients

	HP (+)	HP (-)
HCPM	269 (300, 69-770) ³	10 (72, 0-617)
SDPM	745 (1,104,220-3, 747)	34 (216, 4-1, 422)

3 Median (mean, minimummaximum)

Results

Two patients were excluded from the study because of inadequate biopsy sampling. On histology, 48 patients (65%) were found to be HP Positive, and 25 HP negative.

CLO test

Among the 48 patients evaluated with CLO test, 26 of 32 HP-positive (sensitivity 81% and PPV 86%) and 12 of 16 HPnegative patients (specificity 75% and NPV 66%) were correctly identified. Accuracy was 79% (Tables 1 and 2)

Heliprobe and standard methods

Table 3 shows the spread of the counts with both methods. Counts allowed clear discrimination of HP-positive and negative patients with both methods, the difference being statistically significant in each case ($P < 0.001$).

The Heliprobe method correctly classified 48 of 48 HP-positive patients and 19 of 25 HP-negative patients (sensitivity 100%, specificity 76%). The standard method correctly classified 48 of 48 HP-positive patients and 20 of 25 HP-negative patients (sensitivity 100%, specificity 80%) (Tables 1 and 2).

According to the grading with the Heliprobe method, all of the positive results were grade 2 (infected) and all of the negative results were grade 0 (not infected). No patients were defined as having grade 1 (equivocal) results.

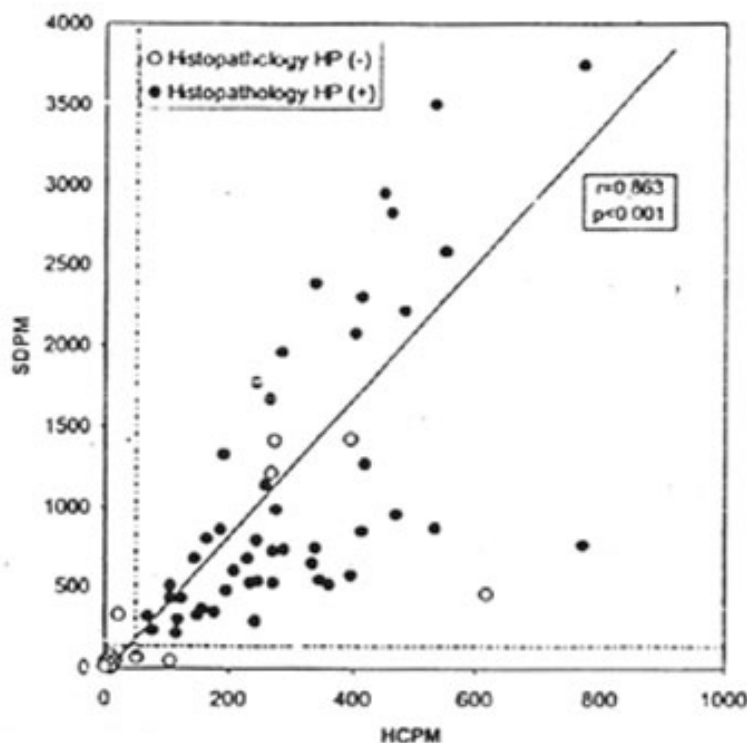


Fig. 1. Comparison of SDPM and HCPM

Heliprobe method versus standard method

A significant correlation was found between HCPM and SDPM counts (r 0.863, $P < 0.001$, Fig.1). According to the ROC analysis, the area under the curve was nearly the same with HCPM (AUC, 0.888; 95% CI, 0.785-0.992) and SDPM (AUC, 0.898; 95% CI, 0.802-0.994) (Fig.2).

Three HP-negative patients showed discordant results with the standard and Heliprobe methods. Two of them had positive test results only with the Heliprobe method (pt.6: HCPM 105, SDPM 52; pt.8: HCPM 52, SDPM 22), whereas one of them was positive only with the standard method (pt.4: SDPM 330, HCPM 21).

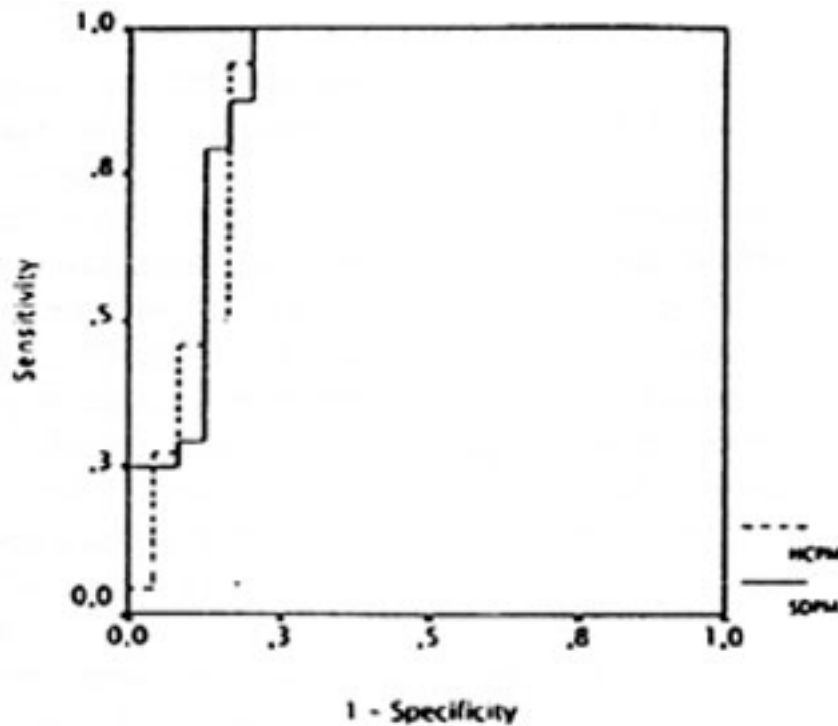


Fig. 2. ROC curve analysis of HCPM and SDPM

Discussion

This study shows that the new, practical Heliprobe ^{14}C -UBT system is highly accurate for the diagnosis of HP infection. Results obtained using the Heliprobe method are comparable to those using standard method [6, 7, 8], and a strong correlation between the methods was found in the present study.

Five patients with the standard method and six with the Heliprobe method had positive results despite negative histology. Since we validated our results against histology, these results were classified as false positive. But owing to the patchy distribution of HP in gastric mucosa, the biopsy-based tests may suffer from sampling error [10, 11]. Furthermore, histological examination is highly dependent on the experience of the pathologist, and high inter-observer variation has been reported [11, 12]. Thus it is likely that these patients were HP positive despite a negative histology. Sources of urease other than HP, such as bacterial overgrowth in the oropharynx, stomach or upper intestine, may rarely cause false-positive test results [6]. However, the reason for differences between the methods is not clear. Capsule dissolution may be slower in some patients, causing relatively lower radioactivity in early breath samples. If this is the case, Heliprobe breath samples may contain lower activity than standard samples, giving rise to a Heliprobe-negative, standard method-positive result.

