



# Breath-Tests:

A Strong  
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“...improved analytical instrumentation and a greater understanding of its limitation have transformed the H<sub>2</sub> breath test from an investigative curiosity to a mainline clinical tool.”

— Noel W. Solomons, MD,  
*Current Concepts in Gastroenterology*,  
Vol. 8/1: 30-34 and 37-40, 1983

## What Do Breath-Tests Have To Do With Digestive Problems?



When some bacteria digest (or ferment) food substances, they produce acids, water and gases. The major gases which are produced by bacteria include, primarily, carbon dioxide (CO<sub>2</sub>), hydrogen (H<sub>2</sub>), methane (CH<sub>4</sub>) and small concentrations of aromatic gases. Carbon dioxide is produced by all cells during metabolism, but only bacteria can produce H<sub>2</sub> and CH<sub>4</sub> as metabolic byproducts, and this is accomplished primarily by bacteria which thrive in the absence of oxygen (called anaerobic bacteria). So, if either H<sub>2</sub> or CH<sub>4</sub> are produced biologically, it tells us that some food substance is exposed to bacterial fermentation.

In the digestive tract, bacteria are normally limited to the colon. Most of the bacteria contained in food are killed by the acidity of the stomach, so the small intestine usually has few bacteria. In some conditions, called “bacterial overgrowth,” bacteria exist in high concentrations in the small intestine. Their presence in that area can interfere with the absorption of some vitamins and other essential foodstuffs, so it’s important to diagnose the condition. The colon is concerned with conserving water and salt by reabsorbing them

from the luminal contents. However, the colon is involved in other functions, some of which depend on having a high bacterial count. Fiber, very popular in breakfast cereals, is not digested in the small intestine, so it undergoes bacterial fermentation in the colon. Short-chain fatty acids (SCFAs) produced by that process are absorbed in the colon, and are beneficial to health. It is becoming apparent that substantial amounts of starch (10-20% of foods like legumes) escape digestion in the small intestine and are broken down in the colon, thus, adding to the efficiency of energy production by such foodstuffs. In addition, colonic bacteria contribute to fecal bulk, and the short-chain fatty acids mentioned above reduce colonic pH. These factors may reduce the likelihood of diarrhea, may confer some degree of protection against other severe colon problems, and may enhance the colonic absorption of metal ions like calcium, magnesium and zinc. Thus, fermentation in the colon is normal, and it is important.

Gases which are produced in the colon are reabsorbed and equilibrated with the blood leaving that area. They appear in the lung

and cross the capillary membrane into the alveoli, from which they are expired during breathing.

### History Of Breath-Testing

Breath trace-gases were first used as an indicator that complex sugars (disaccharides) were not broken down (hydrolyzed) and absorbed in the small intestine during the digestion of foods. Hydrogen (H<sub>2</sub>) was measured in the breath after administering a dose of the sugar to be studied. The widest application of the test was for lactose malabsorption or lactose intolerance, which is related to milk intolerance in a majority of adults, worldwide.

The National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK) of the NIH (USA) at one point estimated that between 30 million and 50 million Americans are lactose intolerant. The hydrogen breath-test (often referred to as the HBT or BHT) replaced a blood test which was based on the absence of a blood glucose response following lactose ingestion. The test is not as reliable as the breath hydrogen test since it produces a greater proportion of false-negative and false-positive tests.

The incidence of lactose malabsorption throughout the world is surprising to most people. Adults who cannot digest milk sugar make up the majority of the world’s population. Those who can drink milk without getting sick are likely to be North Americans, Australians or Northern Europeans. The ability to digest milk beyond the age of 3-5 years is genetically determined, and is a dominant trait. When the reliability and simplicity of the breath-H<sub>2</sub> test was demonstrated with lactose, it was soon applied to other complex sugars like fructose (from fruits), maltose (from some

starches), and sucrose (common table sugar, which is rarely absorbed). It has also been used to indicate that some people are unusually sensitive to sorbitol, an artificial sweetener used in dietetic candy, sugar-free chewing gum and other dietetic foods. Recent studies have shown that methane has been added as a useful trace-gas for the study of digestive problems. Methane (CH<sub>4</sub>) is an important intestinal gas and it should also be measured in studies of carbohydrate malabsorption in order to provide the most comprehensive information to the physician.

