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Hydrogen and Methane-Based Breath Testing in Gastrointestinal Disorders: The North American Consensus

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OBJECTIVES: **Breath tests (BTs) are important for the diagnosis of carbohydrate maldigestion syndromes and small intestinal bacterial overgrowth (SIBO). However, standardization is lacking regarding indications for testing, test methodology and interpretation of results. A consensus meeting of experts was convened to develop guidelines for clinicians and research.**

METHODS: **Pre-meeting survey questions encompassing five domains; indications, preparation, performance, interpretation of results, and knowledge gaps, were sent to 17 clinician-scientists, and 10 attended a live meeting. Using an evidence-based approach, 28 statements were finalized and voted on anonymously by a working group of specialists.**

RESULTS: **Consensus was reached on 26 statements encompassing all five domains. Consensus doses for lactulose, glucose, fructose and lactose BT were 10, 75, 25 and 25 g, respectively. Glucose and lactulose BTs remain the least invasive alternatives to diagnose SIBO. BT is useful in the diagnosis of carbohydrate maldigestion, methane-associated constipation, and evaluation of bloating/gas but not in the assessment of oro-cecal transit. A rise in hydrogen of ≥20 p.p.m. by 90 min during glucose or lactulose BT for SIBO was considered positive. Methane levels ≥10 p.p.m. was considered methane-positive. SIBO should be excluded prior to BT for carbohydrate malabsorption to avoid false positives. A rise in hydrogen of ≥20 p.p.m. from baseline during BT was considered positive for maldigestion.**

CONCLUSIONS: **BT is a useful, inexpensive, simple and safe diagnostic test in the evaluation of common gastroenterology problems. These consensus statements should help to standardize the indications, preparation, performance and interpretation of BT in clinical practice and research.**

SUPPLEMENTARY MATERIAL is linked to the online version of the paper at http://www.nature.com/ajg

Am J Gastroenterol 2017; 112:775–784; doi:10.1038/ajg.2017.46; published online 21 March 2017

INTRODUCTION

Breath test (BT) is performed to aid in the diagnosis of many common gastroenterological conditions including small intestinal bacterial overgrowth (SIBO) and irritable bowel syndrome (IBS)-like symptoms, carbohydrate maldigestion and dysfunction or alterations in oro-cecal transit. Presently in clinical practice, BT is being performed with various substrates (e.g., glucose, lactulose, fructose, sorbitol, sucrose and inulin) using variable doses for a range

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Received 19 August 2016; accepted 22 January 2017

of established and unestablished indications (e.g., SIBO, orocecal-transit time assessment and carbohydrate maldigestion) (1). The number of BTs being ordered by health care professionals is rising (including the use of mail order breath tests), but the lack of standardization regarding the indications, preparation and performance and interpretation of BT has led to considerable heterogeneity between different centers and practitioners. In addition, research gaps are widening due to inter-study variability in methodology. A systematic review (2) found 13 case-control studies (3–15) that used breath testing to diagnose SIBO. Detailed examination of these studies reveals that they used 13 different methodologies to conduct the breath test or interpret the results.

BT relies on measurement of gases produced in the intestine which diffuse into the systemic circulation and are expired through the lungs. There are 4 main sources for intestinal gases: swallowed air and air mixed with food, chemical reactions in the gut, diffusion of gases from the blood stream, and microbial metabolism (16). Healthy subjects have an average of about 100 ml of intestinal gas (ranging from 30 to 200 ml), principally composed of hydrogen (H_2), carbon dioxide (CO_2) and methane (CH_4), with lesser amounts of oxygen (O_2), nitrogen (N_2), hydrogen sulfide (H_2S), indole, skatole and ammonia (NH_3) (17). Of these, H_2 and CH_4 are exclusively produced via microbial fermentation in the gut, which is the principle behind clinical breath testing (17). Gut microbes readily digest carbohydrates, resulting in production of these gases, which then diffuse into the abdominal venous circulation and are transported to the lungs, where they can be detected in the exhaled breath (17).

