

2

Urea Breath Tests for Detection of *Helicobacter pylori*

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Introduction

Helicobacter pylori

The association of *Helicobacter pylori* with peptic ulcer disease and gastric cancer was first proposed by Warren and Marshall in 1983 (Warren and Marshall, 1983). In February 1994, the National Institutes of Health Consensus Development Conference concluded that *H. pylori* infection is the major cause of peptic ulcer disease, and all patients with confirmed peptic ulcer disease associated with *H. pylori* infection should receive treatment with antimicrobial agents (Yamada et al., 1994). The International Agency for Research on Cancer Working Group of the World Health Organization categorized *H. pylori* as a group I, or definite, human carcinogen (Versalovic, 2003). Based on the data retrieved during the National Health Interview Survey of 1989, 10% of adult U.S. residents reported physician-diagnosed ulcer disease, among whom one third had an ulcer in the past year (Sonnenberg and Everhart, 1996). In developing countries, the prevalence of *H. pylori* carriers can be as high as 70–90%. Most patients acquire the infection at childhood. The prevalence of the infection in developed countries is lower, ranging from 25% to 50% (Dunn et al., 1997). Seroprevalence studies demonstrate an increasing rate in adults of 3–4% per decade (Cullen et al., 1993; Sipponen et al., 1996; Kosunen et al., 1997; Versalovic, 2003).

H. pylori-infected patients may develop chronic gastric inflammation that can be asymptomatic. Infection of *H. pylori* is associated with peptic ulcer disease (Dunn et al., 1997). *H. pylori* infection is also associated with gastric adenocarcinoma (Oconnor et al., 1996) and mucosa-associated lymphoid tissue (MALT) lymphoma (Isaacson, 1994). The American Medical Association published guidelines for testing and treatment of *H. pylori*-related disease (Peterson et al., 2000). The panel of experts recommends testing for *H. pylori* in patients with active ulcers, a history of ulcers, or gastric mucosa-associated lymphoid tissue lymphomas, and young patients with ulcer-like dyspepsia and those with family history should also be tested for *H. pylori*. Eradication of the infection leads to cure of the ulcers (Dunn et al., 1997). Treatment of the infection with antibiotics includes twice-daily triple

therapy with a proton pump inhibitor or ranitidine bismuth citrate, clarithromycin, and amoxicillin for 10–14 days (Peterson et al., 2000). A similar recommendation of triple therapy is also recommended by European *Helicobacter pylori* Study Group (Moayyedi, 1999). Multiple therapeutic regimens have been shown to be effective (Harris and Misiewicz, 1996; Dunn et al., 1997; Howden and Hunt, 1998; Gene et al., 2003a, 2003b; Versalovic, 2003). Metronidazole or clarithromycin should be included to achieve higher than 90% eradication rate (Dunn et al., 1997; Versalovic, 2003). The MOC therapy, which includes metronidazole, omeprazole, and clarithromycin for 7–14 days, has also been shown to offer greater than 90% eradication (Versalovic, 2003). The traditional FDA-approved triple therapy includes bismuth subsalicylate (two tablets, 262 mg), metronidazole (250 mg), and tetracycline (500 mg) taken four times daily for 14 days (Dunn et al., 1997). Because of the resistance problems, quadruple therapy (proton pump inhibitor, tetracycline, metronidazole, and a bismuth salt) has been used to improve the efficacy and is associated with fewer side effects (Dunn et al., 1997). However, a later meta-analysis shows only a slightly improved (statistically insignificant) eradication rate of the quadruple therapy compared with the traditional triple therapy, and there are no significant differences in compliance or adverse effects (Gene et al., 2003a).

Laboratory Diagnosis of *H. pylori* Infection

Detection of the Organism in Biopsy Tissue Specimens

Patients infected with *H. pylori* can be diagnosed by examination of biopsy tissue specimens obtained by endoscopy. The organism can be directly demonstrated in silver-stained histology tissue samples or in imprint cytology specimens stained with Giemsa or Gram stain.

