

Nondispersive Infrared Spectrometry: A New Method for the Detection of *Helicobacter pylori* Infection with the ^{13}C -Urea Breath Test

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Nondispersive infrared spectrometry (NDIRS) was used to detect *Helicobacter pylori* infection with the ^{13}C -urea breath test. The results were compared with those of standard isotope ratio mass spectrometry (IRMS). Both methods accurately distinguished between *H. pylori*-positive and *H. pylori*-negative individuals. The results demonstrate that NDIRS technology is accurate and therefore of equal value to standard IRMS for detection of *H. pylori* infection. It can be recommended for routine clinical application. As NDIRS technology is much cheaper than current IRMS machines, we consider the new method extremely useful for clinical applications.

Helicobacter pylori is a gram-negative bacterium that causes infection of the gastric mucosa in humans. A significant amount of evidence has been accumulated in the past decade that shows that *H. pylori* causes chronic superficial gastritis [1] and that there is a strong association between *H. pylori* infection and peptic ulcer disease [2].

A fundamental principle for specific antimicrobial therapy is accurate diagnosis. There are several validated methods for diagnosis of *H. pylori* infection, and they can be divided into invasive and noninvasive tests. The invasive tests include endoscopy, with biopsy specimens examined histologically for *H. pylori*, microbiological culture, and direct detection of urease activity in the gastric tissue. Noninvasive tests include serology and breath tests. ^{13}C -labeled urea is used to detect the presence of *H. pylori* infection by a breath test [3]. The test is highly sensitive and specific, and it is considered by many to be the "gold standard" of noninvasive testing [4, 5]. The ^{13}C -urea breath test has been applied to thousands of patients and healthy volunteers, and excellent epidemiological data have been obtained regarding children and adults as well as patients with peptic ulcer disease [6–9].

Up to now, stable isotope analysis of breath samples of urea breath tests was carried out by very sensitive—but equally expensive—isotope ratio mass spectrometry (IRMS). The high costs of these analyzers and the need for skilled personnel have therefore limited availability of this technology. Recently, a new method was developed to measure $^{13}\text{CO}_2/^{12}\text{CO}_2$ ratios, called isotope-selective, nondispersive infrared spectrometry (NDIRS), with the aim of a broader application of $^{13}\text{CO}_2$ breath

tests in clinical routine [10]. Initial studies suggested that NDIRS appeared to be of equal value to the conventional IRMS for the analysis of $^{13}\text{CO}_2$ in breath samples [11]. Therefore, the aim of the present study was to measure $^{13}\text{CO}_2$ concentrations with a new, commercially available NDIRS analyzer and to compare the results to those of standard IRMS analysis in a series of patients with defined *H. pylori* status.

Methods

Forty patients who were referred for routine $^{13}\text{CO}_2$ urea breath tests after an overnight fast were investigated. For the purpose of this study, the standard IRMS breath test was considered diagnostic of *H. pylori* status [7–9, 11], and no additional endoscopic, histologic, or cultural studies were done. Breath samples were collected in parallel in aluminized breath bags and into 15-mL Vacutainers (Becton Dickinson, Sparks, MD) during fasting and 30 minutes after ingestion of 100 mg of ^{13}C -urea dissolved in 30 mL of water and diluted in 250 mL of orange juice, which was used to delay gastric emptying.

Vacutainers were analyzed by IRMS (VG Isotech, Middlewich, Cheshire, UK). NDIRS analysis was done in duplicate with the bags directly connected to the spectrometer, which allows on-line analysis of breath samples (IRIS Analysator, Wagner Analysen Technik, Worpswede, Germany). For both methods, $^{13}\text{CO}_2/^{12}\text{CO}_2$ ratios were expressed as delta ^{13}C values relative to the Pee Dee Belemnite standard [12]. An increase of the delta ^{13}C value over baseline of more than 5‰ was considered *H. pylori*-positive, as established previously with IRMS in different studies [7–9, 11]. The operators analyzing the breath samples on IRMS and NDIRS, respectively, were not aware of the *H. pylori* status or the results of the other tests. Agreement between the two methods and repeatability were assessed according to Bland and Altman [13].

