Using a Cutoff of <10 ppm for Breath Hydrogen Testing: A Review of Five Years' Experience*

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ABSTRACT

To assess the clinical use of the breath hydrogen test in a large community hospital using a <10 ppm cutoff, we reviewed 222 tests performed over a 5-year period to evaluate patients for disaccharidase deficiency or bacterial overgrowth of the small intestine. Of these, the vast majority (195) were for lactose malabsorption, although fructose (17), sucrose (8) and lactulose (2) were also occasionally administered. One hundred eleven tests (50 percent) were positive, with an increase of at least 10 ppm hydrogen above the fasting level and a maximum value most commonly observed (42.3 percent of the time) at 3 hours post-administration. Only 34 patients (15.3 percent) had symptoms noted during the test, as compared with 185 (83.3 percent) who had experienced persistent intestinal problems prior to the test. Recent conditions which may have caused intestinal distress, such as transient disaccharidase deficiency, infections, surgery or other disorders like Crohn's disease, ulcerative colitis or food poisoning, were recorded in only 14 cases. Patterns consistent with bacterial overgrowth of the small intestine were observed in only 3 cases. Of 111 positives, 9 cases had increases between 10 and 20 ppm hydrogen and 7 showed the increase in the 3-hour sample, possibly reflecting a delayed transit through the intestine. Final diagnoses in 6 of these where information was available were for conditions other than malabsorption. We conclude that using a rise of 10 ppm to interpret a positive test does not contribute significantly to an increased frequency of false positives, but that patients with increases between 10 and 20 ppm probably are not lactase deficient.

Introduction

Since its introduction as a diagnostic test in 1969 by Levitt1 and Calloway,2,3 the breath hydrogen test has gained in popularity for the diagnosis of intestinal disaccharidase deficiency.4,5 It is currently preferred by gastroenterologists and has largely replaced the lactose and sucrose tolerance blood tests for this purpose because it is non-invasive and is considered more sensitive than the traditional blood glucose based tests.6,7 In selected conditions like celiac disease, it may be preferable to enzyme analysis of biopsy because it measures the response of the entire intestinal tract,
not just a small area which may not demonstrate enzyme deficiency in these cases.\textsuperscript{8} Its usefulness in blind loop syndrome has been described by Nose, et al.,\textsuperscript{9} and other applications and considerations relative to performance of the test have been reviewed by Solomons.\textsuperscript{6}

Testing can be done on breath samples collected by any of a variety of devices for the purpose, and studies have shown essentially equivalent results with the postnasal catheter, nasal prong, Rahn-Otis end-tidal sampler, Hal dane-Priestley tube and even a modified party toy, the “Wiggin’s Blowout.”\textsuperscript{10} Because of the relative simplicity of the test and the associated equipment, it is commonly performed in physician offices and may therefore be less familiar to hospital-based laboratorians. Our laboratory has performed this test since 1983 and, for the past 5 years, has been a referral center for area physicians. Data presented here review our experiences during that period, with the effects of using an increase of 10 ppm versus 20 ppm as a cutoff for a positive test.

**Methods**

Breath samples are collected in 60 ml Becton-Dickinson Luer-tip plastic syringes using a nasal prong prepared by removing the needle from a butterfly kit and inserting the tubing end into a small hole cut into the side of a 6 cm segment of 1/8 inch inner diameter Tygon tubing.\textsuperscript{11} The patient holds the prong to one nostril, with the butterfly tubing toward the nostril, while closing off the other nostril and exhaling. The female end of the butterfly is connected to the syringe by means of a stopcock, which is closed after 50 ml is collected. Patients are asked to exhale as long as possible with each breath and only the last 5 ml is drawn into the syringe to minimize the likelihood of contamination by room air. The test requires an overnight fast and usually involves administration of 50 ml of a 20 percent solution of the test substance (10 g) with an upper limit of 50 g. The dose is age-adjusted for the pediatric population.

Specimens collected include a fasting, 30, 60, 90, 120 and 180 minute sample when testing for malabsorption. If bacterial overgrowth is suspected, samples are collected every 10 minutes for the first 70 minutes, then every 20 minutes to 130 minutes, with a final sample at 180 minutes. Collected samples are labeled and tested for both hydrogen and carbon dioxide using a Quintron Model 12 Microlyzer\textsuperscript{a} for hydrogen and a Quintron Alveolyzer\textsuperscript{a} for carbon dioxide. Reliably filling the sample loop of the analyzer requires 20 ml of sample, so a minimum of 45 ml of breath must be collected to make both measurements. Samples received late in the day may be held overnight and tested the next day. The analyzers are calibrated with a standard gas mixture of approximately 100 ppm hydrogen and 4.5 percent carbon dioxide\textsuperscript{b} and a different tank is used as a quality control check.

