Validation of a Simplified Carbon-14-Urea Breath Test for Routine Use for Detecting *Heliobacter Pylori* Noninvasively

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A carbon-14 (^{14}C) urea breath test for detecting *Heliobacter pylori* with multiple breath sampling was developed. Carbon-14-urea (110 kBq) administered orally to 18 normal subjects and to 82 patients with *Heliobacter* infection. The exhaled ^{14}C-labeled CO_2 was trapped at 10-min intervals for 90 min. The total ^{14}C activity exhaled over 90 min was integrated and expressed in %activity of the total dose given. In normals, a mean of 0.59% ± 0.24% was measured, resulting in an upper limit of normal of 1.07%. In 82 patients, a sensitivity of 90.2%, a specificity of 83.8%, and a positive predictive value of 90.2% was found. The single probes at intervals of 40–60 min correlated best with the integrated result, with r ranging from 0.986 to 0.990. The test's diagnostic accuracy did not change at all when reevaluated with the 40-, 50-, or 60-min sample data alone. Thus, the ^{14}C-urea breath test can be applied routinely as a noninvasive, low-cost and one-sample test with high diagnostic accuracy in detecting *Heliobacter pylori* colonization.

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Heliobacter pylori (*H. pylori*) is involved in the pathogenesis of gastritis and ulcer (*1–6*). This has considerably increased interest in this bacterium. Since *H. pylori* produces large amounts of the enzyme urease (*7*), noninvasive breath tests with oral application of carbon-14- (^{14}C) urea and breath sampling of CO_2 have been suggested using varying methodologic approaches. Differences include doses given, gastric preloading, time intervals of the appearance of ^{14}C-labeled CO_2 in the exhaled air, and different modes of quantification. Applied in more than 500 patients so far, this test has been found to be very valuable, with sensitivities ranging from 69% to 100% and specificities ranging from 76% to 100% (*8–15*).

It was our intention to develop a reliable, low-dose, low expense ^{14}C-urea breath test for daily routine use. For this reason, a relatively large-scale test with frequent and long-lasting breath double sampling was primarily validated. Normal range, sensitivity, and specificity were determined. The extensive test data were then analyzed to evaluate the most representative breath specimen and our research allows us to suggest a form of test that is simplified, inexpensive, and easy to perform.

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METHODS

Patient Population

The ^{14}C-urea breath test was performed in 100 subjects from June 1987 to June 1989. All had had gastroscopy with urease quick testing and 23 also had a histologic urease test and histology, including Warthin-Starry-Silver staining, within 1 wk of the breath test. The results of the urease quick test, which showed a 92% concordance with the histologic staining for *H. pylori* in a large scale study including 260 patients previously performed at our clinic (*16*), served as the gold standard. Urease quick testing was performed with a commercial test set (CLO test, Delta West, Perth, Australia) using four biopsy specimens from the antral mucosa (*2,16*). A patient was considered *H. pylori* positive if at least one of the specimens showed a positive reaction in the CLO test. Most of the patients were submitted for primary diagnostic examination without previous treatment. Any antipeptic or antibacterial treatment was withheld for at least 3 wk before the test was performed.

Normals. This group comprised 18 subjects without gastritis, ulcer history, or symptoms of either, who had gastroscopy and biopsy for other reasons, whereby *H. pylori* infection was excluded. There were 13 men and 5 women, aged 31 to 74, with a mean of 56 ± 12 yr.

Patient Group. The patient group comprised 82 patients with symptoms of gastritis or ulcer. There were 52 men and 30 women, with ages ranging from 22 to 79, mean 51 ± 16 yr. In this group, sensitivity, specificity, and predictive values were defined.

Carbon-14-Urea Breath Test

The physiologic considerations for designing a ^{14}C-urea breath test were similar to those for other gastroenterology breath tests, such as the ^{14}C-glycocholate or ^{14}C-aminopyrine

test (*17,18*). A gastric preloading meal is needed to slow down gastric emptying and ^{14}C-labeled CO_2 has to be sampled over a larger time period after oral application, as it was not possible to predict the period of maximum appearance in the exhaled air. The ^{14}C-urea has to be dissolved in a larger volume in order to minimize the relative contamination from nonspecific urease-producing commensale bacteria in the mouth and esophagus. Finally, the dose of ^{14}C to be given has to be small enough to minimize the patients' radiation load, although large enough to obtain measurable amounts in the trapped air.

