Stability of ^{13}C -Urea Breath Test Samples Over Time in the Diagnosis of *Helicobacter pylori*

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Background: The accuracy and repeatability of breath test in the diagnosis of *Helicobacter pylori* infection have not been adequately investigated. Although it has been shown that storage for long periods does not affect the analysis results, no data are available on the effect of repetitive testing. In this study, our aim was to evaluate the repeatability of the analyses of breath samples at room temperature. **Methods:** A total of 202 positive breath samples were collected in duplicates, before and after administration of 75 mg ^{13}C -urea dissolved in 100 ml of orange juice. Breath test results were expressed as delta $^{13}\text{CO}_2$. The cut-off value was 3.5 parts per thousand. Each sample was analyzed in a mass spectrometer 7,

14, 21, and 28 days after collection. The accuracy calculation was based on the comparison of the delta $^{13}\text{CO}_2$ obtained in the three consecutive weeks following the first test run to the delta $^{13}\text{CO}_2$ obtained in the first test run. **Results:** Two hundred (99%), 197 (97.52%), and 196 (97%) of the 202 samples tested positive in the second, third, and fourth test runs, respectively. The accuracy of the delta $^{13}\text{CO}_2$ was 98.6%, 99.2%, and 96.7% in the three consecutive runs, respectively. **Conclusion:** Short-term storage of 1 month does not affect sample stability or the results of ^{13}C -urea breath tests in up to three consecutive repeats. J. Clin. Lab. Anal. 30:231–234, 2016. © 2015 Wiley Periodicals, Inc.

Key words: ^{13}C -urea breath test; reproducibility; test accuracy; *H. pylori* diagnosis; IRMS

INTRODUCTION

Helicobacter pylori (*H. pylori*) is a ubiquitous, flagellated gram-negative bacterium that infects approximately 50% of the world's population with an even higher prevalence in developing countries. Colonization of the gastric mucosa by *H. pylori* may cause chronic gastritis, which may advance to gastric and duodenal ulcers, mucosa-associated lymphoid tissue (MALT) lymphoma, and gastric adenocarcinoma (1, 2).

The exact route of transmission and subsequent infection of *H. pylori* in humans is incompletely understood. It has been suggested that human-to-human transmission is oral–oral, fecal–oral, or both, since *H. pylori* has been isolated from saliva, feces, gastroesophageal refluxate, and vomitus of infected subjects (3–5). It has been hypothesized that the oral–oral transmission is the primary route in developed countries and fecal–oral transmission (primarily via contaminated water sources) is more frequent in developing countries (6).

Diagnosis of *H. pylori* may be managed through invasive and noninvasive methods. Invasive methods include urease test, histology, and bacterial culture, and are based on endoscopy procedure in which biopsy is taken from the infected tissue. Noninvasive methods include serology, which is unreliable in confirming *H. pylori* eradication, due to the slow and unpredictable decline in antibody titer (7), stool antigen and the most reliable ^{13}C -urea breath test (UBT) (8, 9). UBT is also considered a safe laboratory method for *H. pylori* diagnosis, since the use of ^{14}C -labeled urea has been replaced with the ^{13}C , which

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TABLE 1. A Sample of the Test Results From Four Test Runs and the Calculation of the Delta and Accuracy of the UBTs

Sample	First run	Second run	Third run	Fourth run	Delta $^{13}\text{CO}_2$	Delta $^{13}\text{CO}_2$	Delta $^{13}\text{CO}_2$	Delta $^{13}\text{CO}_2$	Accuracy	Accuracy	Accuracy
					First run	Second run	Third run	Fourth run	Second run %	Third run %	Fourth run %
A1 ($T = 0$)	-26.019	-25.521	-25.431	-24.112							
A2 ($T = 30$)	-7.316	-7.132	-7.091	-5.559	18.703	18.389	18.34	18.553	98.32	98.06	99.20

is a nonradioactive carbon isotope. The use of a stable, nonradioactive isotope allows for repetition of the test as required, and is safe even in children and pregnant women (8, 10). Moreover, UBT is also considered highly accurate, with sensitivity and specificity values approaching 100%. Furthermore, UBT is a reliable means of assessing the success of *H. pylori* eradication (6, 10–12).

The interpretation of UBT is carried out using either isotope-ratio mass spectrometer (IRMS) or isotope-selective nondispersive infrared spectroscopy (NDIRS) and it is based on the ability of *H. pylori* to hydrolyze the covalent link between urea and ^{13}C , which is a unique feature of this pathogen (13). The accuracy and repeatability of UBT in the diagnosis of *H. pylori* infection have been a source for debate over recent years. Although it has been shown that storage for long periods does not affect the analysis results, no data are available on the effect of repetitive testing using tubes from the same sample (14–16).

Our laboratory provides UBT for *H. pylori* diagnosis to more than 4 million members of Clalit Health Services (CHS). CHS is the largest health insurer in Israel and the third-largest health maintenance organization in the world. Our laboratory receives samples from the entire country, with an annual turnover of more than 60,000 UBTs. The aim of the present study was to evaluate the repeatability of UBT analyses in our laboratory IRMS system.

METHODS

Sample Acquisition

Breath tests were conducted by dedicated nurses in community CHS clinics. Patients were given 75 mg of ^{13}C -labeled urea mixed with 100 ml of orange juice. Breath samples were collected at two intervals—prior to urea administration (T_0) and 30 min post urea administration (T_{30}). The samples composed of the T_0 and T_{30} collection tubes (Vacutainer, Becton, Dickinson and Company, NJ) were sent to our laboratory for ^{13}C UBT analysis for diagnosis of *H. pylori* infection. The tubes were analyzed by IRMS instruments (AP2003, Analytical Precision, UK).

