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Microdose ^{14}C-Urea Breath Test Offers Diagnosis of *Helicobacter pylori* in 10 Minutes

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Objectives: **The urea breath test diagnoses *Helicobacter pylori* infection of the stomach by identifying the urease enzyme activity of the bacterium. In this "microdose" version of the test, 1 μCi ^{14}C-urea is given orally in a capsule. Our objectives were: *1)* to evaluate a microdose ^{14}C-urea breath test capsule in a gastroenterology outpatient setting, *2)* to determine the diagnostic ranges of the ^{14}C-urea breath test for HP-positive and HP-negative patients, *3)* to define the sensitivity and specificity of the test, and *4)* to see whether breath sample results changed when they were mailed to a remote site for analysis.** ***Methods:*** **In a prospective blinded study, we breath-tested 200 fasted patients before elective outpatient endoscopy. At endoscopy, two gastric biopsy samples were taken and were examined for curved organisms; a third biopsy specimen was evaluated with a rapid urease test (CLOtest). Breath samples were mailed in aluminized balloons to a testing laboratory.** ***Results:*** **Using a single breath sample collected at 10 min, with ≥200 dpm as positive, the breath test correctly classified 63 of 65 HP-positive patients (sensitivity 97%, CI 89–99%), and 128 of 135 HP-negative patients (specificity 95%, CI 90–98%). Radiation exposure from the test equated to natural background received in 1 day. No adverse events were caused by the breath test.** ***Conclusions:*** **The ^{14}C-urea capsule breath test (PYtest) is a convenient noninvasive test for the detection of gastric *H. pylori* infection. Accuracy is equivalent to invasive methods such as histology. Results can be obtained within 15 min if a counting instrument is nearby, or breath samples can be mailed to a testing laboratory for analysis.**

INTRODUCTION

^{14}C-Urea breath testing had been in use at the University of Virginia since 1987 when we first started treating peptic ulcer patients for *Helicobacter pylori* infection. The original test system was adapted from the method of Marshall and Surveyor (1), who devised a test in which a fasting patient was given 10 μCi of ^{14}C-urea solution, and breath samples were collected at frequent intervals for 30 min after ingestion. This test was problematic in that several breath samples were required, and the isotope dose had not been minimized.

We published an abbreviated method in 1990 whereby the isotope dose was halved to 5 μCi, only three breath samples were collected, results were reported simply as counts per minute (cpm), and a diagnosis could usually be made by reading only a single 20-min sample (2).

In this paper, we describe an enhanced test (PYtest) in which the isotope is enclosed in a quick-dissolve capsule. One potential advantage of this delivery method is that urea is no longer exposed to the bacterial flora of the mouth during ingestion. Therefore, urea hydrolysis does not occur unless urease (*H. pylori*) is present in the stomach. This means that in HP-negative subjects, near-background levels of ^{14}C are found in the breath, and the separation of HP-negative and HP-positive is far greater than with liquid-based (urea solution) tests. Therefore, the test can be done with a lower dose of isotope, using a single breath sample at an earlier time point. This more convenient methodology then allows breath samples to be mailed to a centralized testing facility.

The objectives of the study were therefore: *1)* to evaluate a microdose (1 μCi) ^{14}C-urea breath test capsule in a gastroenterology outpatient setting, *2)* to determine the diagnostic ranges of the ^{14}C-urea breath test for HP-positive and HP-negative patients, *3)* to define the sensitivity and specificity of the test, and *4)* to see whether breath sample results changed when they were mailed to a remote site for analysis.

METHODS

Patients

The study was approved by the Investigational Review Boards of the collaborating institutions, and all patients signed

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informed consent. Outpatients referred for elective upper GI endoscopy were interviewed by a research nurse and asked to have a breath test prior to endoscopy. Patients were included if they were between the ages of 18 and 75 yr. Patients were excluded from the study if they had taken bismuth or antibiotics within 4 wk, had taken sucralfate or omeprazole in the past 2 wk, if they had a contraindication to biopsy (*e.g.*, bleeding tendency), or if they had a history of resective gastric surgery.