### Basics Of Hydrogen/ Methane Breath Tests

Hydrogen and methane are produced in the digestive system primarily by the bacterial fermentation of carbohydrates (sugars, starches or vegetable fibers), so when either of these gases appear in the expired air, it is usually a signal that carbohydrates or carbohydrate fragments have been exposed to bacteria, permitting such fermentation to take place.<sup>2</sup> The generation of H<sub>2</sub> and/or CH<sub>4</sub> will result in the reabsorption of some of these gases into the blood stream from the site of their digestion, and they will appear in the expired air. Bacteria are ordinarily not present in significant numbers in the small intestine, where digestion and absorption of sugars take place. Therefore, when a challenge dose (eg. lactose) is ingested, the level of hydrogen in alveolar air will rise significantly within one to two hours (depending on the intestinal transit time) only if the sugar is not digested and, therefore reaches the colon. The breath-H<sub>2</sub> test is a simple non-invasive procedure which is readily accepted by patients and staff,<sup>3</sup> and which has greater reliability

and acceptability than the blood test, according to most reports.<sup>1,4-8</sup> The lower dose of lactose usually does not cause the discomfort and explosive diarrhea frequently seen by malabsorbers who are given the large dose required for the blood test.<sup>9</sup>

A study<sup>10</sup> with over 300 patients showed that GI symptoms after a lactose challenge are strongly associated with the amount of H<sub>2</sub> excreted, and the relationship between blood glucose change and symptom-severity was less evident. False-positive breath-tests are rare, and when they occur they are usually caused by improperly doing the test — allowing the subject to smoke, to sleep or to eat shortly before or

during the test.<sup>11</sup> Bacterial overgrowth (from the colon retrograde into the small intestine) can also produce a false-positive breath-test, but it is usually preceded by an elevated fasting breath-H<sub>2</sub> level and the response is seen soon after the sugar is ingested (within 20-30 minutes). The incidence of false-negative results with the breath-test is well below that seen with the blood test.<sup>1,4,5</sup> False-negative results are reported to be from 5-15% of all lactose malabsorbers,<sup>12-14</sup> due to a variety of causes. Many of the false-negative reports can be avoided by measuring methane in addition to hydrogen<sup>15</sup> because some methanogenic flora convert colonic H<sub>2</sub> to CH<sub>4</sub>.

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# Most Common Patient Prep Guidelines For Breath-Testing



All patients are different and individual patient medical history is unknown to manufacturers of breath testing instrumentation. Physicians and technicians must take into consideration patient medical history prior to and during any breath-testing procedure. The general preparation guidelines can be modified by the physician to accommodate patients with special needs.

The avoidance of foods listed herein are not limited to only the foods listed; they help serve as an umbrella of foods to avoid. Any questions related to preparation should first be asked of the patient's physician(s) or nutritionist(s). Prep guidelines below should be followed for all hydrogen breath-tests unless otherwise noted by the patient's physician:

1. Patients will be NPO (Nothing by mouth) for 12 hours prior to the test. Only water may be consumed.
2. Patients should try to avoid foods listed below 12 hours prior to the NPO request.
3. No smoking, including second-hand smoke, for at least one hour before or at any time during the test.

4. No sleeping or vigorous exercise for at least 1-2 hours before or at any time during the test.
5. Recent antibiotic therapy, runny diarrhea or colonoscopies may affect breath tests, therefore medical staff and physicians should consult with patients about these conditions prior to performing any test. If any of the above conditions apply, rescheduling the patient will most likely be necessary. Drinking water during your breath-test is allowed in moderation. Even though patients are NPO for 12 hours prior to the test, it is also required to avoid certain foods at least another 12 hours prior to the NPO request beginning.

Listed below are generic avoidance groups and are not limited to what is listed. If you and/or the patient are uncertain if something may affect the test, consult the patient's physician. The patient should avoid the product until they get an answer from their physician.