H. pylori can be isolated from clinical tissue specimens (Versalovic and Fox, 2003). Special transport medium, microaerophilic culture environment, and extended incubation time (5–7 days) are required. The organism can be presumptively identified based on its microscopic morphology and positive reactions for catalase, oxidase, and urease tests. *H. pylori* can also be indirectly detected in the gastric biopsy tissue by testing its urease activity. This enzyme (organism) present in the specimen converts urea in the testing medium into ammonia. The elevated pH as a result of the reaction can be observed with a color pH indicator in the testing medium. These methods are reasonably sensitive, specific, and easy to perform. However, invasive procedures are required.

Antibody Detection by Serology Assays

H. pylori specific IgG can be detected in infected patient serum samples by using ELISA assays. IgG-negative patient samples can be followed by detecting specific IgA antibodies. These assays are commercially available in both laboratory-based

and point of care–based formats. They are easy to perform, relatively sensitive, and low cost. The disadvantage is that these antibodies may persist for months or years after eradication of the organism, and test results may need careful interpretation.

Urea Breath Tests

Urea breath tests detect current *H. pylori* infection. This test is based on production by *H. pylori* of powerful urease, an enzyme that converts urea to ammonium and carbon dioxide (CO₂) (Bazzoli et al., 1997; Vakil and Vaira, 2004). When infected with *H. pylori*, high urease activity is present in the stomach. A dose of urea labeled with either ¹³C or ¹⁴C is taken by the subject. The urease-catalyzed reaction then takes place in the mucus layer. The labeled CO₂ diffuses to the epithelial cells and then is carried in the bloodstream and ultimately is released in the exhale. The labeled CO₂ in the subject's breath can be measured. The amount of the labeled CO₂ is related to the urease activity, which indicates the presence or absence of *H. pylori* infection (Bazzoli et al., 1997; Logan, 1993; Vakil and Vaira, 2004). The amounts of the isotopic CO₂ can be measured by various techniques, and the results are expressed relative to the endogenous CO₂ production. The sensitivity and specificity of breath tests range from 95% to 97%, although this method has been reported to be less reliable for patients with gastric surgery or in patients who take proton pump inhibitors or ranitidine (Vakil and Vaira, 2004). In a study involving 20 volunteers, Cutler et al. found that ranitidine at standard dose (150 mg b.i.d.) or high dose (300 b.i.d.) does not decline breath test results reproducibly, and ranitidine does not need to be discontinued before a urea breath test (Cutler et al., 1998).

¹⁴C-Urea Breath Test

Conventionally, patient preparation for the test requires fasting for at least 4 h and oral ingesting of 5 μ Ci ¹⁴C-urea in 20 mL water. Breath is collected 20 min postdosing in a CO₂-absorbing solution (examples are hyamine-methanol solution with a pH indicator or benzethonium hydroxide-methanol with a pH indicator) (Marshall et al., 1991; Desroches et al., 1997; Rollan et al., 1997). Radioactivity in the sample is measured by a scintillation counter, and the result is expressed as counts per minute (cpm) or as specific activity at a specific postdosing time (AS_{time}) (Marshall et al., 1991; Desroches et al., 1997; Rollan et al., 1997).

$$AS_{\text{time}} = (\%^{14}\text{CO}_2 \text{ dose excreted} / \text{mmol of CO}_2) \times \text{weight (kg)}$$

where the ¹⁴C-urea dose is calculated from measurements of standard solutions with known concentrations of ¹⁴C-urea, and ¹⁴CO₂ dose excreted = counts at the specific time – counts at baseline. This parameter is also corrected for the patient's weight (Desroches et al., 1997). The initial ¹⁴C-urea test using β -scintillator is suitable for diagnosis of *H. pylori* as well as confirmation of eradication

of *H. pylori* after antibiotic treatment (Marshall et al., 1991; Desroches et al., 1997; Rollan et al., 1997).

The two parameters that have been subjected to modification are the ^{14}C -urea dose and breath-collection times (Kao et al., 1993; Abukhadir et al., 1998). A reduced dose of ^{14}C -urea to 1 μCi has been shown to be highly sensitive and specific (same as the initial test) for both diagnosis and post-treatment confirmation of eradication. (Hegedus et al., 2002; Raju et al., 1994). Further reduction of the collection time to 10 min post- ^{14}C -urea dosing has been shown to be appropriate for the clinical diagnosis of *H. pylori* (Ozturk et al., 2003; Peura et al., 1996). Though the dose of radioactive ^{14}C -urea is minimal, strict regulations have to be followed to ensure the patient's safety. The test has not been approved for use in pregnant women and children.