Procedures

Two hundred consenting patients who met the entry criteria for the study were given a 1-μCi capsule of ^{14}C-urea with 20 ml of water and an additional 20 ml of water 3 min later. Breath samples (1 mmol CO_2) were collected in benzethonium (hyamine) hydroxide in methanol (1 mmol hyamine hydroxide made up to 2.5 ml in methanol with bromothymol blue as pH indicator, hereafter called "collection fluid") at baseline and then at 5-min intervals for 30 min. The patients blew into a drinking straw through a nonreturn collection trap into the collection fluid vials as previously described (2). Two extra breath samples were collected in aluminized balloons at times 10, 15, and 20 min (six balloon samples in total).

During endoscopy, two antral biopsy samples were taken for histology, and one other sample was taken for CLOtest rapid urease test. Histology samples were fixed in formalin, embedded in wax, sectioned in routine fashion, and stained with Giemsa at Metpath Laboratories. The stained sections were then sent to the University of Virginia Pathology Department where they were read blind by an experienced pathologist (HRF). Presence of curved or spiral organisms on gastric epithelium was graded as follows: 0 = none, 1 = difficult to find but definite curved organisms, 2 = easily found curved organisms in several parts of the specimen, or 3 = many curved organisms throughout the specimen. The CLOtests were held at the study site and were read by the investigator in charge of the patient.

To strengthen the histology data, we subjected all patients with discordant CLOtest, histology, or breath test to a second, blinded, histological examination. For this second examination, stored unstained sections were carefully stained with Giemsa at The University of Virginia and reexamined for spiral organisms. In a subset of specimens without bacteria but with inflammation suggestive of *H. pylori* infection, stored tissue blocks were also recut and examined on a third occasion. We also compared the breath test result with a reference method derived by excluding patients in whom histology did not agree with the CLOtest. In this way, false-positive histology (if it existed) was almost entirely eliminated, because CLOtest is known to be highly specific and is not operator dependent (3).

On the same day as the test, one set of breath samples was transferred from balloons to collection fluid at the study site. The second set was sent by courier to Tri-Med Specialties in Charlottesville, Virginia, where it was transferred to collection fluid no less than 72 h after the test.

TABLE 1
Demographic and Other Characteristics of the Study Population

	HP-Negative		HP-positive		Raw Total %
	No.	%	No.	%	
Gender					
M	57	42	25	38	41
F	78	58	40	62	59
Ethnicity					
Caucasian	121	90	53	82	87
African-American	11	8	11	17	11
Other	3	2	1	2	2
Age (yr)					
<=50	70	52	26	40	48
>50	65	48	39	60	52
Totals	135	67	65	33	

Once the breath samples had been transferred to collection vials, scintillation fluid was added, and they were counted for 5 min. For reading at the study site, the zero time point sample was used as background and was subtracted from the cpm result obtained from each vial. At Tri-Med, a blank sample was counted each day (usually about 55 cpm) and was subtracted from each vial result. The adjusted cpm values thus obtained were converted to disintegrations per minute (dpm) by counting a known standard sample and dividing cpm results by the machine efficiency (usually 0.85). These parameters gave an accuracy ($\pm$2 SD) of $\pm$13% at 50 dpm and $\pm$3% at 1000 dpm.

Statistical analysis was performed using descriptive statistics in the Minitab and Microsoft Excel 5.0.

RESULTS

Patient demography

Two hundred patients completed the study during 1994. In one patient, the histology sample was lost so, in that single case, the CLOtest result (negative) was used as the sole reference method for HP status. In the final analysis, 65 patients (33%) were determined to be HP-positive in the study. Demographic breakdown of the 200 patients is shown in Table 1. There were no side effects from the test and no apparent complications from the study biopsies.