The following test protocol was used. After an overnight fast, 300 ml of a fluid standard meal containing 22.3 g protein, 14.7 g fat, and 55.6 g carbohydrates (Sonana-Formula Diet, Humana, West Germany) was given orally. A baseline breath sample was taken. Another 100 ml of the fluid meal containing 400 mg of unlabeled urea and 110 kBq (3 μCi) of ^{14}C-urea (Amersham, Braunschweig, West Germany) were given 15 min later. Breath samples were taken at 10-min intervals for 90 min in duplicate, with the patient exhaling through a straw into a vessel containing 1 ml of a 1-mol hyaminhydroxide-in-methanol solution and phenolphthalein as an indicator, thereby trapping exactly 1 mmol CO_2 per sample. Baseline samples and breath specimens, together with a standard containing 1100 Bq or 1.0% of the total dose, were measured with a beta liquid scintillation counter. All samples were measured in duplicate and the mean was taken for further calculations. The relative amount of exhaled activity over the entire sampling period of 90 min was integrated from the 10 single measurements data. Since 1.5 mmol CO_2 are exhaled per kg body weight in 10 min (*17*), the mean interval value was multiplied by 1.5 and by body weight and all nine interval fractions were summarized and normalized to the 1.0% standard measurement. The resulting value is the percent of urea degraded over 90 min or, in other words, a measure of urease activity in the upper GI tract. The entire calculation procedure is illustrated in Figure 1.

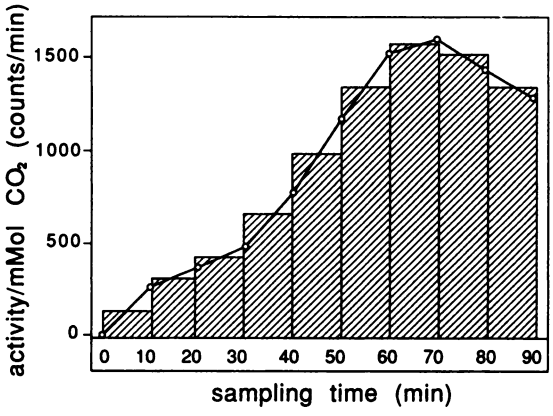

FIGURE 1
Time course of appearance of ^{14}C-labeled CO_2 expressed in cpm/1 mmol CO_2 following oral application of 110 kBq ^{14}C-urea in a patient of 72 kg body weight. The open circles represent the measurements in the breath samples obtained at 10-min intervals. From two adjacent values, a mean value for the respective 10-min interval was calculated (= height of the hatched bars). These values were multiplied by 1.5 and by 72 kg (patient's body weight), since 1.5 mmol of CO_2 is exhaled in 10 min/kg. These data were summarized yielding the integrated total amount of radioactivity exhaled over 90 min after oral application, which was 916.704 cpm for this individual. The counts of the 1.0% standard were 60.125. Thus, in this patient 15.2% of the total dose of ^{14}C-urea was degraded by *H. pylori*.

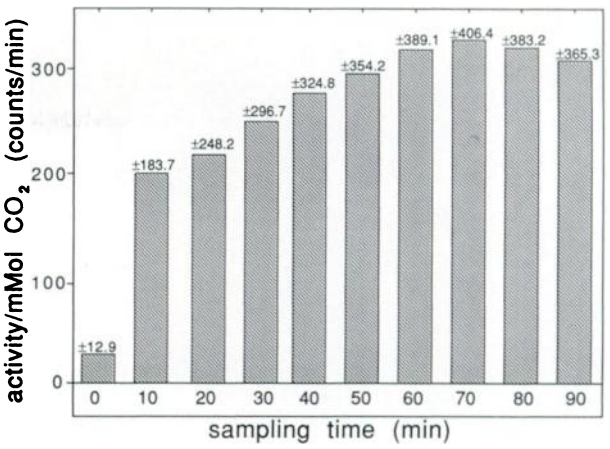

FIGURE 2
Mean of ^{14}C-labeled CO_2 exhalation of 82 patients over a period of time. Note that the maximum of ^{14}C-labeled CO_2 concentration is reached within the sampling period of 90 min.