The CLO test had low sensitivity and specificity in this study. Besides suffering from biopsy sampling error, the CLO test depends greatly on the pH of the media and the amount of the urea in the medium. These factors may vary in different products and thereby influence the results obtained with other tests [5, 10].

Various factors affect the results of the UBT. Several different methodological approaches have been suggested in order to simplify and increase the accuracy of the UBT. The differences concern doses and forms of ^{14}C -urea, patient preparation before the test, the time and number of breath samples, and modes of quantification. Our results showed that most of these steps can be omitted without prejudicing accuracy. The original ^{14}C -UBT system used relatively high activities (200-400 kBq) and multiple breath sampling. Later studies showed that the diagnostic accuracy of ^{14}C -UBT is maintained even with low doses and single breath samples [6, 7].

The UBT indirectly detects gastric HP by measuring urease activity. However, urease-producing bacteria are also present in the oropharynx and may cause false-positive results, especially in early breath samples. Late breath sampling may result in false-negative results because of emptying of urea from the stomach. Several procedures to avoid contamination of breath by the oropharyngeal flora have been suggested, including mouth washing, simultaneous meal to delay gastric emptying, and performance of multiple breath sampling. Another more simple and effective method is use of ^{14}C -urea in a gelatin capsule,

thus bypassing the oropharynx. Hamlet et al reported that when the ^{14}C -urea is supplied in a capsule, a single 10-min breath Sample is highly accurate (100% sensitivity and specificity) for the diagnosis of HP infection. They compared the capsule method with the urea drink method and found the former to be more reliable because no overlapping in activity occurred between HP-positive and negative patients; by contrast, conventional breath testing showed overlapping during the whole 30-min test period. Their study also showed that a fatty test meal lowers the $^{14}\text{CO}_2$ excretion during the first 20 min and may adversely affect the accuracy of a rapid UBT [8]. Other advantages of the capsule form include commercial availability, no risk of spills, shorter test duration and a lower radiation dose.

The expression of results of UBT varies between investigators. Henze et al. and Veldhuyzen van Zanten et al. have used CPM [14, 15]. Because CPM is affected by chemical or colour quenching, chemical changes of the cocktail and methods of sample preparation, Pathak et al. strongly suggested the use of DPM counts [16]. For these reasons we preferred to use DPM counts in the standard method.

Some authors have used formulas to correct for body weight or body surface to account for differences in endogenous CO_2 production, the results being expressed as recovery standard units [(% of administered dose recovered/mmol CO_2 trapped) \times body weight (kg)] [1,7]. However, neither of these factors has been proved to influence the results of the breath test. Indeed, it has even been reported that uncorrected counts result in better distinction between HP-positive and negative patients [8, 15, 16]. For this reason and to simplify the test, we omitted all such calculations. Both tests gave excellent results and a high correlation was found between DPM values of the standard method and CPM values of the Heliprobe method.

Adequate patient preparation is important if accurate results are to be obtained with ^{14}C -UBT. A large number of investigators have reported that the UBT becomes false negative during therapy with proton pump inhibitors. lansoprazole, bismuth compounds, antibiotics and ranitidine [17, 18, 19]. Preliminary reports indicated that addition of citric acid to the urea solution/capsule may diminish the negative effect of acid-inhibitory drugs on the accuracy of UBT [20]. Although we used an acidified ^{14}C -urea capsule, we preferred to discontinue medications before the test for a certain period of time. The exact value of acidified urea needs further verification.

The dry, practical and ready-to-use breath cartridge is an important advantage of this new system. Besides the simple and easy collection of breath, this system prevents accidental ingestion of hazardous organic CO_2 absorber solutions during breath sampling.

Carbon-13 is a no-radioactive isotope, but ^{13}C -UBT is more expensive because it requires mass spectrometry. ^{14}C has a physical half-life of about 5,000 years, raising the question of the risks of radiation exposure. Because nearly the entire ingested isotope is rapidly excreted in urine or breath over the following 72 h and only a small amount of isotope is used, the test actually entails low radiation exposure (3 μSv) [21, 22]. In fact, the dose is less than the natural background radiation in one day. As mentioned by Boivin et al., the debate on safety has revolved only around the radiation does received from ^{14}C -UBT, and it has been generally accepted that there is no or a lower risk with the ^{13}C alternative. On the other hand, ^{13}C -UBT contains more than 30,000 times as much urea as ^{14}C -UBT, and the safety of this amount of urea is also questionable [23]. For this reason, in 1997 the Nuclear Regulatory Commission permitted in vivo diagnostic use of capsules containing $1\mu\text{Ci}$ of ^{14}C -urea without a license [24]. Additional advantages of the Heliprobe system are the shorter test time and the low cost. Breath samples are analysed with a β -scintillation counter in ^{14}C -UBT and with a mass spectrometer in ^{13}C -UBT. Because both items of equipment are expensive, analysis can be done in an external laboratory by mail order and results are usually obtained a few days later. In contrast, with the Heliprobe system the results are obtained in half an hour on-site and the analyzer is much cheaper than either a β -scintillation counter or a mass spectrometer.

In conclusion, the new Heliprobe ^{14}C -UBT is a simple, rapid, practical, safe, cheap and highly accurate system for the diagnosis of HP infection. The main advantages of the system are commercial availability, no risk of spills, reduced interference by oropharyngeal flora, shorter test duration, low radiation dose, simple and safe breath collection, and a practical and cheap counting system.