Choice of a diagnostic sample

Breath samples collected and read immediately at the study site were almost always identical to those sent to Tri-Med and read at 72 h (Fig. 1). We therefore chose to use samples read blind at Tri-Med for the accuracy analysis (rather than the duplicate samples read at the study site by the investigators). At Tri-Med, three samples (10, 15, and 20 min) were read from each patient, but the diagnostic accuracy of each single sample was the same. We therefore chose the earliest single time point (10 min) for accuracy analysis of the breath test.

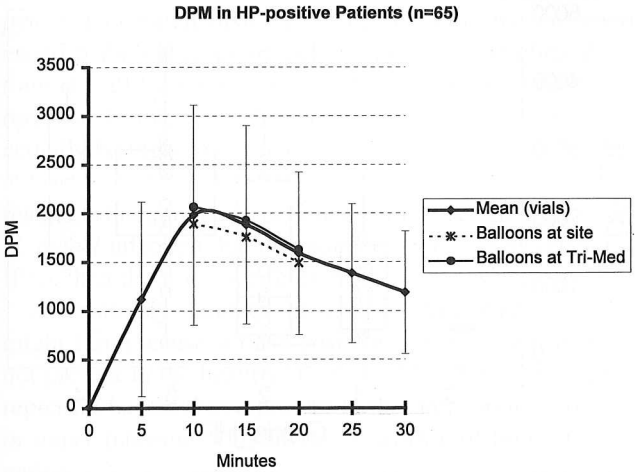

FIG. 1. ^{14}C excretion in breath for patients who were HP-positive. *Major curve* shows mean and *bars* show SD for HP-positive samples collected directly into vials and read at the study site (seven time points were available for vials). *Secondary lines* represent values of three samples collected into balloons and read at the study site (*dotted*) or at Tri-Med Specialties (*thin solid line*) 72 h later.

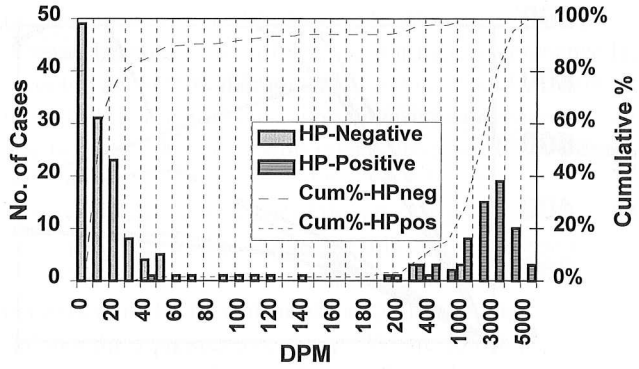

FIG. 2. Frequency distribution of 10-min breath test results (n = 200). *Interrupted lines* show cumulative % of patients (as value on *second Y-axis*). Percent of HP-negative cases correctly detected is shown by the *dashed line* at the top of the graph. The *lower dotted line* is equivalent to (1-sensitivity). Optimal cutoff for the test is where the separation of the two lines and specificity is greatest, *i.e.*, 200 dpm.

Criteria for a positive breath test

When the study was first initiated, we had chosen ≥50 dpm in a 10-min breath sample as the lower limit of the positive range. It became clear from receiver operator curve (ROC) analysis that ≥200 dpm was a more appropriate positive range. We therefore used the higher cutoff point (positive ≥200 dpm) for the accuracy analysis. The rationale for this can be seen in the frequency analysis graph shown in Figure 2. The figure demonstrates that there were very few patients who gave dpm results between 50 and 200, and most of these were HP-negative cases. It was appropriate therefore to use 200 dpm as the start of the positive range, rather than the value of 50 dpm, which we had previously determined by statistical analysis during a very small pilot study (4).

Histology versus breath test

Histology "first look" detected *H. pylori* in 61 of the 200 cases. We then performed a second, blinded review of histology from patients with conflicting CLOtest, histology, and breath test results. In 15 saved sections and 11 resectioned tissue blocks, the pathologist was able to detect four more cases of *H. pylori* in patients previously classified as HP-negative. There were no patients whose status was changed from HP-positive to HP-negative.