Data Analysis

The normal range of %urea degradation was computed from the mean ± 2 s.d.s in the normal group. The upper limit of normal was determined by mean + 2 s.d.s. Sensitivity, specificity, and a positive predictive value were obtained in the patient group by the equations: sensitivity = true-positives/(true-positives + false-negatives); specificity = true-negatives/(true-negatives + false-positives); and the predictive value in a positive test result by true-positives/(true-positives + false-positives).

For comparing the various group data, the Student's t-test for non-paired data was applied. In order to find out whether one or more out of the 10 single-breath specimens might be representative of the whole integrated test result, the 10 single-breath samples of all 100 subjects were correlated to the integrated %-value. Further data analysis included the calculation of the time of maximum appearance of ^{14}C-labeled CO_2 and the calculation of the upper normal limit of the validated single measurements between 40 and 60 min (see Discussion).

RESULTS

Of primary importance was whether maximum ^{14}C-labeled CO_2 release had occurred within the measured period. That this was the case is shown in Figure 2; the maximum of ^{14}C-labeled CO_2 concentration in the exhaled air was reached at 70 min.

Normal Range. The results obtained in normals are plotted in Figure 3. The amount of degraded ^{14}C-urea in the 18 normals ranged from 0.17% to 1.18% and showed a Gaussian distribution with a mean of 0.59% and a s.d. of 0.24%, resulting in an upper limit of 1.07%. For reasons of simplicity, an upper limit of normal of 1% was used.

Patients. In the patient group, the enzymatic degra-

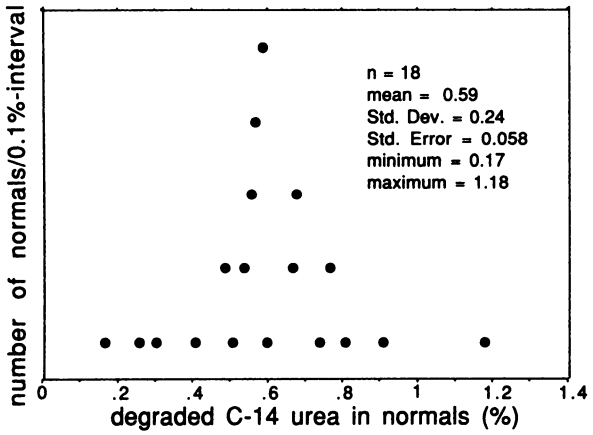

FIGURE 3
Distribution frequency of ^{14}C-urea degradation in normals, plotted in 0.1% increments of integrated ^{14}C-labeled CO_2 showing Gaussian distribution.

dation of ^{14}C-urea ranged from 0.16% to as high as 24.9%. Fifty-one patients were CLO positive, as revealed by gastroscopy, whereas in 31 patients no evidence of *H. pylori* colonization was found invasively. In the infected subset, 46 had a true-positive breath test and 5 had a false-negative test, resulting in a sensitivity of 90.2%. In the noninfected subset, 26 patients were true-negative and 5 were false-positive, giving a specificity of 83.8%. The predictive value in a positive test was 90.2%. The individual data are shown as a frequency distribution plot with the false-positive and the false-negative cases indicated in Figure 4.