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References:

- 1.Desroches JJ, Lahaie RG, Picard M, Morais J, Dumont A, Gaudreau C, Picard D, Chartrand R. Methodological validation and clinical usefulness of carbon-14-urea breath test for documentation of presence and eradication of Helicobacter pylori infection. J Nucl Med 1997; 38:1141-1145.
- 2.Reilly TG, Poxon V, Sanders DSA, Elliott TSJ, Walt RP. Comparison of serum, salivary and rapid whole blood diagnostic tests for Helicobacter pylori and their validation against endoscopy based tests. Gut 1997;40:454-458.
- 3.Sipponen P, Hyvarinen H. Role of Helicobacter pylori in the pathogenesis of gastritis, peptic ulcer and gastric cancer. Scand J Gastroenterol 1993;28 Suppl 196:3-6.
- 4.Newell DG, Hawtin PR, Stacey AR, MacDougall MH, Ruddle in an asymptomatic elderly population comparing [^{14}C] urea breath test and serology. J. Clin Pathol 1991;44:385-387.
- 5.Thijs JC, Van Zwet AA, Thijs WJ, Oey HB, Karrenbeld A, Stellaard F, Luijt DS, Meyer BC, Kleibeuker JH. Diagnostic tests for Helicobacter pylori: a prospective evaluation of their accuracy, without selecting a single test as the gold standard. Am J Gastroenterol 1996;91:2125-2129.
- 6.Peura DA, Pambianco DJ, Dye KR, Lind C, Frierson HF, Hoffman SR, Combs MJ, Guilfoyle E, Marshall BJ. Microdose ^{14}C -urea breath test offers diagnostic of Helicobacter pylori in 10 minutes. Am J Gastroenterol 1996;91:233-238.
- 7.Raju GS, Smith MJ, Morton D, Bardhan KD. Mini-dose (1-microCi) ^{14}C -urea breath test for the detection of Helicobacter pylori Am J Gastroenterol 1994;89:1027-1031.
- 8.Hamlet AK, Erlandsson KI, Olbe L, Svennerholm AM, Backman VE, Pettersson AB. A simple, rapid and highly reliable capsule-based ^{14}C -urea breath test for diagnosis of Helicobacter pylori infection. Scand J Gastroenterol 1995;30:1058-1063.
- 9.Ahuja V, Bal CS, Sharma MP. Can the C-14 urea breath test replace follow-up endoscopic biopsies in patients treated for Helicobacter pylori infection? Clin Nucl Med 1998;25:815-819.

10. Andersen LP, Kiilerick S, Pedersen G, Thoreson AC, Jorgensen F, Rath J, Larsen NE, Borup O, Krogfelt K, Scheibel J, Rune S. An analysis of seven different methods to diagnose *Helicobacter pylori* infections. *Scand J Gastroenterol* 1998;22:24-30.
11. Morris A, Ali MR, Brown P, Lane M, Patton K. *Campylobacter pylori* infection in biopsy specimens of gastric antrum: laboratory diagnosis and estimation of sampling error. *J Clin Pathol* 1989;42:727-732.
12. Christensen AH, Gjørup T, Hilden J, Fenger C, Henriksen B, Vyberg M, Ostergaard K, Hansen BH, Observer homogeneity in the histologic diagnosis of *Helicobacter pylori*: latent class analysis, kappa coefficient and repeat frequency. *Scand J Gastroenterol* 1992;27:933-939.
13. MacOni G, Vago L, Galletta G, Imbesi V, Sangaletti O, Parente F, Cucino C, Bonetto S, Porro GB. Is routine histological evaluation an accurate test for *Helicobacter pylori* infection? *Aliment Pharmacol Ther* 1999;13:327-331.
14. Henze E, Malferteiner P, Clausen M, Burkhardt H, Adam WE. Validation of a simplified carbon-14-urea breath test for routine use for detecting *Helicobacter pylori* noninvasively. *J Nucl Med* 1990;31:1940-1944.
15. Veldhuyzen van Zanten SJ, Tytgat KM, Hollingsworth J, Jalali S, Rshid FA, Bowen BM, Goldie J, Goodacre RL, Riddell RH, Hunt RH. ¹⁴C-urea breath test for the detection of *Helicobacter pylori*. *Am J Gastroenterol* 1990;85:399-403.
16. Pathak CM, Panigrahi D, Bhasin DK, Rana SV, Malik AK, Mehta SK. Advantage of use of DPM for ¹⁴C-urea breath test for the detection of *Helicobacter pylori*. *Am J Gastroenterol* 1992;87:1887-1888.
17. Laine L, Estrada R, Trujillo M, Knigge K, Fennerty MB. Effect of proton-pump inhibitor therapy on diagnostic testing for *Helicobacter pylori*. *Ann Intern Med* 1998; 129:547-550.
18. Bravo LE, Realpe JL, Campo C, Mera R, Correa P. Effects of acid suppression and bismuth medications on the performance of diagnostic tests for *Helicobacter pylori* infection. *Am J Gastroenterol* 1999; 94:2380-2383.
19. Chey WD, Woods M, Scheiman JM, Nostrant TT, DelValle J. Lansoprazole and ranitidine affect the accuracy of the ¹⁴C-urea breath test by a pH-dependent mechanism. *Am J Gastroenterol* 1997;92:446-450.
20. Chey WD, Chathadi KV, Montague J, et al. Intra-gastric acidification reduces occurrence of false-negative urea breath test results in patients taking a proton pump inhibitor. *Am J Gastroenterol* 2001;96:1028-1032.
21. Stubbs JB, Marshall B. Radiation dose estimates for the carbon-14-labeled urea breath test. *J Nucl Med* 1993; 34:821-825.
22. Leide-Svegborn S, Stenstrom K, Olofsson M, Mattsson S, Nilsson LE, Nosslin B, Pau K, Johansson L, Erlandsson B, Hellborg R, Skog G. Biokinetics and radiation doses for carbon-14 urea in adults and children undergoing the *Helicobacter pylori* breath test. *Eur J Nucl Med* 1999; 26:573-580.
23. Boivin C. ¹³C-urea versus ¹⁴C-urea breath test which is the safer? *Nucl Med Commun* 1999;20:978.
24. Nuclear Radioactive Committee, USA, 10 CFR §30.21. Radioactive drug: capsules containing carbon-14 urea for "in vivo" diagnostic use for humans.

EHSG 2003- European Helicobacter Study Group XVIth International Workshop, September 3-6, 2003, Stockholm, Sweden

Validation of a new portable near patient urea breath test; Heliprobe system W. A. de Boer¹, C. van Alfen¹, J. Ryden²;
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Background: Test & Treat is the evidence-based optimal strategy for the dyspepsia in primary care. According to the Maastricht guidelines urea breath tests (UBT) are the preferred non-invasive initial *Helicobacter* test. Usually breath samples need to be mailed to a central facility. We tested a new portable "near patient" ¹⁴C-UBT designed for primary care, thus obviating the need to refer the patient.

Methods: Between April 2000 - January 2002 endoscoped patients in whom biopsies were taken for *Helicobacter* were asked to return for UBT. They received 1 μ Ci (37kBq) of ¹⁴C-urea (Helicap capsule) with citric acid. After 10 minutes the patients exhaled into a breathcard. After saturation it was inserted into the Heliprobe machine. Results take 5 minutes. Infection status was based on number of detected ¹⁴C counts per measurement (d): infected if d \geq 50, not-infected if d \leq 25, and indeterminate for d-values in-between.