Sample Analysis

A random sample of 202 positive and 22 negative samples corresponding to 448 tubes were selected for analysis. The results are expressed as the difference between the two scores—delta over baseline. The cutoff $\frac{^{13}\text{C}}{^{12}\text{C}}$ at $T_{30} - T_0$ was 3.5 parts per thousand (PPT), according to referral laboratories and the manufacturer's instructions. Any increase above 3.5 PPT was considered *H. pylori* positive.

Each pair of test tubes was analyzed by the IRMS system 7 days after collection, and then at intervals of 7 days for an additional 3 weeks. The accuracy calculation was based on the comparison of the results of the delta $^{13}\text{CO}_2$ obtained in the three consecutive weeks following the first run to the delta $^{13}\text{CO}_2$ obtained in the first run. Samples were stored in our laboratory at room temperature between each measurement.

Statistical Analysis

The results of the delta $^{13}\text{CO}_2$ from the first test run were considered as the baseline for evaluation. For every test run, the delta was calculated for each sample ($T_{30} - T_0$) in absolute numbers. The accuracy percentage was calculated by dividing each delta value from the second, third, and fourth test runs by the delta value of the first run and then multiplying the result by 100 (Table 1). The final accuracy values were obtained by calculating the mean of the total accuracy values for each of the samples in the same test run (Fig. 1, Table 2).

RESULTS

Test Accuracy and Reproducibility

Two hundred and two samples with delta value above 3.5 PPT and 22 samples with delta value equal to or lower than 3.5 PPT were selected for examination of reproducibility and test–retest accuracy. Of the 202 positive samples, 200 samples in the second test run were reproducible and the accuracy of the delta $^{13}\text{CO}_2$ measurement calculated was 98.6% when compared to the delta $^{13}\text{CO}_2$ of the first test run. In the third test run, 197 of the original 202 samples were reproducible and the accuracy was 99.2%. In the fourth and final test run 196 of the

Fig. 1. Summary of accuracy values of three consecutive UBT runs over baseline. The delta ¹³CO₂ of each of the positive and negative samples in the three consecutive test runs was compared to the delta ¹³CO₂ of the first test run (baseline). The accuracy values represent the mean of the total accuracy values for each of the samples in the same test run.

TABLE 2. Reproducibility of Consecutive Testing of Paired UBT Samples

	First run	Second run	Third run	Fourth run
Total positive samples (N)	202	200	197	196
Accuracy (%)	100	98.6	99.2	96.7
Reproducibility (%)	100	99.0	97.5	97.0
Total negative samples (N)	22	22	22	22
Accuracy (%)	100	96.4	99.2	98.0
Reproducibility (%)	100	100	100	100

original 202 samples were reproducible and the accuracy was 96.7% (Table 2, Fig. 1).

Of the 22 negative samples, 22 samples were reproducible in all three consecutive test runs post baseline. The accuracy values were 96.4%, 99.2%, and 98.0% in the second, third, and fourth test runs, respectively. (Table 2, Fig. 1).

Unreadable Samples

Of the 202 positive samples collected, two samples became unreadable by the IRMS instrument after the first

test run reducing the reproducibility percentage to 99%. Of the remaining 200 samples, an additional three samples became unreadable after the third test run, reducing the reproducibility percentage to 97.5%. Finally, after the fourth test run another single sample became unreadable, reducing the total reproducibility percentage to 97.0% (Table 2, Fig. 1). Interestingly, all negative samples were readable during the three consecutive test runs post baseline, preserving a reproducibility rate of 100% (Table 2, Fig. 1).

DISCUSSION

H. pylori remains a widespread bacterium throughout the world, and therefore accurate diagnosis followed by a successful eradication of the bacterium is an important objective of health-care providers and patients alike (1–3). UBT is considered an accurate and noninvasive laboratory procedure (8,9), and thus serves as the de facto gold standard for *H. pylori* diagnosis worldwide.

Consequently, *H. pylori* diagnosis in Israel is primarily achieved using UBTs that are sent to our laboratory for analysis in subjects prior to treatment and for confirmation of eradication. In contrast to other major laboratory systems, our high-yield IRMS instruments consume only

a small portion of the exhaled CO₂ in the sample tubes and, therefore, repetition of UBT on the same sample is feasible. In this aspect it is important to mention that NDIRS instruments, although quite accurate, are somewhat limited by their high consumption of gas in the UBT tubes. This allows for a maximum of two runs per sample (17).

Despite the fact that our measurements demonstrated high reproducibility values, five positive samples became unreadable by the IRMS instrument during the four consecutive test runs. We speculate that these samples had an insufficient concentration of exhaled CO₂ due to improper completion of the breath test at the remote clinics in the community. It is possible that the patient did not exhale enough CO₂ into either or both test tubes or they were not sealed properly. In fact, the only test-limiting variable is insufficient CO₂ concentration in the samples test tubes, thus proper examination and test tube handling should be exercised.

In this study we have evaluated the repeatability of UBT analysis over a period of 1 month, using 7-day intervals between measurements. According to our findings, it is apparent that 1-month storage at room temperature does not affect the sample's stability and does not significantly affect the test's diagnostic accuracy. More importantly, storage for 1 month does not affect the repeatability of the test, at least in three consecutive repeats. In conclusion, the UBT is a valid laboratory method for *H. pylori* diagnosis and eradication evaluation and it has high test-retest reliability.

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