^{13}C -Urea Breath Test

^{13}C -urea breath test is considered a standard noninvasive test for both initial diagnosis and eradication confirmation. Compared with the ^{14}C -urea breath test, ^{13}C -urea is a nonradioactive substance, and no special handling is necessary (Logan, 1993). The general procedure is to take a simple test meal to delay gastric emptying and maximize the distribution of ^{13}C -urea after fasting followed by ingesting the ^{13}C -urea dose in water or tablets. If the ^{13}C -urea dose is taken in water solution, immediate mouth-rinsing with water is recommended to prevent false-positive results caused by oral bacteria with urease activity (Epple et al., 1997; Liao et al., 2002; Ohara et al., 2004; Oksanen et al., 1997; Peng et al., 2001). This mouth-rinsing step can be eliminated by taking a film-coated tablet-formulated ^{13}C -urea dose that is not soluble in the oral cavity but readily soluble in the stomach (Ohara et al., 2004). A breath sample is then taken at both baseline and the specified postdose time points, usually at 20 or 30 min. The conventional detection of the breath is by isotope ratio mass spectrometer (IRMS) that differentiates $^{13}\text{CO}_2$ and $^{12}\text{CO}_2$. Less expensive gas chromatography-mass spectrometry (GC-MS) has also been used to measure the specimens (Lee et al., 1998). The ^{13}C element is a nonradioactive isotope of ^{12}C with a natural relative abundance of 1.11% (Silverstein and Webster, 1998). The delta over the baseline of $^{13}\text{CO}_2$ excess is used as the diagnostic parameter. The formula is expressed as the following (Oksanen et al., 1997):

$$\delta = \frac{(R_{\text{sample}} - R_{\text{ref}})}{R_{\text{ref}}} \times 1000\text{‰}$$

where R is the ratio of $^{13}\text{CO}_2$ to $^{12}\text{CO}_2$ in the sample and in a reference gas. The reference gas is an international primary standard, PD belemnite calcium carbonate (Logan, 1993). The test results are expressed as the difference in relative enrichment between predose and postdose breath samples (delta over baseline, or DOB) (Oksanen et al., 1997). Cutoff values vary with various ^{13}C -urea doses, different test administration methods including formulation of ^{13}C -urea and test meals, sample collection time, and detection techniques.

Other detection techniques have been developed to reduce the initial cost of mass spectrometry. Based on the slightly different absorption spectra between $^{13}\text{CO}_2$ and $^{12}\text{CO}_2$, the ratio of $^{13}\text{CO}_2/^{12}\text{CO}_2$ can be accurately determined by nondispersive isotope-selective infrared spectrometer (NISIR). The sensitivity and specificity of the ^{13}C -urea breath test using NISIR are comparable with those measured by mass spectrometer (Braden et al., 1999; Savarino et al., 1999; Isomoto et al., 2003; Kato et al., 2004). This detection technique is less expensive compared with mass spectrometry. It can also be placed in a regular laboratory, clinics, and even in a doctor's office (see "FDA Approved Tests," below).

Another technique to detect ratio of $^{13}\text{CO}_2/^{12}\text{CO}_2$ is laser-assisted ratio analyzer (LARA). The detection principle is based on the optogalvanic effect, which is an electrical signal in response to optical stimulation of a resonance transition in an electrical discharge species. The optogalvanic effect is due to changes in the effective electrical impedance of the gas discharge, which results from an optically induced change in the electron energy distribution function in the molecules. The laser-induced stimulation modifies ionization rate in the discharge cell, which enables measurement of electron energy to determine the gas concentration in the specimen (Braden et al., 2001; Murnick and Peer, 1994). The LARA is based on two unique light sources: $^{13}\text{CO}_2$ and $^{12}\text{CO}_2$ charging lamps. The use of the two charging lamps ensures that light absorption is due to the existence of $^{13}\text{CO}_2$ and $^{12}\text{CO}_2$ only in the gas mixture. It also reduces the background radiation leading to a highly sensitive and specific technique (Shirin et al., 2001). The application of this technique has been proved to be an effective alternative to the traditional IRMS (Minoli et al., 1998; Cave et al., 1999; Savarino et al., 2000; Braden et al., 2001; Shirin et al., 2001).