Thus, on initial examination, histology detected 61/65 HP-positive patients and was 94% sensitive [95% confidence interval (CI) 85–98%]. The breath test identified 63/65 patients as HP-positive and was 97% sensitive (CI 89–99%). The breath test also correctly classified 128/135 HP-negative patients. Assuming that the reference method (histology) was 100% sensitive, this made the breath test 95% specific (CI 90–98%). The differences between breath test accuracy and histology accuracy were not statistically significant; *i.e.*, the accuracy of the breath test was similar to histology. Complete data results are summarized in Table 2.

TABLE 2
Final Analysis, Breath Test versus Histology, ≥200 dpm as HP-Positive

		Histology		Total
		HP Pos	HP Neg	
Breath test result at 10 min	Pos	63	7	70
	Neg	2	128*	130
	Total	65	135	200

Sensitivity = 0.97 (CI 0.89–0.99), specificity = 0.95 (CI 0.90–0.98), positive predictive value = 0.90 (CI 0.81–0.96), negative predictive value = 0.98 (CI 0.96–0.99).

* CLOtest result was used here (negative) for the one patient with missing histology.

Evaluation of the breath test by the improved reference method

There were 185 patients in whom the histology result agreed with the CLOtest result. When the 15 discordant cases were excluded, the breath test correctly identified 52/54 patients as HP-positive (sensitivity 96%) and 127/131 patients as HP-negative (specificity 97%).

Individual analysis of incorrect breath test results

Analysis of the incorrect results revealed that, of two false-negative tests, one was clearly negative (dpm = 36) and the other was high negative at 10 min (dpm = 182) but positive at 15 min (dpm = 606). By setting the positive range 50 dpm lower at 10 min, we could have raised sensitivity to 98%, but specificity would have decreased.

Detailed analysis of seven "false-positive" cases as shown in Figure 3 suggested that at least half of these were actually HP-positive individuals with false-negative histology. If so, then the specificity of the breath test could have been as high as 98% (132/135).

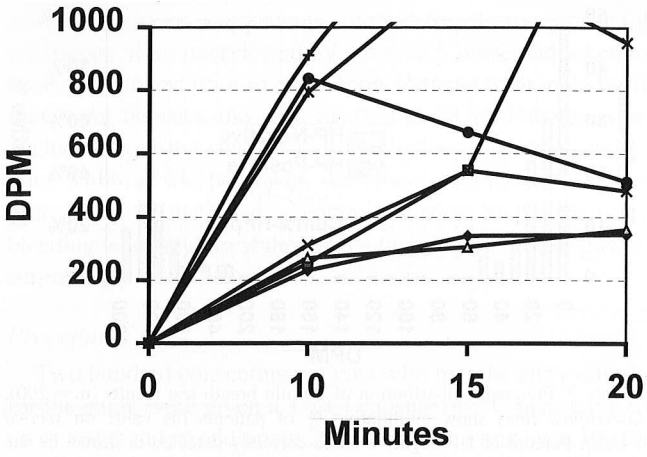

FIG. 3. Cases with positive breath test but negative histology. Four of the seven tests had dpm well into the positive range, with counts above 800 at 10, 15, or 20 min. These may have been true positive results in patients with incorrect "negative" histology.

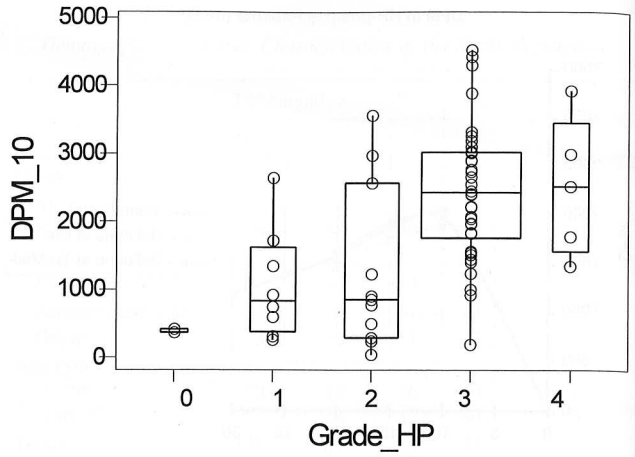

FIG. 4. Means, upper and lower quartiles for HP grades 1–4. *Boxplots* show means, upper and lower quartiles; *circles* show location of individuals. Only HP-positive patients are shown. See text for explanation of grading scale.