Results

The individual test results, expressed as delta over basal (DOB) (‰) and analyzed by both IRMS and NDIRS, are given

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in figure 1. The fact that the results analyzed by both methods lie close along the line of identity is not surprising, as both methods quantify the same samples. More important is the clinically relevant fact that both methods accurately distinguish between *H. pylori*-positive and *H. pylori*-negative subjects. Neither method produced false-negative or false-positive results as judged by a cutoff value of 5‰; the subject with an arbitrary DOB value of 5.1‰ as analyzed by IRMS showed a clearly positive value of 6.7‰ by NDIRS (figure 1).

To more precisely compare the new NDIRS with the established IRMS method, the sensitivity of which is higher than needed for clinical purposes, the measured differences of individual results were plotted against the mean of both results (figure 2). The middle line (-0.23%) depicts the mean of all differences, whereas the upper and lower lines indicate ± 2 standard deviations of the differences. If the differences follow a normal distribution, 95% of the differences have to lie between ± 2 standard deviations, which is the case with our data, as only two of 40 values are slightly outside these limits. Provided differences within mean ± 2 standard deviations would not be clinically relevant, both methods, IRMS and NDIRS, can be used as measurement methods to analyze $^{13}\text{CO}_2$ breath samples. We refer to these differences as the "limits of agreement." For the results the mean difference is -0.23 DOB‰ and the standard deviation is 1.11 DOB‰.

Thus, both methods provide results that are largely acceptable for clinical purposes. Although there is a tendency of the

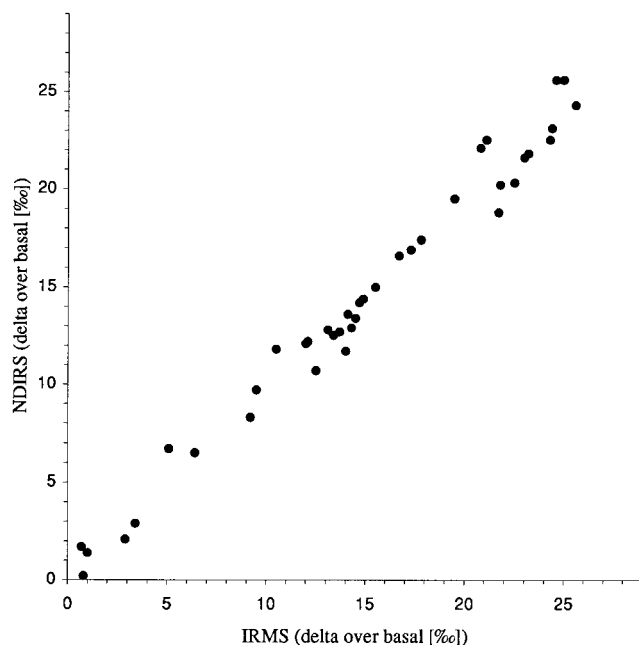


Figure 1. Individual results of ^{13}C -urea breath tests analyzed by IRMS (isotope ratio mass spectrometry) and NDIRS (nondispersive infrared spectrometry). Data are expressed as delta over basal (DOB‰). Values below 5‰ are considered as representative of *H. pylori*-negativity.

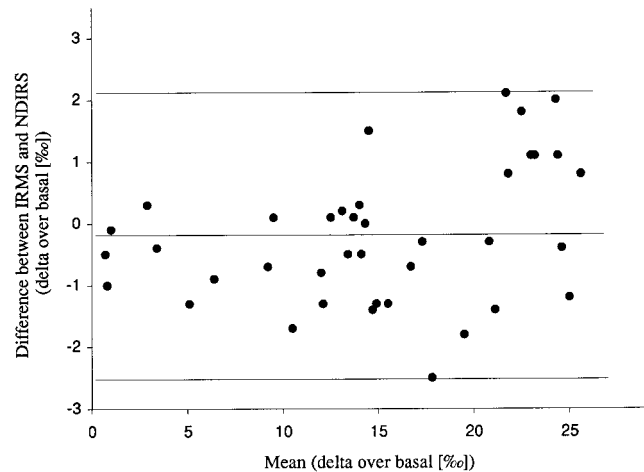


Figure 2. Agreement of the two different methods. The difference between the results of the same breath sample analyzed by IRMS (isotope ratio mass spectrometry) and NDIRS (nondispersive infrared spectrometry) is plotted against the mean value of both results. The middle line represents the mean of the differences; the upper and lower lines represent ± 2 SD. Data are expressed as delta over basal (DOB‰).

difference to increase with higher values of the average DOB value (figure 2), this potential bias does not interfere in the more important lower region of values (i.e., DOB 5‰), where the measurements have to discriminate between baseline and elevated values, thus discriminating between *H. pylori*-positive and *H. pylori*-negative individuals.