Since its description using 350 mg of ^{13}C -urea (Graham et al., 1987) the test has been modified extensively on two major areas to reduce the cost and increase the comfort level: ^{13}C -urea dose and duration of the test. Reduction of ^{13}C -urea dose to 100 mg for a test duration of 30 min without a test meal has been shown to be highly sensitive and specific (Oksanen et al., 1997). Tests employing a dose of 100 mg or 75 mg ^{13}C -urea for duration of 30 min have been proved to be as accurate and less expensive compared with larger doses (Epple et al., 1997; Labenz et al., 1996; Liao et al., 2002; Oksanen et al., 1997). The test meal can be milk, orange juice, or a citric acid solution (Epple et al., 1997; Hamlet et al., 1999; Labenz et al., 1996; Liao et al., 2002). Reduction of dose to 50 mg ^{13}C -urea and test duration to 15 min have also proved to be sufficient (Liao et al., 2002). Further modification using a tablet containing 50 mg ^{13}C -urea and 456 mg citric acid without a test meal for duration of as short as 10 min provides sufficient sensitivity and specificity when endoscope was used as a "gold standard" diagnosis of *H. pylori* infection (Gatta et al., 2003; Wong et al., 2003). Ingestion of 100 mg ^{13}C -urea in 50 mL water with no test meal after 6 h fasting, the earliest optimal time for discriminating *H. pylori*-positive and -negative patients is 2 min with endoscopic administration and 6 min with conventional method of administration (Peng et al., 2001). Another study involving 202 patients shows no significant difference between the conventional tests (75 mg ^{13}C -urea in 50 mL water) with and without a test meal (200 mL 0.1 N citric acid) (Wong et al., 2000).

A further modification incorporating the endoscope technique shows highly accurate diagnosis of *H. pylori* and confirmation of eradication (Suto et al., 1999). The most important feature of the technique (endoscopic ^{13}C -urea breath test; EUBT) is the direct spray of ^{13}C -urea over the entire gastric mucosa under observation endoscopically. However, this technique requires a lot of patient preparation, including oral intake of 80 mg dimethylpolysiloxane to remove adherent gastric mucus 10 min before the endoscope, oral intake of 200 mg lidocaine to anesthetize the pharyngeal areas, and intramuscular injection of 20 mg scopolamine butylbromide 5 min before the endoscopy (Suto et al., 1999).

The ^{13}C -urea breath test is not affected by bleeding peptic ulcers, whereas the sensitivity of the rapid urease test is decreased significantly (Wildner-Christensen et al., 2002). One drawback with the ^{13}C -urea breath test is that equivocal or false-negative results often occur in patients on antisecretory medications. This problem could be resolved by taking the ^{13}C -urea in a tablet formulation supplemented with citric acid (Hamlet et al., 1999).

The diagnosis of *H. pylori* using a ^{13}C -urea breath test has been explored in infants and adolescents. The commonly accepted method using 75 mg ^{13}C -urea with breath samples taken at baseline, 20 min, and 30 min was shown to be highly sensitive (100%). The specificity is lower in children less than 6 years of age (88.1% vs. 97.8%) compared with the older group. Because of some overlap, definition of a gray zone seems to be appropriate (Kindermann et al., 2000). This method has also been shown to have excellent sensitivity and specificity for confirmation of eradication of *H. pylori* (100%) in 72 children aged 3–18 years. The diagnostic specificity (95%) and sensitivity (100%) have also been shown to be comparable with histology, rapid urease test, and serology (Yoshimura et al., 2001). Reduction of ^{13}C -urea dose to 50 mg in children is sufficient for diagnosis of *H. pylori* (Bazzoli et al., 2000; Kawakami et al., 2002; Canete et al., 2003). A fatty test meal and 50 mg ^{13}C -urea with breath sampled at 30 min have been shown to give the best sensitivity (98%) and specificity (98%) in a multicenter study (Bazzoli et al., 2000).

FDA-Approved Tests

As shown in Table 2.1, urea breath tests from two companies have been approved by the FDA for *H. pylori* diagnosis (U.S. Food and Drug Administration, 2004).