Correlation between grade of H. pylori on histology and breath test result

A correlation matrix between breath samples and measures of *H. pylori* colonization showed that the best predictor of the breath test result was constructed by adding the initial histology grade (0–3) to the result of the CLOtest (0–1) to give a total score of 0–4. In this analysis, HP-negative patients were excluded because almost all of them had histology grades and dpm near zero. As shown in Figure 4, the breath test tended to be more strongly positive in patients with heavier colonization with *H. pylori*. The graph shows mean with upper and lower quartiles for each grade of colonization (0–4). The correlation between dpm at 10 min and the histology grade was 0.528 (Pearson *r*). There was very little overlap between grade 1–2 and grades 3–4. To obtain a grade of 1, the histology must have shown 1 + HP, and the CLOtest must have been HP-negative. To obtain a grade of 2, either the histology was 1+ and CLOtest was positive, or histology was 2+ and CLOtest was negative. In either case, grades 1–2 meant that that HP was patchy and/or sparse. Grades 3–4 indicated heavy and consistent colonization. The relationship was highly significant ($p < 0.001$, $R^2 = 0.279$).

Choice of a single breath sample for diagnosis of H. pylori

The breath $^{14}CO_2$ excretion curves for HP-positive patients are shown in Figure 1. Note that in HP-positive subjects, $^{14}CO_2$ excretion peak occurs between 10 and 15 min so that a single sample taken anywhere between these two time points gives optimal diagnostic accuracy. In Figure 1, the average for HP-negative subjects could not be displayed adequately on the same scale.

Amalgamation of results

In this study, a breath sample collected at 10 min detected *H. pylori* at presentation with 97% sensitivity, a value numerically superior to that of histology. Although we collected numerous breath samples, diagnosis could nearly always be made on a single sample collected at 10–15 min (the exact collection time between these two points was not critical). In our 200-patient study, we initially expected the negative range to be less than 50 dpm in the 10-min sample. When receiver-operator-curve analysis was performed on the results however we determined that accuracy was optimal when the positive range was ≥200 dpm. The mean value for negative samples was only 16 dpm, and the mean value for positive samples was at least 100 times greater at 1500–2000 dpm. Values between 150 and 200 were uncommon, so it was not difficult to discriminate between positive and negative samples, even with a brief counting time (5 min is recommended). With a positive range of ≥200 dpm, the sensitivity and specificity were 97% and 95%, respectively. This trial used a batch of ^{14}C-urea capsules with an average potency of 1.17 μCi. Using a linear correction, the commercial version of the 1 μCi test would give a positive range starting at 170 dpm (200 ÷ 1.17).

DISCUSSION

The microdose ^{14}C-urea breath test is a simple, quick and accurate test to diagnose *H. pylori*. To perform the test, fasting patients ingested a ^{14}C-urea capsule with 20 ml of water, then drank a further 20 ml of water after 3 min. To ensure adequate CO_2 content of the breath sample, patients held their breath for 10 s, then blew through a drinking straw into a 2 L aluminized balloon. The neck of the balloon was tied in a knot, identifying documents were attached, and the sample was mailed to the testing laboratory. At the laboratory, 1 mmol of CO_2 was removed from the balloon and assayed for β activity. The entire test could be done in 15 min, and results could have been available 5 min later if a scintillation counter was located at the testing site.