The mean of degraded ^{14}C-urea in the 31 Heliobacter-negative patients, reflecting noninfected gastritis and

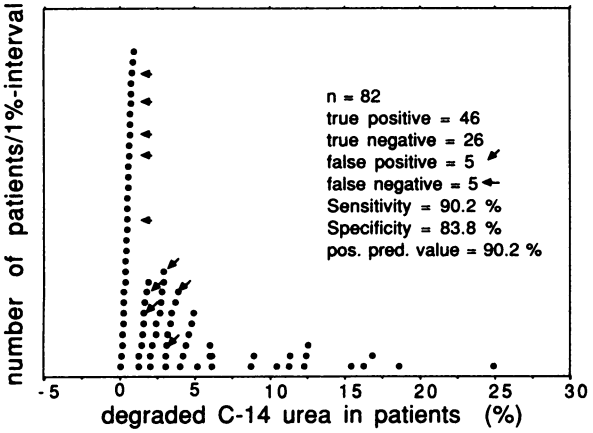

FIGURE 4
Distribution frequency of ^{14}C-urea degradation in all 82 patients plotted in 1% increments of integrated ^{14}C-labeled CO_2 exhaled over 90 min. The false-positives and false-negatives are indicated by arrows. Thus, Heliobacter-positive cases (CLO test) can be identified as dots above the 1% range plus the false-negatives minus the false-positives, whereas the Heliobacter-negative cases are represented by the dots within the 1% normal range plus the false-positives minus the false-negatives.

TABLE 1
Correlation of the Degraded ^{14}C-urea (%) as Calculated by Integrating all Data over 90 min (y) Versus the Single Measurements (x)

Sampling time (min)	Correlation equation (y = ax + b)	Correlation coefficient (r)
0	y = −0.02x − 3.94	0.001
10	y = 0.02x − 0.54	0.837
20	y = 0.02x − 0.09	0.934
30	y = 0.02x − 0.05	0.962
40	y = 0.01x − 0.19	0.986
50	y = 0.01x − 0.14	0.994
60	y = 0.01x − 0.05	0.990
70	y = 0.01x − 0.01	0.978
80	y = 0.01x − 0.04	0.968
90	y = 0.01x − 0.01	0.949

ulcer patients, was 0.96% ± 1.1% and was not significantly different from the control group, with $p < 0.5$. The patient subset of 51 cases with a positive CLO test had a mean of 5.82% ± 5.41% of ^{14}C-urea degradation and was very different from both the noninfected subset and the normal control group, each having $p < 0.001$.

By correlating the single breath samples, at each time interval, with the integrated value of all 82 patient studies employing linear regression analysis, the correlation equations and coefficients printed in Table 1 were obtained. Optimum correlation was reached between 40 and 60 min after oral administration, with the 50 min correlation coefficient being as high as 0.994, as illustrated in Figure 5. The upper limits of normal for the 40-, 50-, and 60-min single specimens computed from the normal group were 12.5, 14.8, and 17.1 ppm, respectively. When reevaluating all patient data, using the 40-, 50-, or 60-min measures alone, no change in sensitivity and specificity occurred at all. Since the 40-, 50-, and 60-min data are strongly linear, following the

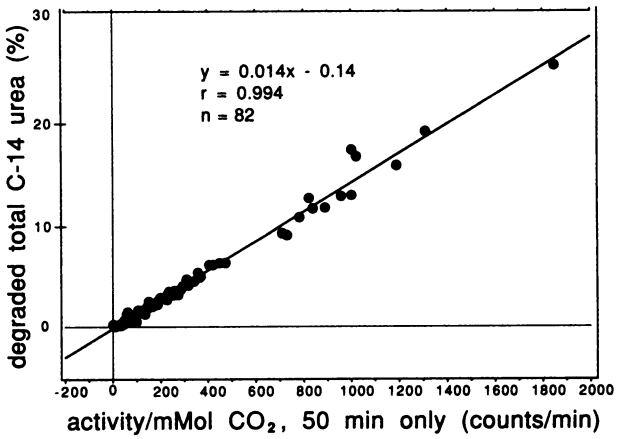

FIGURE 5
Linear regression analysis between the integrated measurement of ^{14}C-labeled CO_2 and the single measurement at 50 min of all 82 patient studies.

equation, ppm = 0.23 × min + 3.3, any upper limit of exhaled part/million of ^{14}C-labeled CO_2 can be interpolated for a breath sample taken at any time between 40 and 60 min. The upper limits for this validated time period are plotted in Table 2. In other words, a single breath sample of 1 mmol CO_2, obtained between 40 and 60 min after oral administration, is sufficient for accurate diagnosis and has the same validity as multiple sampling. This simplification will make the test more flexible, less expensive and, thus, applicable for practical routine use.