**Grain products:** Whole-grain products, including cereals and melba toast, bran or high-fiber cereals.

**Fruits:** Fruit juices, applesauce,

apricots, bananas, cantaloupe, canned fruit cocktail, grapes, honeydew melon, peaches, watermelon. Raw and dried fruits like raisins and berries. Yogurt which contains fruit.

**Vegetables:** Vegetable juices, potatoes, alfalfa sprouts, beets, green/yellow beans, carrots, celery, cucumber, eggplant, lettuce, mushrooms, green/red peppers, squash, zucchini. Vegetables from the cruciferous family: Broccoli, cauliflower, Brussels sprouts, cabbage, kale, swiss chard, beans, lentils, etc.

**Nuts, Seeds, Beans:** All nuts, seeds and beans, as well as foods that may contain seeds.

**All Dairy Products** (except eggs): Milk, cheese, ice cream, yogurt, butter. Suggestions for the patient's last meal to consume prior to NPO can be:

**Baked or broiled chicken or turkey** (salt and pepper only)

**Baked or broiled fish** (salt and pepper only)

**Rice** (plain steamed white rice)

**Eggs**

**Broth** (clear chicken or beef broth)

## Which Patients Are Good Candidates For Breath Testing?

Patient's reasons for good or bad candidacy may not be apparent at first glance. However patients with unspecified abdominal cramping, gas, bloating, diarrhea or constipation are excellent candidates for breath tests. In more recent surveys, offices are testing their patients with three tests: Small Intestinal Bacterial Overgrowth (SIBO), Fructose Malabsorption, and Lactose Malabsorption. Ruling out any of these tests as a cause of the patient's discomfort on the front-end of testing is non-invasive and patients are typically more acceptable and tolerable to perform these tests as opposed to having a colonoscopy.

## Using The Proper Instrumentation And Collection Techniques

Determining if your facility uses the right instrumentation and techniques can be quite burdening sometimes. There are multiple ways to collect breath samples from patients and many variables and choices that need review before any breath-test is performed. It has been found that other companies that distribute or manufacture hydrogen breath-testing instrumentation and products do not fully understand the principle and science behind how the test works. Choosing the right company to work with can also be one of the most important decisions to make. The company of choice for almost all hydrogen breath testing equipment and supplies is QuinTron Instrument Company, Inc. in Milwaukee, Wisc. QuinTron is the long-standing gold-standard in breath-testing instrumentation and supplies since 1962. QuinTron develops the only solid-state instrumentation that can measure Hydrogen, Methane and Carbon Dioxide in a single breath sample; breath collection kits for adults, pediatrics, veterinary sciences, and custom collection devices to suit almost any type of breath sampling that may be desired. The representatives at QuinTron can help technicians with preparation questions, breath collection techniques and instrumentation guidance. If you are not currently offering the hydrogen breath-test at your office or facility, the customer service specialists will help you



# BreathTracker™

DIGITAL MICROLYZER

determine what instrument will best suit your practice's needs and budget.

### Thinking About Breath-Testing But Aren't Sure If It's Right For Your Practice?

More and more patients are searching for help to find answers to their IBS symptoms. In addition, more and more research has validated that breath-testing, especially for bacterial overgrowth, can allow your office to be the answer for these patients. Most offices that currently provide the hydrogen breath-test to their patients perform 3-20 in a week. Imagine if you had one patient who you tested who then referred your office to another five of their closest friends or family. Now imagine how word can spread from those five people to five of their friends. When you become the office that finally provides the answers these patients have been longing for with a simple breath-test, they most likely will tell everyone they know about where they got the test and that your office "is the place to go."

### What Can Adversely Affect Your Breath-Tests?

Although breath-testing can be simple, attention to detail is critical for valid trace-gas breath analysis. Breath-testing is a sensitive application. Technicians and facilities should not attempt to modify any breath collection systems or techniques. Minute changes or use of products not supplied by the manufacturers of the equipment or collection kits may result in improper

analysis results because materials/solvents/lubricants in the manufacturing process adversely affect the gases that are analyzed in the breath samples. Offices should not develop or modify any collection techniques or devices without consulting the manufacturers of the products to ensure that you do not compromise the validity of your test, the products, or your instrumentation.