To conduct a BT, fasted subject ingests a carbohydrate substrate and breath samples are procured for the measurement of H_2 and CH_4 levels at set intervals over the next several hours. Currently, there are two consensus documents available regarding breath testing published by the Italian H_2-breath Testing Consensus Conference Working Group (1) and the German Society of Neurogastromotility (18) in 2009 and 2005, respectively. Since then the body of evidence, especially in the field of the microbiome, has evolved exponentially. For instance, these statements do not provide guidance on measurement and interpretation of methane gas levels, false-positivity of carbohydrate malabsorption testing in the setting of SIBO, and the effects of anti-acids on BT. There is no North American consensus document, despite more data emerging on the utility of BT in the North American population. Therefore, a consensus meeting was convened with the goals of providing easy-to-follow guidelines for physicians performing BT and to lay the foundation for future consensus guidelines, as well as identifying gaps of knowledge in breath testing and to direct future research initiatives.

METHODS

Consensus development process

The process for the development of the consensus statements is outlined in **Figure 1**. Specific topics for discussion including indications for breath testing, the preparation and performance of the test, and the interpretation of the results were identified

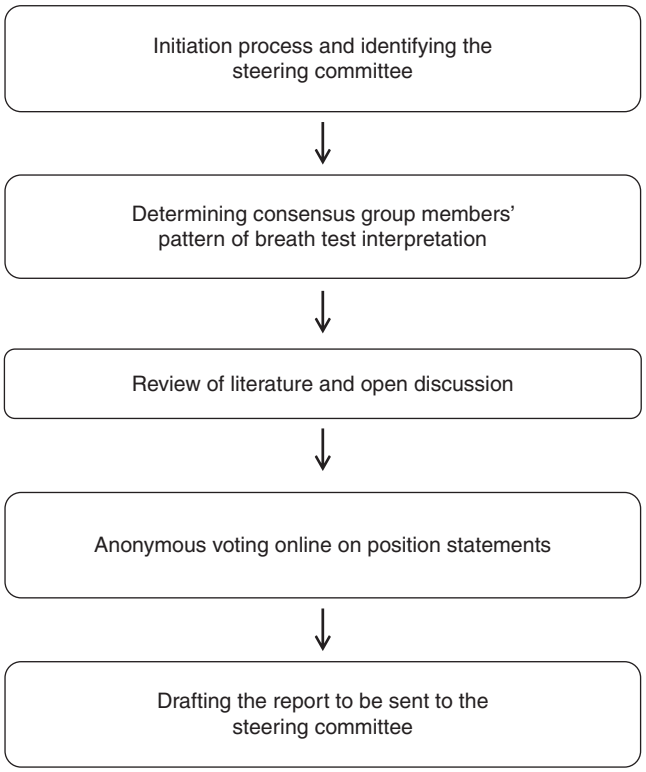

Figure 1. Consensus development process.

on the basis of literature reviews. Pre-meeting survey questions (**Supplementary Information S1** online) were designed to further delineate the current knowledge gaps in breath testing and were sent to the consensus group members. The survey results were collated and summarized prior to the consensus meeting.

Selection of the physician-scientists for this meeting were based on a number of criteria. First, was to be sure that attendees represented those with active and recent research in the area of breath testing research. This involved a literature search to understand those in North America with active publications in the area. Many of these included physicians from major academic motility programs. The second was to also have representation from clinician scientists from high volume breath testing referral centers to ensure those with a vast experience in the use and interpretation of breath tests. The third was to ensure representation from various regions in the US as well as Mexico and Canada. An email was sent to those meeting these criteria. A total of 17 clinician scientists from North America were invited to participate in the survey and attend the consensus meeting, of whom 10 were able to attend the meeting in person. The half day meeting was held on 16 May 2015 in Washington, DC. At the consensus group meeting, results of the survey and comprehensive literature review were presented for discussion among the group members.