Using the 30- or 20-min data only, sensitivity and specificity declined slightly by 5% and 7% for the 30-min measurements, and by 9% and 11% for the 20-min measurements, respectively.

In the 23 patients who had been examined histologically, an excellent concordance with the breath test was found. In 11 out of 12 patients with positive histology, the breath test was also positive and in 10 out of 11 it was true-negative. In all 23 cases, there was 100% concordance of the histology findings with the CLO test, confirming our previous results (*16*).

DISCUSSION

In this study, a new noninvasive test for directly measuring urease activity and, thus, indirectly measuring *H. pylori* colonization in the upper GI tract was developed, validated, and analyzed. The test has good sensitivity and specificity and predictive diagnostic values. The excellent diagnostic performance found in this study is also in agreement with the published results (*8*) or preliminary reports of others (*9,11,13–15*). Thus, the test can be considered reliable, accurate, and efficient in terms of radiation burden on the patient (*8,17, 18*), with an estimated whole-body radiation dose of 50 to 100 μSv per test (*17*). Even the critical organs, such as fatty tissue, the skeleton and the lungs, only receive doses as low as 44, 180, and 3.6 μGy, respectively, even when using doses three times higher than the dose used in this study (*8*). Furthermore, small doses such as 110 kBq (3 μCi) of beta-radiating ^{14}C can be stored and administered in any medical institution without the need for sophisticated radiation protection measures.

TABLE 2
Upper Limits of Normal for Single-Sample Measurements for the Time Period 40–60 Min After Oral Application of ^{14}C-urea Interpolated from the Validated Data at 40, 50, and 60 Min (expressed in ppm/mmol CO_2) of the Total Dose

Sampling time (min)	Exhaled activity (ppm/mmol CO_2)	Sampling time (min)	Exhaled activity (ppm/mmol CO_2)
40	12.5	50	14.8
41	12.7	51	15.0
42	12.9	52	15.2
43	13.2	53	15.5
44	13.4	54	15.7
45	13.6	55	15.9
46	13.8	56	16.0
47	14.1	57	16.3
48	14.3	58	16.6
49	14.6	59	16.8
50	14.8	60	17.1

The use of the nonradiating ^{13}C-labeled urea instead of the ^{14}C isotope has also been demonstrated to be a successful approach (*19,20*). However, this procedure requires the somewhat more sophisticated and less accurate measurement of gaseous samples on a mass spectrometer.

The good agreement in terms of accuracy of the ^{14}C- or ^{13}C-urea breath test by different laboratories was reached despite different methodologic approaches. In the literature (*9,11,13–15*), these differences include different preloading meals, doses, dilution volume, sampling time, intervals and, finally, result calculations.

In order to make the ^{14}C-urea accessible to all who are interested, standardization and, more importantly, simplification are desirable. An analysis of the methods and results of the ^{14}C-urea test available so far (*8,9,11, 13–15*), together with the results of this study, allows the following suggestions to be made: The 110-kBq dose of ^{14}C-urea used in this and other studies (*9,13*) is definitely sufficient. Smaller doses such as 37 kBq will require longer breath sampling (*11*) but may be considered.

One major drawback of all the tests reported is the multiple sampling/multiple measuring of the beta-radiating ^{14}C-labeled CO_2 specimens, requiring technologic time for supervising the patient and a liquid scintillation counter. As documented in this study, the entire procedure can be reduced to a single-sample test without reducing the test accuracy as shown in Tables 1–2 and in Figure 5. This also reduces the handling of radioactive samples to only those that could be sent by ordinary mail to the next center with a liquid scintillation facility since they contain ^{14}C only in the part per million range of the dose. Thus, the simplified test could be performed in any medical institution, including private practice, without nuclear medicine facilities.

In conclusion, the ^{14}C-urea breath test for detecting *H. pylori* colonization in the upper GI tract is a reliable, accurate, and noninvasive test. It can be reduced to a single-sample test without loss in diagnostic accuracy and is amenable to standardization, widespread use, and possible commercial distribution.

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