Results: 107 pts participated, 1 was excluded due to indeterminate result. In all pts 7 biopsies were taken (antrum: 2 histology, 1 culture, 1 CLO. Corpus: 2 histology, 1 CLO). Combined biopsy results served as gold standard. Prevalence was 39%. The Heliprobe System was easy to use and results were obtained within 20 minutes. Results: Sensitivity 95% (40/42) (95%CI 84-99), and specificity 100% (64/64) (95%CI 94-100). There were no adverse events.

Conclusion: The Heliprobe ¹⁴C-UBT system is a very reliable, easy to use, near patient *Helicobacter* test which can be used for test & treat in primary health care. It is extremely reliable in patients not taking acid suppressants.

HELICOBACTER PYLORI:

Diagnostics: a ^{14}C -urea breath test

Therapy: less than 7 days; a systematic overview.

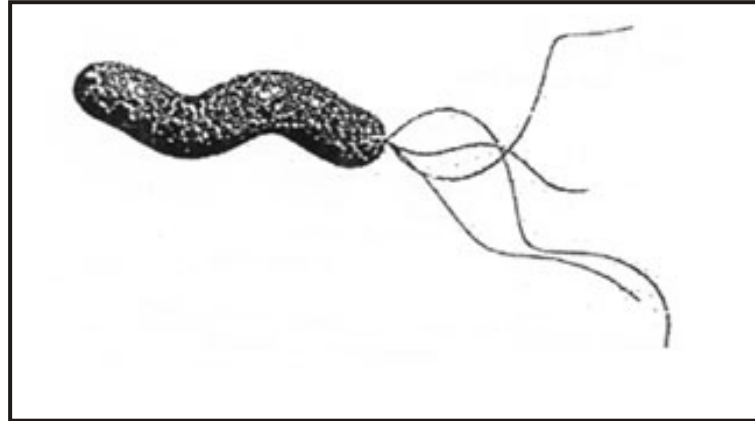
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Summary

The ^{14}C -urea breath test is a non-invasive method for the detection of H. pylori. In this research this test is compared with the biopsies taken during gastroscopy. A second group of patients is also subjected to the ^{14}C -urea breath test in addition to the ^{13}C -urea breath test, after informed consent. It appears from the results that the ^{14}C -urea breath test is a good non-invasive alternative test, which can be applied easily.

Two extra biopsies were taken from the antrum during a gastroscopy, in addition to the Golden standard. These two extra biopsies have been used for the GUT-test a new, fast urease test. The GUT-test is compared with the CLO-test, the most used urease test, and separately with the culture and the histology of the antrum.

The results from the GUT-test and the CLO-test are the same, with a sensitivity of 100% and a specificity of 100%. In comparison to the histology and the culture, the sensitivity and specificity are respectively 94.1% and 96.6%.

The difference is particularly related to the costs and the fact that the GUT-test already provides a reliable result after 60

minutes.

Presently the treatment of *Helicobacter pylori* consists of a combination of medicines: a PPI with two or three antibiotics, being prescribed for 7 days. The therapies achieve good results in clinical trials, but in practice it appears that the success percentage is lower. This has got to do with the compliance.

The object of this study is to determine the effect of a treatment duration of less than 7 days on the effectiveness of therapies for *H. pylori* eradication, by means of a systematic literature review.

It is shown from the results that a quadruple therapy already has sufficient effect within 2 to 4 days. A modern triple therapy only has a sufficient effect after 5 days. These results show that it is possible to stop at an earlier stage. However, this is dependent on which therapy a patient has obtained. A quadruple and a modern PPI triple therapy does make this possible but the old bismuth triple therapy has to be applied for at least 7 days.

Preface

When choosing a subject for my research training period I immediately thought of one of the subjects regarding *Helicobacter pylori*. I have always been interested in this bacterium. Previously I had given a presentation about it. Thus I considered this training period to connect well with my interests. I have chosen a combined training period, which means a part consisting of patient directed research and a part consisting of literature searching. It was my intention to look at both aspects of research.

The practice directed part I have accomplished in the Bernhoven hospital in Oss. I have asked patients if they were willing to voluntarily do a breath test to show the existence of the bacterium. Most patients are very curious as to what it is and how it works. Therefore most of them were willing to co-operate with the research. I have enjoyed doing the literature searching, although it is not always easy, because it takes a lot of time. But the results obtained make most of it very satisfying. I have enjoyed doing both training periods and have learned a lot from them. Furthermore I would like to express my gratitude to my supervisors for the support and time they have given me after the passing away of my mother.

Part 1: Validation of the Heliprobe and the GUT-test

Section 1: General Introduction

Helicobacter pylori is a Gram negative spirally bacterium that exclusively exists in the mucosa of the stomach, particularly in the antrum. But in addition to this it also exists in the corpus and fundus.^{1,2}

H. pylori causes gastritis in anyone who is infected by the bacterium. The prevalence of *H. pylori* in the entire population is 30-50% in the western world; in developing countries it is up to 90%. Eradication of *H. pylori* results in the cure of gastritis.³

5-20% of the persons with a *H. pylori*-infection get a peptic ulcer.

The correlation the other ways around between a duodenal ulcer and *H. pylori* is 95-100%. The correlation between an ventricular ulcer and *H. pylori* is 70-90%.³ This infection also gives an increased risk of a stomach carcinoma.⁴ The risk of getting a stomach carcinoma concerns persons with a *H. pylori*-associated gastritis and is approx. 6 times higher than for persons without *H. pylori* gastritis.

An infection with *H. pylori* can be shown in different ways. Invasive and non-invasive tests^{5,6} exist. All tests have advantages as well as disadvantages, which is why in effect a screening has never been set up for *Helicobacter pylori*.

The Golden standard for the diagnostics consists of: a gastroscopy with biopsies for a direct urease test (for example the CLO test), a biopsy for culture and biopsies for histology⁷. These biopsies are taken both from the antrum and the corpus. Thus this is an invasive test. The non-invasive tests are a ¹³C-urea breath test, a ¹⁴C-urea breath test, specific *H. pylori* serology or a faecal antigen test.^{8,9} The urea breath tests are considered presently as reference methods for the non-invasive diagnosis of *H. pylori* infection and undergoing the therapy.^{10,11} IgG-antibodies are shown during serology with the aid of ELISA. The sensitivity varies between 80 and 95%, the same applies for the specificity. This test can only be carried out with untreated patients, because after eradication therapy the titres are only decreasing slowly, and a sero-conversion mostly is not forthcoming. If one would like to do a follow up with serology then 2 sera have to be collected, one from before the treatment and one from at least 6 months after the treatment. A decrease of titre of more than 40% proves eradication.⁹ The faeces antigen test has a specificity of 90% and a sensitivity of 80-90%. *H. pylori* antigens are shown in the stools with this test. This can be both before and after eradication therapy.