The new microdose breath test detected more cases of *H.*

pylori than initial histological examination by an experienced pathologist, so we believe that in most clinical settings it will be more sensitive than currently available biopsy for histology. The specificity of the breath test may actually be higher than our detected value of 95%, because at least four of the "false-positive" cases had quite high dpm results, which, in our experience, almost certainly indicated *H. pylori* infection that had not been detected by histology. If so, then the true specificity of the test was actually 98%.

Alternatively, sources of urease other than *H. pylori* might rarely cause a false-positive test. Because urease is not present in the healthy stomach, this phenomenon could represent bacterial overgrowth in the oropharynx, stomach, or upper intestine. This might be worthy of future investigation.

We looked for and could demonstrate a correlation between numbers of *H. pylori* seen on biopsy and the result of the breath test, but we do not think this is an important feature of the test. The test could discriminate between heavy and very light colonization in groups of patients but, according to our study, changes in the test cannot reliably predict subtle changes in *H. pylori* density in individuals. This may have been because the histological grade of "2" covered such a wide range of colonization states defined as "easily found curved organisms in several parts of the specimen," and the histology only sampled antral mucosa. The microdose ^{14}C-urea capsule usually samples several square inches of greater curve mucosa. It is likely that breath counts depend more on where the capsule lodges in the stomach than on the relative density of *H. pylori* organisms in antral biopsy material.

We tested mailed breath samples 72 h or longer after collection. As expected, breath samples did not change when sent by courier to a central testing site. In other studies, we have encountered occasional lost or damaged balloon samples, but these losses were usually secondary to gross mishandling of the mailing boxes rather than fragility.

Now that several different tests are available for diagnosis of *H. pylori*, the physician can choose the most appropriate test for each patient. Serology is the most convenient test, because no preparation is necessary and arrangements for sample delivery are already in place at all medical practices. Serology is less than 100% accurate, however, and some patients invariably fall into a "borderline" or indeterminate zone in which the diagnosis of *H. pylori* is uncertain (5). A breath test can be used in these patients to confirm *H. pylori* infection (6). After treatment of *H. pylori* infection, antibodies remain for many months, so that serology remains positive in most patients (7, 8). If patients are feeling well, then sequential serological samples over 3–12 months can be used to determine whether *H. pylori* has been eradicated (7), but usually all samples must be run on the same plate to detect small changes in titer (9). On the other hand, if patients are still unwell after antibiotic therapy for *H. pylori*, it will be necessary to know immediately whether or not the bacterium has been eradicated. This might also be important in patients who have had complications of peptic ulcer disease, because they will need to continue maintenance H2 receptor antagonist therapy if *H. pylori* is still present. In these follow-up situations, a noninvasive convenient breath test is cost-competitive with quantitative serological examination (2–3 serum tests) and has the advantage of giving a positive result within 4 wk of therapy (10). In this paper, we have not presented data on use of this test to confirm cure of *H. pylori*, because with current therapies large numbers of patients must be treated in order to have enough treatment failures for a prospective study. We are informed by other investigators that the test has performed similarly in the posttreatment situation [William Chey (MI), David Metz (PA), Simmy Bank (NY), personal communications].

Some might consider the small radiation dose a handicap for the ^{14}C-urea breath test. On the other hand, this naturally occurring isotope allows rapid, inexpensive, and extremely sensitive counting methods. Our earlier data and data from others have shown that nearly all of the ingested isotope is excreted in urine or breath over the next 72 h so that radiation exposure from the test is trivial (1, 11, 12). With the current method, the amount of isotope is so small that the test actually gives less exposure than normal people receive from background radiation in one day (0.3 mrem) and is hundreds or thousands of times less than well-accepted procedures such as mammograms and upper GI series x-rays (11, 13).

In conclusion, we have shown that the microdose capsule ^{14}C-urea breath test is a convenient and accurate method for detecting *H. pylori* infection. Its sensitivity and specificity are comparable to histology, and breath samples can be read locally or mailed to a testing laboratory for analysis.

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