### Why Breath-Testing Requires Attention To Detail

Breath testing is the measurement of trace concentrations of breath gases. The tiny concentrations found in the breath can be compromised in many different ways: they can be diluted through improper collection or handling of the samples after collection. It can be affected by contact with materials or substances that can alter the unique gases present in the breath. The analysis can be compromised by improper set-up or usage of the very sensitive equipment required to analyze your samples. Inadequate preparation of the patient prior to testing can result in a false-positive interpretation.

The accuracy and reliability of breath-testing is dependent on the care applied to collecting the sample and the technique used in transferring it to the instrument for analysis. If it has been incorrectly collected or contaminated with room air prior to the analysis, the resultant error cannot be remedied by paying attention to how the sample is analyzed in any instrument except for instrumentation that has built-in capabilities of detecting and

correcting for contaminations like the BreathTracker SC or BreathTracker H<sub>2</sub>+ breath-testing equipment.

The general lack of detail about sample collection procedures in the literature leads one to suspect that many gastroenterologists and their staff members do not understand or appreciate the importance of eliminating dead-space air from samples to be analyzed as “alveolar air.” A variable volume of the first portion of an exhaled tidal breath is simply a wash-out of the airways which was filled with the last portion of room air inhaled with the preceding breath. That volume can be approximated as one mL per pound of body weight, usually generalized as being about 150mL for the average adult. With a normal tidal volume of about 500mL/breath, the first 1/3 of so is dead space air. Because of the laminar pattern of airflow through the major airways, roughly twice that volume must be exhaled before all of the dead-space air is washed out, to avoid contaminating alveolar air with a portion of slowly mixed dead space air.

The problem is even greater with neonates, in whom dead space volume is represented by close to 50% of the tidal volume because of the disproportionately larger airway diameter in babies, as compared with adults. Most published articles describing the use of breath trace-gases simply indicate that “expired air” or “end-expired air” samples were analyzed, without a description of the methods and precautions used for the sample collection. Since that step is so critical to the success of the test, a description of the process should be included in the reported methodology, including the application of a correction factor for sample contamination if it is applied.

### What types of breath collection devices and kits are available?

**AlveoSampler™ System:** An economical and disposable device used to collect alveolar air



samples for subsequent analysis. The alveolar sample is drawn into a syringe from the end-expiratory air blown through the device. During expiration through the mouthpiece, a vented polyethylene bag with a medium-resistance leak is filled to indicate that adequate dead space volume has been exhaled. As exhalation continues, air is then steadily drawn into the syringe by the operator. The bag serves as a check-valve to prevent contamination of the syringe sample with atmospheric air as long as the patient keeps the AlveoSampler mouthpiece in his/her mouth. Use of the AlveoSampler removes the danger of inter-patient cross-infection since they are single-patient use only, and will save time and money by eliminating the costs of cleaning and sterilizing reusable products.

**GaSampler™ System:** Can be handled by untrained technicians (or even by a patient without supervision, after having its operation explained) to collect a sample of up to 750 mL of alveolar air for subsequent analysis. The GaSampler system consists of two collapsible bags, a Tee-connector,

mouthpiece and one-way flutter valve. The first portion of an expired breath, containing “dead-space” air, is directed into the Discard Bag and alveolar air is then diverted to the Collection Bag, where the sample can be removed for analysis (or transferred to the Sample Holding Bag) for later subsequent analysis. After the alveolar air sample is collected and the Collection Bag cap is securely in place, the sample can be immediately analyzed by withdrawing the sample from the stopcock attached to the small port.



**KidSampler™ System** (GaSampler system for small children): Is designed to collect up to 250ml of alveolar air from children who can follow simple verbal instructions. For children who cannot blow voluntarily in though a mouthpiece you may alternatively attach a face mask to the Tee-Piece in lieu of a mouthpiece.