Distinct topics of discussion included indications, preparation, performance, interpretations of results, and future directions in breath testing. Based on the results of these discussions, a series of draft consensus statements was compiled and sent to committee

Table 1. Preparation before breath testing

Consensus statement	Percentage of agreement	Quality of evidence (GRADE)
1. We recommend that antibiotics should be avoided for 4 weeks prior to the breath test.	Agree (88.9% agree, 0% uncertain, 11.1% disagree)	⊕⊕⊕⊕
2. A firm position statement cannot be reached due to lack of conclusive data on stopping or continuing pro/prebiotics prior to breath testing.	Uncertain (44.4% agree, 44.4% uncertain, 11.1% disagree)	⊕⊙⊙⊙
3. We suggest that, if tolerated by the patient, promotility drugs and laxatives should be stopped at least 1 week prior to breath testing.	Agree (77.8% agree, 11.1% uncertain, 11.1% disagree)	⊕⊙⊙⊙
4. We suggest that fermentable foods such as complex carbohydrates should be avoided on the day prior to breath testing.	Agree (100% agree, 0% uncertain, 0% disagree)	⊕⊕⊕⊙
5. We suggest that the fasting period for breath testing as part of preparation should be 8–12 h.	Agree (77.8% agree, 0% uncertain, 22.2% disagree)	⊕⊕⊙⊙
6. We recommend that smoking should be avoided on the day of breath testing.	Agree (100% agree, 0% uncertain, 0% disagree)	⊕⊕⊕⊕
7. We recommend that physical activity should be limited during breath testing.	Agree (100% agree, 0% uncertain, 0% disagree)	⊕⊕⊕⊕
8. We suggest that it is not necessary to stop proton pump inhibitors prior to breath testing.	Agree (77.8% agree, 11.1% uncertain, 11.1% disagree)	⊕⊕⊙⊙

Table 2. Indications for breath testing

Consensus statement	Percentage of agreement	Quality of evidence (GRADE)
1. Current small bowel culture techniques are not satisfactory for the assessment of SIBO.	Agree (88.9% agree, 0% uncertain, 11.1% disagree)	⊕⊕⊙⊙
2. If culture is considered for diagnosis of SIBO, based on the current evidence, we suggest the threshold of $>10^3$ c.f.u./ml for the definition of SIBO	Agree (77.8% agree, 11.1% uncertain, 11.1% disagree)	⊕⊕⊙⊙
3. We suggest breath testing in the diagnosis of small intestinal bacterial overgrowth.	Agree (100% agree, 0% uncertain, 0% disagree)	⊕⊕⊕⊙
4. Until a true gold standard is established, we suggest breath testing in assessing the presence of antibiotic-responsive microbial colonization of the gastrointestinal tract.	Agree (77.8% agree, 11.1% uncertain, 11.1% disagree)	⊕⊕⊕⊙
5. We suggest to evaluate for excessive methane excretion on breath test in association with clinical constipation and slowing of gastrointestinal transit.	Agree (88.9% agree, 0% uncertain, 11.1% disagree)	⊕⊕⊕⊙
6. We suggest that breath testing should not be used for assessment of orocecal transit time.	Agree (77.8% agree, 11.1% uncertain, 11.1% disagree)	⊕⊕⊕⊙
7. We suggest breath testing for the diagnosis of carbohydrate maldigestion syndromes.	Agree (88.9% agree, 11.1% uncertain, 0% disagree)	⊕⊕⊕⊙
8. We suggest breath testing in the assessment of conditions with bloating.	Agree (88.9% agree, 11.1% uncertain, 0% disagree)	⊕⊕⊙⊙