Section 2; Validation of Heliprobe

In this study I have reviewed a ¹⁴C-urea breath test, which makes use of the HeliCap[®] capsule as a source for the labeled urea, and the breath samples are analysed in the Heliprobe[™] analyser.^{12,13}

The Heliprobe[™] method is based on the fact that the bacterium, *H. pylori*, produces an enzyme. This enzyme, urease, catalyses the conversion of urea into ammonium and CO₂. *H. pylori* needs this ammonium in order to create an optimal local mucous environment in the acid stomach, thus increasing the pH. The urea can be labeled with a ¹³C or ¹⁴C-atom. The Heliprobe makes use of the isotope ¹⁴C, which has a very low amount of β-radiation. The radioactive amount used here is 1 μCurie. This is many thousands of times less than the radiation load of a Stomach x-Ray or a Thoracic x-Ray. The test can be used both for the primary diagnosis and for the follow-up after eradication therapy, minimum 4 weeks after the end of the therapy. Thus this ¹⁴C-urea breath test is a new non-invasive method to prove *H. pylori*. In this research we have compared this new breath test with the Golden standard and in addition to it also with a validated, commercial ¹³C-urea breath test, the HELICO STAT[™] with the BreathID analyzer. We would like to know if this test is reliable and safe and if the test would be a good alternative in daily practice for the tests presently in use.

Section 2.1: Method

Patients, who have undergone a gastroscopy and from whom biopsies were taken, were asked to also undergo the ^{14}C -urea breath test on a voluntary basis.

The indications for a gastroscopy are mostly ulcer complaints not responding to acid inhibition within 4-8 weeks, primary recrudescence ulcer complaints, secondary recrudescence reflux complaints and secondary recrudescence non-specific stomach complaints within 1 year.

Following gastroscopy informed consent was obtained from most patients and we made a new appointment for them. Before the gastroscopy and the breath test they had stopped swallowing a proton pump inhibitor and antibiotics for a minimum of 1 week. Before commencement of the test the patients were asked again if they were taken a stomach acid inhibitor and if they had indeed stopped using it. Furthermore the patients had fasted since the previous midnight.

The Helicap[®], a small capsule containing $1\mu\text{Ci}$ labeled ^{14}C -urea and citric acid, is ingested with 50 ml water. If an infection with *H. pylori* exists, then the bacteria produce urease, which convert labeled urea. The $^{14}\text{CO}_2$ is absorbed in the blood via the mucosa and transported to the lungs where it is exhaled. By exhaling into a BreathCard[®], the quantity of $^{14}\text{CO}_2$ present in the exhaled air can be measured with the aid of the HeliprobeTM apparatus. The BreathCard[®] consists of 2 pads containing a concentrated quantity of alkaline. The pads become saturated with exhaled air, which is to stay with a certain amount of CO_2 . After approx. 1 to 2 minutes the BreathCard[®] is saturated. This can be seen because an indicator on the BreathCard[®] changes colour from yellow to orange.

Table 1: Definition of Golden Standard

	Histology (PA)	Culture	CLO-test
Patient <i>H. pylori</i> +	+	+	+
Patient <i>H. pylori</i> +	+	+	-
Patient <i>H. pylori</i> +	+	-	+
Patient <i>H. pylori</i> +	-	+	+
Patient <i>H. pylori</i> +	-	-	+
Patient <i>H. pylori</i> +	-	+	-
Patient <i>H. pylori</i> +	+	-	-
Patient <i>H. pylori</i> -	-	-	-

A second patient group had undergone a regular ^{13}C -urea breath test, the HelicoSTATTM with the BreathID analyzer, and those people were asked to voluntarily participate in the ^{14}C -urea breath test as well. This as a comparison between both breath tests. The patients who are participating in a ^{13}C -urea breath test are always told that they have to fast starting from the previous evening. They also should not have taken, for at least the previous week, any stomach acid inhibitors or antibiotics. For the version of the urea breath test used in Oss, patients are connected via a flexible nose tube with the BreathID analyzer. Then the basic measurements starts, that is to say that the baseline is determined for the quantity of $^{13}\text{CO}_2$ present in the exhaled air. Then the patient is asked to drink water with 75 mg ^{13}C -urea and citric acid dissolved in it. The quantity of $^{13}\text{CO}_2$ in the exhaled air is determined again and the apparatus measures if a change has occurred. A graph will appear on the screen showing whether a patient has been infected with the bacterium or not. The test takes a minimum of 20 minutes. The cut-off value of the graph is 5 DOB. That means that all patients with a value above the 5 DOB are Helicobacter pylori positive.

If informed consent was obtained, first the ^{14}C -urea breath test was carried out because the quantity of urea, $1\mu\text{Ci}$ labeled ^{14}C -urea, which is used in this test is many times less than the 75 mg labeled ^{13}C -urea breath test. Conversely, there is a strong possibility that the bacteria are already saturated with the labeled ^{13}C -urea and thus will not absorb ^{14}C -urea anymore. Thus there is a larger theoretical possibility of false-negative results.

Section 2.2: Results

2.2.1; Comparison of ^{14}C -urea breath test with the gastroscopic biopsies.

In total 77 patients participated in the first part of the research in which the breath test was compared with the biopsies. Twenty two patients from the Canisius Wilhelmina hospital in Nijmegen and 55 patients from the Bernhoven hospital in Oss were examined.

The research sample consisted of 52 men with an age between 25 and 83 years and 25 women with an age between 54 and 89 years. Thirteen patients used a proton pump inhibitor at the moment they were examined and 64 patients did not use a stomach acid inhibitor.

The d-values varied between -21 and 516 cpm. These values originated only from the patients in Oss. In the positive group the d-values were between 94 and 516 cpm. In the negative group they were between -21 and 19 cpm. For one patient (1.3%), a man of 76 years of age, the result from the ^{14}C -urea breath test was a 1, doubtful positive, and both the CLO-test and the culture and histology were positive. Thus the patient had received treatment. The d-value was 48, just below the cut-off value of the test. Thus he is probably infected with the bacterium.

He uses a PPI, possibly he did not stop using it in time. It is already known from previous research that the d-value for patients with a proton pump inhibitor decreases. And thus the test can yield false negative results. This patient was excluded by us on the basis of the doubtful breath test results, and so the definitive patient sample consisted of 76 patients. Biopsies were taken from these patients in order to prove H. pylori, by means of the Golden standard. The ¹⁴C-urea breath test was positive for 21 patients, negative for 55 patients. The number of patients having a positive Golden Standard was in total 23. Moreover, 17 patients had both a positive CLO-test and a positive culture and positive histology. Four patients had a positive CLO-test and a positive histology, but the culture was negative. For 2 patients only the CLO-test was positive. 53 patients had a negative Golden Standard, that is to say all 3 test were negative. The results from the Golden standard and the ¹⁴C-urea breath test are listed in table 2.