**EasySampler™ System** (US Patent # 5,467,776): Designed to provide a method for filling vacuum tubes with a sample suitable for analysis with the BreathTracker and MicroLyzer instrumentation which have the ability to detect Carbon Dioxide (CO<sub>2</sub>). It is necessary to use instrumentation which can measure the CO<sub>2</sub> because there are sources of sample dilution which require correction when the samples are analyzed. There is a residual volume of air in the tube, though it is evacuated as far as practical in its preparation. There may be a slight contamination with dead space room air during sample collection. Since the volume of the tube is limited to about 12mL and the sample loop (internal to the MicroLyzer SC or BreathTracker SC

and H<sub>2</sub>+) may not completely flush, some residual carrier gas (room air) may dilute the sample in the sample loop. The EasySampler is preferred by clinics and laboratories which analyze samples collected elsewhere and are mailed in for analysis. This is because of the convenience of the tube for handling, the stability of the sample in a glass tube which allows longer-term storage, and the simple, straight-forward technique of using the EasySampler. Sample dilution is not a problem because the BreathTracker SC and H<sub>2</sub>+ and Microlyzer SC are designed to correct for dilution of the trace gases by reference to the decreased concentration of CO<sub>2</sub>.



### Properly Storing Samples

QuinTron's Sample Holding Bag is a small foil-laminate bag of about 250mL capacity, which is used to hold samples until the analyses can be completed. When using a GaSampler system for sample collection, you can transfer samples directly from the Collection Bag into the Sample Holding Bag through a male-male connector which is included with the Sample Holding Bags. When using the AlveoSampler System to collect samples, the samples can be transferred into the Sample Holding Bags via the syringe used for sample collection and analysis at a later time. The Sample Holding Bag volume is sufficient to allow several analyses with the BreathTracker/MicroLyzer

instrumentation, and they are inexpensive enough to be used for the collection of additional samples so they can all be analyzed at the same setting. Storage Information for the Sample Holding Bags:

- Prior to transferring samples into Sample Holding Bags from whichever collection device you used for collection, it is recommended to prolong storage capabilities of the breath sample, to dry the patient sample prior to storage by using the Patient Sample Drying Tube filled with an approved drying agent like Drierite®.
- Each Sample Holding Bag is designed to store a single breath sample for as long as two weeks with minimal loss in sample integrity. These bags do not come in contact with patients directly and can be reused across multiple patients.
- When it is time to analyze the sample, withdraw the sample from the small port with a syringe/stopcock.
- After the sample is analyzed, flatten the bag and withdraw any remaining sample out with a syringe until you feel back

pressure on the syringe plunger.

- You may wipe the outside of the bag off with mild soap and water to clean, but use caution to not get any water inside the Sample Holding Bag.

### Patients with High Baseline Values

Sometimes elevated control values (above 10 ppm) for H<sub>2</sub> can be observed in normal patients or carbohydrate malabsorbers who did not fast properly or ate slowly-digesting, fiber-containing foods the day before the test. High baseline (control) levels in H<sub>2</sub> (usually above 20 ppm) can also sometimes be seen with patients who have small intestinal bacterial overgrowth (SIBO). In those patients, it may increase modestly from the elevated baseline soon after the ingestion of a sugar such as lactose (within 15-45 minutes), and fall back near control levels later in the test (perhaps by the three-hour sample). Elevated CH<sub>4</sub> levels can also be seen with bacterial overgrowth,<sup>16</sup> though it may not change as much as H<sub>2</sub> does in response to the challenge dose of sugar.

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## Non-Responders/Antibiotics/ Laxatives/Enemas/Use of Methane (CH<sub>4</sub>)/False-Negatives

A common but smaller percentage of patients taking a carbohydrate breath test (i.e. Lactose, Fructose, Sucrose) do not produce H<sub>2</sub> after ingesting the substrate for the test, and some of them even excrete no hydrogen after ingesting a non-absorbable sugar, such as lactulose. The incidence of false-negative tests with the breath-test is well below that seen with the blood-test.<sup>1-3</sup> False-negative results are reported in 5-18% of all lactose malabsorbers.<sup>4-7</sup> There are numerous conditions which can lead to false-negative reports (erroneously suggesting no evidence for malabsorption).