members for online voting using SurveyMonkey (SurveyMonkey, Palo Alto, CA) with revision as necessary. Using a modified Delphi process (19,20), group members anonymously voted on their level of agreement with each statement on a scale of 1–3 (disagree, uncertain and agree, respectively). A statement was accepted if >70% of participants voted 3 (agree), and it was rejected if >50% of participants voted 1 (disagree). If neither of these criteria were reached, it was stated that "a firm position statement could not be reached due to lack of conclusive data". The strength of recommendation for each statement was assigned by the consensus group as either strong ("we recommend...") or weak ("we suggest..."). The strength of each statement was based on resource and cost benefit, patients' values, risk/benefit balance and quality of evidence. The quality of evidence for each consensus statement was classified as high (⊕⊕⊕⊕), moderate (⊕⊕⊙⊙), low (⊕⊕⊙⊙), or very low (⊕⊙⊙⊙) based on Grading of Recommendations, Assessment, Development and Evaluations (GRADE) system (21). The manuscript was circulated to all group members for review, revisions and approval.

RESULTS

The consensus statements (**Tables 1–4**) achieved for each of the five broad domains are summarized below:

Preparation of patients for breath testing

1. We recommend that antibiotics should be avoided for 4 weeks prior to the breath test.
2. A firm position statement could not be reached due to lack of conclusive data on stopping or continuing probiotics or prebiotics prior to breath testing.
3. We suggest that, if tolerated by the patient, promotility drugs and laxatives should be stopped at least one week prior to breath testing.
4. We suggest that fermentable foods such as complex carbohydrates should be avoided on the day prior to breath testing.
5. We suggest that the fasting period prior to breath testing should be 8–12 h.

Table 3. Performance of breath tests

Consensus statement	Percentage of agreement	Quality of evidence (GRADE)
1. We suggest that the correct dose of lactulose for breath testing is 10 g with or followed by one cup of water.	Agree (100% agree, 0% uncertain, 0% disagree)	⊕⊕⊕⊙
2. We suggest that the correct dose of glucose for breath testing is 75 g mixed with or followed by one cup of water.	Agree (88.9% agree, 11.1% uncertain, 0% disagree)	⊕⊕⊙⊙
3. We suggest that the correct dose of lactose for breath testing is 25 g mixed with or followed by one cup of water.	Agree (88.9% agree, 0% uncertain, 11.1% disagree)	⊕⊕⊙⊙
4. We suggest that the correct dose of fructose for breathe testing is 25 g mixed with or followed by one cup of water.	Agree (88.9% agree, 0% uncertain, 11.1% disagree)	⊕⊕⊕⊙
5. We suggest that fructose and lactose breath test should be performed for at least 3 hours.	Agree (100% agree, 0% uncertain, 0% disagree)	⊕⊕⊕⊙
6. We suggest that the presence of bacterial overgrowth should be ruled out before performing lactose or fructose breath testing.	Agree (100% agree, 0% uncertain, 0% disagree)	⊕⊕⊕⊙
7. We recommend that hydrogen, methane and carbon dioxide should all be measured simultaneously during breath testing.	Agree (77.8% agree, 22.2% uncertain, 0% disagree)	⊕⊕⊕⊕

Table 4. Interpretation of breath testing

Consensus statement	Percentage of agreement	Quality of evidence (GRADE)
1. We suggest that a rise of ≥20 p.p.m. from baseline in hydrogen during the test should be considered positive for fructose and lactose breath testing.	Agree (100% agree, 0% uncertain, 0% disagree)	⊕⊕⊙⊙
2. We suggest that until better data are available, for clinical and research purposes, a rise of ≥20 p.p.m. from baseline in hydrogen by 90 min should be considered a positive test to suggest the presence of SIBO.	Agree (77.8% agree, 11.1% uncertain, 11.1% disagree)	⊕⊕⊙⊙
3. We suggest that two peaks on breath test are not required for the diagnosis of SIBO.	Agree (88.9% agree, 0% uncertain, 11.1% disagree)	⊕⊕⊙⊙
4. Until further data is available, we suggest that a level of ≥10 p.p.m. be considered positive for methane on a breath test.	Agree (88.9% agree, 0% uncertain, 11.1% disagree)	⊕⊕⊙⊙
5. A firm position statement cannot be reached due to lack of conclusive data on the definition of abnormal methane on to be ≥3 p.p.m.	Uncertain (44.4% agree, 44.4% uncertain, 11.1% disagree)	⊕⊕⊙⊙

6. We recommend that smoking should be avoided on the day of breath testing.
7. We recommend that physical activity should be limited during breath testing.
8. We suggest that it is not necessary to stop proton pump inhibitors prior to breath testing.