BreathTek (Meretek Diagnostics, Inc., Lafayette, CO, USA) is an FDA cleared and CLIA nonregulated test (Meretek Diagnostics, 2004). It is claimed to be simple, with no special in-office licenses or personnel needed to perform the test. The test can be administered in a doctor's office, clinic, or patient service center. The patient should abstain from antibiotics, proton pump inhibitors, and bismuth 14 days before the initial testing or 4 weeks prior to testing for confirmation of eradication. Though H_2 antagonists are not in the list, discontinuation of H_2 antagonists 24 h prior to the testing is recommended. The patient is also required not to have anything in his or her mouth 1 h prior to the testing. Immediately after a baseline breath sample is collected by blowing into a collection bag (or duplicate collection

TABLE 2.1. Comparison of ^{13}C -urea breath tests for *H. pylori* based on information at manufacturers' Web sites.

Test name	FDA status	Fasting	Detection	Sample collection time	Instrument time	Sensitivity ^a	Specificity ^a	Manufacturer
BreathTek	IVD ^b	1 h	GIRMS, or UBiT-IR300	0 and 15 min	Sent to specialty lab (GIRMS) 5.5 min (UBiT-IR300)	95%	95%	Meretek
Helikit	IVD ^b	4 h	IRMS, or ISOMAX 2002	30 min	Sent to specialty lab (IRMS) not available (ISOMAX)	98%	95%	Isodiagnostika

^a Based on statements in manufacturers' product inserts.

^b For in vitro diagnostic use.

tubes for GIRMA) to determine the initial ratio of $^{13}\text{CO}_2$ and $^{12}\text{CO}_2$, the patient is given a lemon-flavored Pranactin-Citric solution by mouth. Each 3-g dose of the Pranactin-Citric powder is supplied in a polyethylene-lined foil pouch containing 75 mg ^{13}C -urea, citric acid, aspartame, and mannitol. The second breath sample is then collected 15 min after the dose ingestion by blowing into the second collection bag (or duplicate collection tubes for GIRMA). Urease produced by *H. pylori* hydrolyzes ^{13}C -Panactin-Citric to form $^{13}\text{CO}_2$, which is expelled and detectable in the second breath sample. The system uses a Gas Isotope Ratio Mass Spectrometer (GIRMS) or an UBiT-IR300 Infrared Spectrometer for the measurement of $^{13}\text{CO}_2$ and $^{12}\text{CO}_2$ in breath samples. GIRMS assay has to be performed by Meretek Clinical Laboratory or other qualified laboratories licensed by Meretek. Quality checks have to be performed on all final results: each specimen must contain at least 1.5% volume CO_2 to assure adequate breath for analysis; the relative abundance of the baseline has to be in the range of -27.0 to -17.0 delta per milliliter; the DOB result must be greater than -1.0 . Analysis by UBiT-IR300 spectrometer can be set up and operated by each individual laboratory or test facility. The result is provided as delta over baseline, which is defined as the difference between the ratio $^{13}\text{CO}_2$ and $^{12}\text{CO}_2$ in the postdose specimen and the corresponding ratio in the baseline specimen. A cutoff of 2.4 is for both initial diagnosis and post-treatment monitoring of *H. pylori*. However, the test performance of persons under 18 years of age has not been established. There is also no established correlation between the number of *H. pylori* organisms in the stomach and the breath test results (Meretek Diagnostics, 2004).

Helikit (Isodiagnostika, Edmonton, Alberta, Canada) also incorporates ^{13}C -urea formulation with possibilities of both IRMS and infrared point-of-care (ISO-MAX2002) detections. The postdose breath collection is set at 30 min, and the sensitivity and specificity are claimed to be 98% and 95%, respectively (Isodiagnostika, 2004).

BreathID (Oridion BreathID Ltd., Jerusalem, Israel) has been considered as a test for investigational purposes. The detection of $^{13}\text{C}/^{12}\text{C}$ is achieved by LARA via continuous breath sampling at a point-of-care environment. The BreathID technology enables health care providers to perform the breath test by pushing a single button, and results are printed within 10 min in most cases. It is also claimed that this technology is suitable for pediatric testing (Oridion BreathID Ltd., 2004).

In summary, urea breath tests for diagnosis of *H. pylori* detect active infection. They are noninvasive and highly accurate. Newer assay formats and instruments are much simpler, more cost effective, and more user friendly and thus are the alternative choices for clinical diagnosis.

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