Tests are interpreted as positive for lactose malabsorption if they show a rise in breath hydrogen of at least 10 ppm above the fasting level. Interpretive criteria using fructose, glucose or lactulose as the test substance are not as well standardized. Bacterial overgrowth is consistent with a 10 ppm rise over the fasting level using glucose as the test substance, or an early peak at 20, 40, or 60 minutes using lactulose as the test substance, which is clearly distinguishable from a later peak representing arrival of substance at the colon. Hydrogen levels are corrected using a factor generated by the Alveolyzer, which is the ratio of the theoretical alveolar CO\textsubscript{2} of 5.0 percent over the actual CO\textsubscript{2} value measured for that sample. Correcting hydrogen values to a standard alveolar carbon dioxide level this way compensates for poor collection or shallow breathing by a patient who has difficulty cooperating with the collection.

**Results**

Of 222 breath tests done during the past 5 years, 195 were for lactose, 17 were for fructose.

\textsuperscript{a}Quintron Instrument Co., Inc., Division of E. F. Brewer Co., 13901 Main Street, Menomonee, WI 53051

\textsuperscript{b}QuinGas, Part # QT07031-G, Quintron Instrument Co., Inc.
tose and 8 were for sucrose malabsorption. Only 2 involved lactulose as the test substance. The majority of tests were done in females, 136 versus 86 in males, and exactly half (111) showed positive results for malabsorption. A history of persistent intestinal difficulty was noted in 185 cases, but only 34 patients had symptoms recorded during the test, which may reflect incomplete recording. In some cases, patients complained only of nausea or dizziness, which could be effects of a dumping syndrome on the duodenum and not due to the underlying disease.

Figure 1 illustrates the distribution of patients by age and indicates that 50 percent (111) are in the pediatric age range, with the largest number in the group less than 11 years of age. Lactose intolerance is believed to be a common cause of abdominal pain and diarrhea in pediatric patients. Regardless of age, lactose is the predominant substance administered because lactase deficiency may develop in childhood, or at older ages in patients previously tolerant of lactose. Table I shows data on the frequency of positive results with each of the substances tested and indicates that lactose is by far the most prevalent substance tested in our patient set. In practice, a physician would almost always begin an investigation of chronic intestinal distress with a lactose breath hydrogen test and possibly proceed to other disaccharides if that were negative. Although the normal response for fructose is not well established, the diagnostic usefulness of fructose testing appears to be significantly

<table>
<thead>
<tr>
<th>Substance</th>
<th>Total Number</th>
<th>Positive Number</th>
<th>Positive Percent</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lactose</td>
<td>195</td>
<td>94</td>
<td>48</td>
</tr>
<tr>
<td>Fructose</td>
<td>17</td>
<td>14</td>
<td>82</td>
</tr>
<tr>
<td>Sucrose</td>
<td>8</td>
<td>2</td>
<td>25</td>
</tr>
<tr>
<td>Lactulose</td>
<td>2</td>
<td>1</td>
<td>50</td>
</tr>
</tbody>
</table>

**TABLE I**

Frequency of Positives

**Figure 1.** Age distribution of patients for breath hydrogen testing.
higher than that for the other substances with positives in 82 percent of cases versus only 48 percent for lactose. Based on our experience, the frequency of bacterial overgrowth testing is quite low.

Figure 2 graphs the timing of the peak breath hydrogen level in positive tests and shows that the majority occur after 1.5 hours, with the largest number at 3 hours. Two of 3 cases with peak values at one-half hour were reported as possible bacterial overgrowth, one of which had short bowel syndrome, and the third case had a flu at the time of testing. Table II presents specimen stability data gathered during parallel testing and compares results from our laboratory on specimens held overnight before testing, to those sent to a reference laboratory considered to be the gold standard for the test. Reference specimens were sent in evacuated, stoppered glass tubes and assayed on receipt by the reference laboratory. Although there are quantitative differences in the levels, the correlation of results indicates that specimens remain stable in the closed syringes for at least 18 hours.