Table2: ¹⁴C-urea breath test in comparison with the Golden standard

	Golden Standard +	Golden Standard -
¹⁴ C-urea breath test +	21	0
¹⁴ C-urea breath test -	2	53

Sensitivity 91%, Specificity 100%, Accuracy 97.3%

PPI+

	Golden Standard +	Golden Standard -
¹⁴ C-urea breath test +	1	0
¹⁴ C-urea breath test -	1	10

Sensitivity 50%, Specificity 100%, Accuracy 91.7%

PPI-

	Golden Standard +	Golden Standard -
¹⁴ C-urea breath test +	20	0
¹⁴ C-urea breath test -	1	43

Sensitivity 95%, Specificity 100%, Accuracy 98.4%

For 21 out of the 76 patients both the Golden Standard and the ¹⁴C-urea breath test were positive. The sensitivity is 91% and the specificity 100%.

If a distinction is made between patients who do use PPI and those who don't use PPI, then the results are as follows:

With PPI: sensitivity 50%, specificity 100%

Without PPI: sensitivity 95%, specificity 100%

For a patient, a man of 61 years of age, the results from the Golden standard (both the culture and the histology were positive) did not agree with the results from the ¹⁴C-urea breath test. The d-value is not known. This patient does not use PPI. Maybe he had not fasted, or his bacteria count is so slow that it was false-negative in this test. For another man, 78 years of age, the breath test was also negative while the histology and the CLO-test were positive. The d-value was 0 cpm. He uses a PPI, but stopped using it in time for the examination.

2.2.2: Comparison of the ¹⁴C-urea breath test with the ¹³C-urea breath test

In a second patient group both the ¹⁴C-urea breath test and the ¹³C-urea breath test were carried out. In total 69 patients participated it, all originating from the Bernhoven hospital in Oss.

There were 29 women with an age between 21 and 80, and 40 men with an age between 18 and 83.

Twenty six out of the 69 patients had a positive ¹⁴C-urea breath test and 43 a negative ¹⁴C-urea breath test. Twenty seven patients had a positive ¹³C-urea breath test and 42 a negative ¹³C-urea breath test.

All patients who use stomach acid inhibitors stopped them at least 1 week before the examination. At the time of the examination the patients did not use antibiotics.

The results are shown in table 3.

Table 3: ¹⁴C-urea breath test in comparison with ¹³C-urea breath test

Total

	¹³ C-urea breath test +	¹³ C-urea breath test -
¹⁴ C-urea breath test +	26	0
¹⁴ C-urea breath test -	1	42

Sensitivity 96%, Specificity 100%

For 26 out of the 69 patients both the ^{14}C -urea breath test and the ^{13}C -urea breath test were positive and for 42 out of the 69 both tests were negative. This results in a sensitivity of 96% and a specificity of 100%.

For one patient, a man of 77 years of age, the ^{13}C -urea breath test was positive and the ^{14}C -urea breath test negative. The d-value of the ^{14}C -urea breath test was 0 cpm and the value of the ^{13}C -urea breath test was 7 DOB. The bacteria count for this patient is probably too low. This man used a PPI, so it is possible that he did not stop using it in time. It has been shown in previous research that the ^{13}C -urea breath test used by us is less sensitive for the effect of a PPI.¹⁴ For patients who use a PPI, false negative breath tests results are often obtained. For that reason, we advise the patient to stop using a PPI one week before the examination. However, some researchers advise a period of 14 days. In that way it can be explained why the ^{14}C -urea breath test is false-negative and the ^{13}C -urea breath test is (true) positive.

Section 2.3: Discussion

For patients with stomach complaints the general practitioners can choose from multiple strategies. Recently a new NHG-Standard for Stomach Complaints has been published in which advice is provided for the general

Practitioner regarding the policy to be followed.^{2,15} Elderly patients, above 55 years of age are advised to undergo an endoscopy, in connection with the risk of malignancy. Patients with typical reflux complaints first undergo a test treatment with a PPI. For all other patients the “test & treat” strategy is advised.¹⁶

This “test & treat” strategy, by means of a non-invasive diagnostic test, determines if the bacterium, *H. pylori*, is present. Patients with positive results can obtain immediately an eradication therapy. Stomach ulcer disease is excluded in patients with negative results. An endoscopy is medically not necessary for *Helicobacter*-negative patients who are younger than 55 years of age, and without alarm symptoms. Only a small number of patients are still advised to undergo gastroscopy after the “test & treat” protocol.¹⁷

In the directives from the CBO and NHG about stomach complaints², the Urea Breath Test is indicated as the non-invasive test method with the best test characteristics. Therefore this method is preferred to other non-invasive methods. For that reason I have analysed further, in the Bernhoven Hospital in Oss, the value of the Urea Breath Tests.

My results show that the ^{14}C -urea breath test, executed with the Heliprobe TM system, is a good and reliable non-invasive method to show *H. pylori*, in comparison with the Golden standard (endoscopic biopsies). It is a method that can easily be carried out and moreover is not expensive. This test does not have to be carried out in a hospital environment, but can also be carried out in the general practitioner's office. Here the test can be used optimally within the framework of the “test & treat” strategy. When comparing the ^{14}C -urea breath test with the previously evaluated ^{13}C -urea breath test, in fact the same results are obtained. Both tests are equally effective in tracing *H. pylori* infection. But patients do have to stop using acid inhibitors and antibiotics in time.

The Heliprobe TM system is less expensive than the ^{13}C -urea Breath Test and easier to use. It is only a small portable apparatus. The patients do not find it inconvenient to exhale into a BreathCard, they don't have to blow hard. The tablets and the BreathCards have a long shelf life. The only, particularly emotional disadvantage is that an extremely small quantity of radioactive β -radiation is used. Working with the capsule does not pose a problem either, because it concerns a very low amount of β -radiation, which cannot get outside the capsule because of its low penetrating capacity.

The test is much less radioactive than an X-ray photo. But the word radioactivity deters patients, because they are still afraid that it can pose a danger to their health. The radiation load is equal to drinking 3-4 glasses of orange juice; therefore in America the test does not come under the stipulations of the law regarding working with radioactivity.

An analyzer, which is an expensive purchase, is necessary for the ^{13}C -urea breath test. In this case many different tests have also been marketed, but in Oss use is made of the BreathID analyzer from Oridion. This test can only be applied in a cost-effective manner if many tests per month are being carried out. In a general practitioner's practice that will not be feasible, and patients will still be referred to the hospital or to the general practitioner's surgery.