With regards to preparation for breath testing, the use of antibiotics has been clearly shown to alter the H_2 and CH_4 composition of the exhaled breath (22,23) and although no clear data exist, a 4-week gap between the cessation of antibiotic therapy and performance of the test is generally recommended (1). While the effect of antibiotics on hydrogen and methane production is well known (24,25), the extent of this effect based on bioavailability and pharmacodynamics of antibiotics is not well understood. This timeline between breath testing and antibiotic use may depend on the purpose for which the test is being performed; for example, breath tests may be performed shortly after cessation of antibiotic therapy to confirm eradication.

Although probiotics have been shown to affect H_2 levels on breath test (26,27), a firm position statement could not be reached due to lack of conclusive data on stopping or continuing probiotics or prebiotics prior to breath testing.

Prokinetic drugs such as tegaserod and other laxatives such as magnesium compounds can also affect the composition of the breath gases (28). Faster transit time caused by promotility drugs will lead to an earlier delivery of the substrate to colon and will increase the chance of a false positive result. Again, no clear data exist, but a 4-week gap between cessation of prokinetics and breath testing has previously been recommended (1). However, a 4-week gap may not be practical in patients with constipation or gastroparesis; hence, we propose discontinuing laxatives and promotility agents for a week and only if tolerated by patients.

A low fasting level of breath H_2 is essential for interpreting the breath test results, as these are directly affected by consumption of fermentable complex carbohydrates (29,30). As a result, avoidance of complex carbohydrates and dairy products the evening or day before a breath test (meat and rice do not appear to affect the breath test (31)) and overnight fasting is also recommended (1), although the precise duration requires to be determined in future studies.

Smoking affects breath test results by increasing exhaled H_2 and CO_2 content of the exhaled breath (32,33) and should be avoided on the day of the test. Smoking also increases gastric transit time (34). As hyperventilation inversely affects H_2 levels (35), excessive physical activity should be avoided during the breath test. Conflicting results exist in the literature in regards to the effect of proton pump inhibitors (PPIs) on breath test results (36,37). Stopping PPIs may not lead to more accurate measurements of exhaled

breath gas levels and recurrence of reflux symptoms may not be tolerable to all patients. Currently, stopping anti-acid medications prior to breath testing is not necessary.

Indications for breath testing

1. Current small bowel culture techniques are not satisfactory for the assessment of SIBO.
2. If culture is considered for diagnosis of SIBO, based on the current evidence, we suggest the threshold of $>10^3$ c.f.u./ml for the definition of SIBO.
3. We suggest breath testing in the diagnosis of small intestinal bacterial overgrowth.
4. Until a true gold standard is established, we suggest breath testing in assessing the presence of antibiotic-responsive microbial colonization of the gastrointestinal tract.
5. We suggest to evaluate for excessive methane excretion on breath test in association with clinical constipation and slowing of gastrointestinal transit.
6. We suggest that breath testing should not be used for assessment of orocecal transit time.
7. We suggest breath testing for the diagnosis of carbohydrate maldigestion syndromes.
8. We suggest breath testing in the assessment of conditions associated with bloating.