Administering a course of antibiotics may sterilize the colon, so the colony-count of bacteria is low or non-existent.<sup>8-9</sup> However, not all antibiotics have this effect on the breath-test; it was reported that Neomycin may even increase H<sub>2</sub> excretion,<sup>10</sup> presumably due to the selective inhibition of hydrogen-consuming bacteria by the antibiotic. Laxatives and enemas can decrease the H<sub>2</sub> and CH<sub>4</sub> response in malabsorbers.<sup>11</sup> The decrease may result from reducing the resident time for carbohydrate in the colon, leading to reduced time for fermentation. The reduced gas production may also result from a change in the concentration of the bacteria and from a change in the environment, such as increasing the acidity, which could

inhibit bacterial action.<sup>12</sup> Inherent rapid transit of the carbohydrate through the colon due to hypermotility will also reduce the exposure time and decrease H<sub>2</sub> production in the colon. H<sub>2</sub> production is affected by colonic pH. A decrease in stool pH from

7.0 to 5.5 will cause a drop in H<sub>2</sub> generation to ¼ the former rate.<sup>13</sup> The rate is returned promptly by increasing the pH. Thus, severe diarrhea and/or hyperacidic colon contents may inhibit the generation of hydrogen, or cause the generation of methane in addition to,<sup>13,14</sup> or instead of,<sup>11,12</sup> hydrogen by colonic bacteria. The majority of malabsorbers who do not produce H<sub>2</sub> when exposed to a carbohydrate (i.e. Lactose, Fructose, etc.) will generate CH<sub>4</sub> instead. These patients will be properly diagnosed if Methane (CH<sub>4</sub>) is measured as part of the routine test.

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## False-Positive Breath-Hydrogen/ Methane Tests



Falsely high H<sub>2</sub>-levels in response to the ingestion of lactose (false-positive tests)<sup>1</sup> may occur, but they are almost always the result of improper preparation for the test or improper conduct of the test. Such errors may include:

- a. Improper preparation of the patient** — The inappropriate choice or incomplete avoidance of food by the patient on the night before the test will provide a high, but gradually falling level of H<sub>2</sub> or CH<sub>4</sub> on which the test will be superimposed. This is because the amount of fiber in the colon will be elevated at the beginning of the test, and will fall during the hours of measurement. Even if H<sub>2</sub> is produced from the challenge-dose of carbohydrate, it may not exceed the initial baseline level enough to be classified as a positive test. Breath levels of CH<sub>4</sub> are not as affected by the ingestion of food as are levels of H<sub>2</sub>. The reasons for high CH<sub>4</sub> levels is not completely clear, but may be related to endogenous mucopolysaccharides or other residual material in the colon, though sugars and other carbohydrates quite obviously can change CH<sub>4</sub> levels.
- b. Smoking in the area of the test** — Tobacco smoke contains high levels of H<sub>2</sub>, so smoking (by the patient or by anyone in the area) will produce high H<sub>2</sub> levels and will cause extreme instability of the instrument, since it

uses room air as a standard against which breath samples are measured. Smoking by the patient should be avoided for at least one hour before any sample is taken, and there should be no smoking at any time in the vicinity of the instrumentation.

- a. Sleeping** — Allowing the patient to sleep during the test will cause an increase in breath-H<sub>2</sub>, and probably in CH<sub>4</sub>. There are two reasons for such increases. Hypoventilation, which is an inadequate rate of air turnover in the lung, slows down the rate of H<sub>2</sub>-removal from the blood. Sleep also decreases intestinal motility, which slows down

the movement of carbohydrates through the colon and allows a longer time for the accumulation of H<sub>2</sub>. Thus, intermittent sleeping during the test will interfere, and should not be allowed. High fasting levels of H<sub>2</sub> (and perhaps of CH<sub>4</sub>) at the beginning of the test may suggest that the patient did not follow instructions for the complete avoidance of carbohydrate and fiber the night before; but it also may suggest that the patient has bacterial overgrowth, which is defined as the presence of bacteria in the small intestine. Bacterial overgrowth exposes the complex sugars and other soluble carbohydrates in the small intestine to bacterial fermentation instead of allowing them to be hydrolyzed enzymatically and absorbed in the relatively sterile intestine.

### References:

1. Lembke, B.; Honig, M.; Caspary, W.F. Different actions of neomycin and metronidazole on breath hydrogen (H<sub>2</sub>) exhalation. *Z Gastroenterol.* 1980 (Mar); 18(3):155-60

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