Thus the ^{14}C -urea breath test appears to be a good alternative for the ^{13}C -urea breath test. It can be used as a reliable non-invasive Golden standard for the detection of *H. pylori*. The ^{14}C -urea breath test is a valid possible choice for the “test & treat” strategy, which can be applied well in a general practitioner's practice. This test can be carried out completely autonomously by the general practitioner in his own practice. A disadvantage is that the ^{14}C -urea capsule is not yet registered in the Netherlands and the ^{13}C -urea capsule with 75 mg urea is.

Annexe 1: I

^{14}C -urea breath test versus Golden Standard

				CLO	CLO			
Gender	Date of birth	14C	14C D-value	Antrum	Corpus	Culture	Histology	PPI
M	31/07/49	0	12	-	-	-	-	+
M	23/02/60	0		-	-	-	-	+
M	10/02/30	0		-	-	-	-	-
F	07/01/45	2	325	+	+	+	+	-
F	08/11/37	0	7	-	-	-	-	-
M	10/02/46	0	4	-	-	-	-	+
F	02/11/24	0		-	-	-	-	-
M	24/08/39	0	-3	-	-	-	-	+
M	14/08/48	0	9	-	-	-	-	-
F	29/03/26	2	260	+	+	-	+	-
M	03/05/25	0	-6	-	-	-	-	-
M	08/07/79	2	468	+	+	-	+	-
M	28/07/37	0	19	-	-	-	-	-
M	23/04/59	0	15	-	-	-	-	-
M	05/04/26	0	0	+	+	-	-	+
F	31/07/34	0	-21	-	-	-	-	-
M	10/06/36	0	-21	-	-	-	-	-
M	26/09/35	2	335	+	+	-	+	-
F	05/06/43	2	410	+	+	+	+	-
M	04/04/65	0	-3	-	-	-	-	+
F	12/10/48	0	6	-	-	-	-	-
M	28/03/38	2	257	+	+	-	+	-
M	31/10/24	0	-9	-	-	-	-	-
M	05/09/29	0	-10	-	-	-	-	+
M	27/09/57	0	-3	-	-	-	-	-
F	04/12/45	2	317	+	+	+	+	-
M	24/10/37	1	48	-	+	+	+	+
M	24/10/35	2	443	+	+	+	+	-
M	10/02/40	0	-4	-	-	-	-	-
M	29/06/42	2	268	+	+	+	+	-
F	21/02/50	0	12	-	-	-	-	-
M	20/06/48	2	267	+	+	-	+	-
F	10/07/45	2	151	+	+	+	+	-
M	27/03/39	2	122	+	+	+	+	-
M	20/05/30	2	94	+	+	-	+	-
F	18/11/46	0	15	-	-	-	-	+
M	28/05/22	2	516	+	+	+	+	-
M	02/12/34	0	5	-	-	-	-	-
F	30/10/23	0		-	-	-	-	-
F	18/04/32	0		-	-	-	-	-
M	24/05/56	0		-	-	-	-	-
M	23/04/53	0		-	-	-	-	-

F	07/10/35	0		-	-	-	-	-
M	29/11/37	2		+	+	+	+	-
M	04/09/21	0		-	-	-	-	+
F	28/04/24	0		-	-	-	-	-
M	03/08/46	0		-	-	-	-	-
F	19/12/19	0		-	-	-	-	-
M	23/10/69	0		-	-	-	-	-
F	02/07/34	2		+	+	+	+	-
M	24/04/62	2		+	+	+	+	-
M	07/05/55	0		-	-	-	-	-
M	02/07/40	0		-	-	-	-	-
F	23/08/15	0		-	-	-	-	-
M	05/02/37	0		-	-	-	-	-

¹⁴C urea breath test in CWZ versus Golden Standard

F	31/03/46	0				-	-	-
M	16/01/37	0				-	-	+
M	10/07/76	0				-	-	-
M	16/06/45	2				+	+	-
M	10/02/41	2				+	+	-
M	15/01/47	0				-	-	-
M	16/04/38	2				+	+	+
F	04/07/45	0				-	-	+
F	29/01/40	2				+	+	-
M	23/06/56	0				-	-	-
F	21/05/60	0				-	-	-
M	24/08/59	0				-	-	-
M	24/02/70	0				-	-	-
M	02/09/52	0				-	-	-
M	20/04/41	0				-	-	-
M	25/06/42	0				+	+	-
M	14/06/66	0				-	-	-
F	13/11/38	0				-	-	-
F	06/06/42	0				-	-	-
M	01/11/45	0				-	-	-
M	15/01/44	0				-	-	-
F	13/02/36	0				-	-	-

Annexe2;

¹⁴C-urea breath test versus ¹³C-urea breath test

Gender	Date of birth	14C results	14C D-value	113C results	13C DOB
F	07/09/79	2		+	12.4
M	31/08/58	0		-	0.4
M	27/12/51	0		-	1.5
F	27/10/40	2		+	21.4
M	20/09/61	2		+	6.6
F	30/07/55	0		-	0.3
M	11/10/54	0		-	0.2
F	05/05/64	2	284	+	7.6
F	14/09/70	2	491	+	26.6
M	30/08/21	0	-8	-	0.1
F	20/07/72	0	10	-	2.3
F	11/01/60	2	534	+	11.3
M	02/11/86	0	1	-	0.6
M	28/09/36	0	-8	-	0.7
M	25/01/69	2	191	+	8.1
M	09/01/42	0	-10	-	0.7
F	25/06/59	0	-20	-	1.6
F	10/01/81	0	1	-	1.7
F	29/09/59	2	678	+	16.6
F	26/06/52	0	1	-	0.1
M	21/09/56	0	3	-	0.2
M	08/01/43	2	320	+	7.6
F	25/01/51	2	400	+	27.2
F	02/08/45	0	7	-	0.6
M	03/03/35	2	80	+	7.2
M	14/06/57	2	81	+	8.2
F	27/06/53	2	228	+	8.3
F	28/09/58	0	-1	-	0.6
F	22/01/54	0	3	-	0.3
M	14/09/36	0	13	-	1
M	31/07/49	0	12	-	2
M	23/02/60	0	0	-	1
M	10/02/30	0	0	-	2
M	03/04/64	2	180	+	10
F	07/01/45	2	325	+	21
F	08/11/37	0	7	-	0
M	10/02/46	0	4	-	1
F	02/11/24	0	0	-	0
M	24/08/39	0	-3	-	-1
M	14/08/48	0	9	-	3
M	13/12/61	0	12	-	0
F	29/03/26	2	260	+	27
M	03/05/25	0	-6	-	2
M	08/07/79	2	468	+	11