Another important parameter of the quality of a method is the repeatability of measurements. Again, we expect 95% of the differences to be less than 2 standard deviations, which is the definition of the coefficient of repeatability according to the British Standards Institution [14]. Figure 3 shows the differences between two measurements of the same breath sample analyzed by NDIRS, plotted against the mean of both values. There does not appear to be any relation between the difference and the absolute value of the DOB measurements. The mean was close to zero (-0.14%) and the standard deviation was 0.82‰, resulting in a coefficient of repeatability of 1.64‰ for NDIRS.

Discussion

The urea breath test is probably at present the most popular breath test used in clinical medicine. It is most useful for epidemiological studies of the prevalence of *H. pylori* and for evaluating the therapeutic success of antimicrobial treatment of *H. pylori*-infected patients with ulcers. However, the high costs and complexity of IRMS equipment have limited application of the test.

The development of a simple and rapid technique therefore offers new possibilities. NDIRS has several advantages com-

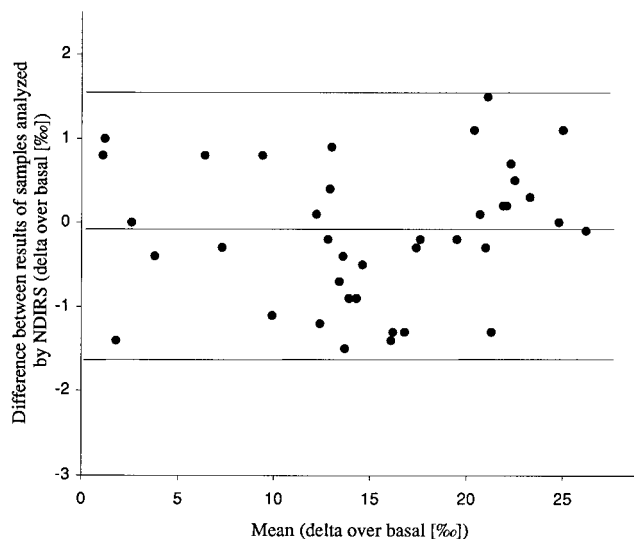


Figure 3. Repeatability of measurements by NDIRS (nondispersive infrared spectrometry). The difference between two measurements of the same breath sample analyzed by NDIRS is plotted against the mean value of both results. The middle line represents the mean of the differences; the upper and lower lines represent ± 2 SD. Data are expressed as delta over basal (DOB%).

pared with IRMS analysis: it gives on-line results, the tabletop analyzer needs only a plug and can be operated with minimal training, and it costs less than conventional IRMS analytcs. The immediate availability of the test results makes it especially effective for routine clinical practice. Koletzko and co-workers have compared the procedure to H₂-breath testing for lactose intolerance, underlying this comparison with a statement about how easy the procedure can be [11].

Summary

The present study confirms and extends previous results and clearly demonstrates that the method is sensitive enough to identify *H. pylori*-positive and *H. pylori*-negative individuals. Furthermore, because of the agreement of the results measured with different methods and the coefficient of repeatability, NDIRS is therefore of equal value to the standard IRMS technology for routine clinical application. It can be foreseen that further applications of ¹³C breath tests such as gastric emptying studies or liver function tests, which require frequent analysis of breath samples, are excellent possibilities for NDIRS.

The main disadvantage of NDIRS is the amount of gas required for analysis: presently 500 mL of air is necessary for a continuous flow, which makes it difficult to mail samples or to use the method for small children. Furthermore, NDIRS can

be used only for breath tests involving ¹³C-labeled substrates. However, this is not a real disadvantage, as all presently available substrates used in clinical tests are based on ¹³C compounds. We therefore consider NDIRS extremely useful for clinical practice.

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