The instrument response using serial dilutions of the calibrating gases with room air is linear between 0 and the calibrating value of

<table>
<thead>
<tr>
<th>Specimen Time</th>
<th>ppm Hydrogen</th>
<th>Sendout</th>
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</thead>
<tbody>
<tr>
<td>10:00</td>
<td>12</td>
<td>8</td>
</tr>
<tr>
<td>10:45</td>
<td>6</td>
<td>6</td>
</tr>
<tr>
<td>11:15</td>
<td>67</td>
<td>65</td>
</tr>
<tr>
<td>11:45</td>
<td>193</td>
<td>166</td>
</tr>
<tr>
<td>12:15</td>
<td>159</td>
<td>120</td>
</tr>
<tr>
<td>1:15</td>
<td>112</td>
<td>78</td>
</tr>
</tbody>
</table>

*18 hour delay.

![Figure 2](image-url)
103 ppm for hydrogen and 4.9 percent for CO₂ (data not shown). Readings on duplicates for hydrogen repeat to within +/- 1 ppm at both 3 ppm and 100 ppm during such a study. The standard deviation obtained for 10 replicate hydrogen measurements determined over 30 minutes was 0.8 ppm with a mean of 103.3 ppm, and for carbon dioxide it was 0.02 percent with a mean of 4.91 percent. These data indicate that both the imprecision and the stability of the instruments over a typical measurement time for a set of 6 samples are adequate to track changes in the breath hydrogen level accurately.

Figure 3 shows patterns observed in patients from this study which represent typical normal (negative) and malabsorption (positive) cases evaluated with lactose, as well as a bacterial overgrowth case evaluated with lactulose as the test substance. Negative tests almost always have baseline values <20 ppm and it has been our observation that subsequent values often decrease from the fasting level, as shown here. While there is no apparent physiologic reason for this, the decrease is more than can be explained by the slight downward drift of 3 to 5 ppm sometimes seen in the instrument calibration during the course of the test.

With only few exceptions, values show a substantial rise from baseline in a positive test for malabsorption. Occasional problems in which one or more of the samples may be poorly collected do not negate the usefulness of the test, since a single sample showing a rise of >10 ppm is adequate for interpretation. Bacterial overgrowth cases show an early peak representing bacterial metabolism in the small bowel, as well as a later peak representing arrival of the test substance at the colon. These cases also test positive for hydrogen production with glucose, due to bacterial metabolism of the glucose in the upper intestine before it is completely absorbed.

Of 34 patients who had symptoms including abdominal pain, cramps, diarrhea, gas, dizziness or vomiting noted during the test, 14 had

![Figure 3. Representative patterns in normal, lactose malabsorption and bacterial overgrowth patients.](image-url)
negative breath hydrogen test results. In 12 other patients, conditions known to affect the lining of the intestine and therefore, possibly, the test result, such as Crohn’s disease (3), food poisoning (1) and use of antibiotics (6 infections and 2 recent surgeries) were noted. All of these were lactose tests; 7 were negative, 4 were positive and 2 were borderline positive with increases of 10 and 15 ppm hydrogen above the fasting level.

Discussion

The breath hydrogen test has been widely used for the evaluation of lactose intolerance (lactase deficiency) and less frequently for sucrose (sucrase deficiency), or fructose intolerance in both children and adults. It is preferred by gastroenterologists over the older tolerance tests, which relied on an increase in blood glucose, because it is non-invasive and is considered more analytically sensitive, requiring only 2 grams of carbohydrate in the distal intestinal tract to produce a significant increase in the level of breath hydrogen. Despite its wide application and relatively long history of use, certain aspects of the test remain unsettled, including fundamentals such as the appropriate cutoff for interpretation. Studies by Solomons, et al, suggest that far fewer false positives and better correlation with symptoms are seen if a rise of 20 ppm hydrogen, instead of 10 ppm, is used to interpret a test as positive. A review of our 111 positive cases revealed that only 9 would be considered borderline positives with a rise between 10 and 20 ppm. In 7 of these, the rise occurred entirely in the 3-hour specimen and may reflect longer transit times in these patients. In fact, physicians routinely inquire whether patients experienced symptoms after completion of the test, since onset of symptoms can be delayed beyond the time of sample collection. Of the 9 borderline results, final diagnoses available in 6 cases included 4 irritable bowel syndrome, one dyspepsia and one esophageal reflux. While these cases do represent false positives, they also illustrate that physicians do not necessarily consider a borderline positive as consistent with lactose malabsorption. All the other positive tests showed increases greater than 20 ppm, so this study would not suggest a significant difference in results by using a 20 ppm cutoff.