M	28/07/37	0	19	-	1
M	23/04/59	0	15	-	1
M	05/04/26	0	0	+	7
F	31/07/34	0	-21	-	0
M	10/06/36	0	-21	-	-1
M	26/09/35	2	335	+	9
F	05/06/43	2	410	+	12
M	04/04/65	0	-3	-	1
F	12/10/48	0	6	-	-1
M	28/03/38	2	257	+	13
M	31/10/24	0	-9	-	2
M	05/09/29	0	-10	-	0
M	27/09/57	0	-3	-	3
F	09/06/47	2	77	+	1
M	21/12/59	2	175	+	8
M	20/08/60	2	244	+	9
F	20/08/49	2	535	+	25
F	01/10/62	0	6	-	0
M	05/08/60	0	-5	-	1
M	08/01/61	0	17	-	0
M	05/02/63	0	8	-	1
F	08/07/83	2	113	+	9
F	11/01/48	0	-4	-	0
M	03/08/62	2	534	+	32
F	04/06/35	0	0	-	1

Selling against HISTOLOGY

Advantages:

- + Type of Helicobacter strain
- + Sensitivity / specificity
- + Other information is given (not only if the patient is infected with Hp)

Disadvantages:

- Requires expertise
- Expensive
- Invasive (trying for the patient)
- Have to wait for result)

Selling Against ¹³C

Advantages:

- + No need for baseline
- + Shorter postprandial period
- + Time

Disadvantages:

- Expensive
- Complexity
- Service & Maintenance

Selling Against BIOPSY TEST

Advantages:

Disadvantages:

- Invasive (trying for the patient)
- Operator dependent
- Time consuming
- Costly

Selling Against SEROLOGY

Advantages:

Disadvantages:

- Only antibody
- No quick test
- No follow up

BREATH TEST

Advantages:

- + Sensitivity / specificity
- + Cheap
- + Non invasive
- + Test results immediately
- + Layperson can do the test (easy to use)

Disadvantage:

- No information about strain

RISK ASSESSMENT

The risk immanent to the use of a radiopharmaceutical is the exposure to radiation either of patients, of health personnel or of the public. The risk assessment of the use of HeliCap capsules containing 37 kBq (^{14}C) was given in the toxicological/pharmacological documentation (Part III). The points considered were and are the following:

1. Risk to patients: The physical half-life of (^{14}C) is 5730 years. Studies on the biological half-time have, however, demonstrated that ingested (^{14}C) urea, administered e.g. as one HeliCap capsule, is eliminated quickly, and that determined pharmacokinetic parameters clearly indicate a negligible risk to no risk at all:

-Munster et al investigated 18 subjects who received either 185 kBq or 37 kBq (^{14}C) urea. Elimination via breath and urine were examined up to 72 hours. Maximum recoveries of (^{14}C) were between 1 and 2 hours after ingestion. Overall elimination of (^{14}C) independent of the amount ingested (185 kBq vs 37 kBq) was ca 87% in "high expirers" and ca 99% in "low expirers". Long-term retention was low. When compared to daily exposure to natural sources of radiation which on average figure 3.7 kBq/day, then the remaining activity 3 days after ingestion of a HeliCap capsule is not more and even less than the average natural daily exposition to radiation.

-Like Munster et al, the detailed studies by Leide-Svegborn et al. also conclude that the exposure from a test dose of 110 kBq and of 55 kBq in children both correspond to about a day of natural radiation from the environment. The majority of (^{14}C) excreted in urine was found in the first 24 hours, and peak expiration of (^{14}C) occurred within the first hour after ingestion. Leide-Svegborn et al also conclude that there is no reason for restrictions on even repeated screening investigations with ^{14}C -urea in whole families, including children when administering a dose of 55 kBq ^{14}C -urea (48% more than what is administered with the HeliCap).

-Further, exposure to radioactivity associated with the use of HeliCap is hundreds to thousands of times less than well accepted procedures performed in departments of radiology.

2. Risk to health personnel: The council directive 96/29/Euroatom, Article 9(1) states that the limit on effective dose for exposed workers shall be 100mSv in a consecutive five year period, subjected to a maximum effective dose of 50mSv in any single year. A positive patient taking a HeliCap is exposed to a maximum effective dose of 0.003mSv. By definition the effective dose for a health personnel carrying out the test being at risk for contamination from the patient must be much less. This is confirmed by the analysis performed by the US Nuclear Regulatory Commission.

-The analysis made by the US Nuclear Regulatory Commission on exposure of workers administrating the ^{14}C -urea breath test led to the conclusion that a full-time worker administrating 8000 capsules per year containing 37 kBq ^{14}C -urea followed by breath testing would get exposed to 0.007 mSv per year which is by magnitudes below the annually permitted effective dose stipulated by Council Directive 96/29/Euroatom (maximum effective dose of 50 mSv in any single year).

-The analysis made by the US Nuclear Regulatory Commission also included analysis of exposure due to potential accidents in the administration facility: They calculated that rupture of a capsule causing skin contamination of the worker or the patient (100 cm² exposed for one hour prior to washing resulting in 2.775 kBq skin absorption) would lead to an effective dose of 0.00029 mSv.

3. Risk to the public: The Council Directive 96/29/Euroatom, Article 13(2) states that the limit for effective dose for members of the public shall be 1 mSv in a year. The analysis made by the US Nuclear Regulatory Commission on the environmental impact 1017 Bq (equivalent to the activity of 3.8 x 10¹² breath tests), which is in addition to the huge inventory of about 8.9 x 10¹⁸ Bq in the world's oceans" Further, "the current world inventory of naturally occurring ^{14}C results in an average dose to the public of about 0.0125 mSv per year, and the release of 2,22 x 10¹⁰ Bq of ^{14}C from total 600 000 tests would result in an additional average annual dose of 2.0 x 10⁻⁹ mSv per year". This amount is negligible compared to the 1 mSv limit stated in the above mentioned European Council Directive.

-Taking the European Commission Publication Radiation Protection 97. Radiation Protection following Iodine-131 therapy (exposures due to out-patients or discharged in-patients) into consideration, which states that the following dose constraints will be applied:

-Children (including unborn children)	1 mSv	
-Adults (under 60 years of age)		3 mSv
-Adults (60 years of age and older)	15 mSv	
-Other persons (members of the public)		0.3mSv

Then the maximum radiation dose of 3 microSv following one diagnostic test in a Helicobacter pylori positive patient is still 100 times less than even the tightest dose constraint stipulated for the public in the above mentioned Radiation Protection Publication to safeguard them from radiation exposition through Iodine-131 treated patients.

Noster System AB's overall conclusion concerning radiation risk and the use of HeliCap is: For patients, health personnel and the public there is potentially no radiation risk involved in the use of HeliCap.