Proper interpretation of the test can only be done with consideration of the patient’s long-term and short-term history. Recent conditions or therapeutic treatments can affect results by altering the bacterial flora, lumen pH or intestinal mucosa. Temporary lactose or other disaccharide intolerance due to transient loss of enzyme activity can follow certain disorders like intestinal infection. Because of these effects, it should be recommended to requesting physicians that the patient be off antibiotics for at least 10 days before having this test. Of 12 patients with recent conditions which could affect the test, all were lactose tolerance tests. Two were borderline positive and included food poisoning and pneumonia; 3 were strong positives including sinus infection, upper respiratory infection (URI) and Crohn’s disease; and 7 were negative and included Crohn’s disease, recent surgery, URI or other infection, and a ruptured appendix. There was no correlation observed between type of condition and test result in this set of patients.

Because this test is done primarily on outpatients referred from physician offices and on whom the diagnosis is not yet made, final diagnoses were not readily available to us in most cases. The 14 patients who had negative test results, but experienced symptoms (abdominal pain, gas, diarrhea, nausea or vomiting) during the test, were particularly intriguing and attempts to obtain diagnoses were successful in 9 cases. Two were fructose, two were sucrose and the remainder were lactose tests. Two had cow’s milk protein intolerance, 4 had irritable bowel syndrome, one had mold allergies and cramps due to yeast in milk, one had immune deficiency and one had sensitivity to dairy products and fruit. Four cases, one irritable bowel syndrome, another intolerance to cow’s milk protein, and two unknown diagnoses, experienced nausea or dizziness and/or
abdominal pain as the only symptoms, which may have been due to the osmotic effect of the test substance on the duodenum (dumping syndrome) and not to intestinal bacteria. This would be especially true in 2 of these cases involving fructose, which does not require an enzyme for absorption. The immune deficiency case was a 20-month-old with a past Giardia infection and multiple watery bowel movements per day since birth. The case with dairy and fruit sensitivity was a sucrose breath test which had demonstrated normal lactase and sucrase activity on biopsy, but had a previous positive lactose breath test.

The presence of gas pain or diarrhea with negative breath hydrogen results could be due to production of methane by non-hydrogen producing bacteria or the lack of hydrogen producing bacteria in the colon. False negative tests have been reported in 5 to 20 percent of cases due to the lack of hydrogen-producing bacteria in the gut or to the presence of hydrogen-scavenging bacteria which prevent its absorption and excretion by the lung. Adding measurement of methane to the test, along with hydrogen, has been reported to improve the measurement precision in methane producers and may help reduce the percentage of false negatives in the future by adding a second indicator of bacterial metabolism which is not scavenged.

The application of the breath hydrogen test to evaluating bacterial overgrowth of the small intestine is more controversial than the previously discussed applications and has been questioned in the elderly population by MacMahon, et al. In their study, they found only 75 percent sensitivity and 30 percent specificity in a group of 20 culture positive elderly patients. Corazza, et al, found only 62 percent and 68 percent sensitivity and 83 percent and 44 percent specificity for the glucose and lactulose breath hydrogen test versus jejunal culture in 77 cases of suspected bacterial overgrowth, while Davidson, et al, found lactose malabsorption, established by breath hydrogen testing, was corrected by antibiotics in 9 of 9 pediatric cases in which duodenal cultures were all questionable. Our study included only 3 cases with results consistent with bacterial overgrowth and, therefore, cannot shed light of any significance on this question.

Conclusions

A review of 222 breath hydrogen test results shows the largest application of the test is for evaluation of lactose intolerance with essentially equal representation of patients in all age groups. Samples collected in plastic. Luer-tip syringes remain stable for at least 18 hours and may be held overnight, if necessary, for assay. Using a 10 ppm increase in breath hydrogen as a cutoff does not appear to increase the false positive rate significantly, as compared to a 20 ppm increase. And, finally, proper conversion of test results into a diagnosis can only be done by integrating other information about the patient’s recent and long-term conditions into the process.

Acknowledgments

The authors wish to state that they have no financial interest in the Quintron Instrument Company, nor have they received any financial support from any source for this study.

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