SIBO is a condition in which the small bowel is colonized by excessive numbers of aerobic and anaerobic microbes that are normally found in the large intestine (38). Aspiration of small bowel fluid, followed by culture and bacterial count is considered to be the current gold standard for diagnosis of SIBO (39). Unlike breath testing, small bowel aspiration is invasive, time-consuming and costly. In addition, small bowel aspiration has multiple shortcomings. Mid and distal segments of small bowel are beyond the reach of regular endoscopes; therefore, proximal small bowel aspirates may be falsely negative (40). Although aseptic techniques for small bowel aspirates have been described (41), contamination with oral and esophageal flora may lead to a significant number of false positive results (42). Quantitative PCR studies have been used to diagnose SIBO with promising results (43,44); however, such techniques are not readily available for clinical practice.

Historically, a bacterial concentration $\geq 10^5$ colony forming units (c.f.u.)/ml has been used for identification of any infection in the small bowel including SIBO. However, this cut-off is not well-validated and has been a point of controversy. A systematic review of literature on the diagnosis of SIBO observed that healthy controls have a bacterial concentration of $\leq 10^3$ c.f.u./ml while concentrations $\geq 10^5$ c.f.u. are mostly seen in patients with blind loop syndrome such as patients with Billroth II procedure (2). Currently a bacterial concentration of $>10^3$ c.f.u./ml is generally considered significant (41,42,44,45).

Glucose is a monosaccharide which is absorbed in the proximal small bowel, whereas lactulose is a non-digestible disaccharide that reaches the colon. Determination of the exact test characteristics of glucose and lactulose breath testing in diagnosing SIBO is difficult to achieve, due to the variability between studies in interpretation of small bowel bacterial concentration as the gold standard test and lack of uniformity in defining a positive breath test (2). In fact, such variability was one of the main motivations for the current consensus document. In a systematic review, Khoshini *et al.* (2) found 11 studies that have attempted to validate the accuracy of breath testing in diagnosis of SIBO. The sensitivity of glucose breath testing varied from 20 to 93% and specificity from 30 to 86%. The sensitivity of lactulose breath testing ranged from 31 to 68% and specificity ranged from 44 to 100%. There were striking differences in methodology in the included studies (2). In a recent comparative study, duodenal culture was positive in 62/139 (45%) patients with unexplained gas, bloating and diarrhea and negative endoscopy, whereas GBT was positive in 38/139 (27%) patients. The sensitivity and specificity of GBT were 42% and 84%, respectively (41).

A recent retrospective study of subjects undergoing concurrent glucose breath testing with scintigraphy concluded that glucose breath testing has a high false positive rate due to arrival of the scintigraphy in the cecum prior to a rise in hydrogen or methane on testing (46). This is similar to conclusions previously published pertaining to lactulose breath testing (47). However, both of these studies rely on the arrival of technetium to the cecum as a determinant of oro-cecal transit—either 5% arrival of technetium in the cecum (47), or the "head of the labeled bolus" (46). If 5% of the lactulose or "the head of the bolus" is in the cecum, the rest of the medium is still in the small intestine; hence, the rise of breath gases is not necessarily due fermentation in the cecum (48). Moreover, the lag time between colonic flora exposure to sugar and peak of bacterial fermentation was not taken into account in these studies (48), and as such, they do not yet provide a clear answer on the validity of these breath tests.

Methane gas has been shown to inhibit intestinal transit in dogs by 59% (ref. 49) and in human subjects CH_4 positivity on breath test has been associated with constipation (50–53). Patients with CH_4-predominant bacterial overgrowth usually present with bloating and abdominal distention. However, they are 5 times more likely to have constipation as opposed to H_2-predominant overgrowth (53). Moreover, the severity of constipation directly correlated with the methane level (50) and the choice of antibiotics for the treatment of SIBO differs between these two groups as the predominant methanogen in the human gut, *Methanobrevibacter smithii*, is resistant to many antibiotics (22,38,54).

Lactulose and inulin breath testing have been used to assess oro-cecal transit time (i.e., gastric emptying time plus small bowel transit time). Variable criteria have been used to estimate the transit time including time to the second peak of hydrogen or rise of 5–10 p.p.m., and different substrates, including a liquid meal containing lactulose, or a solid meal containing fermentable food such as baked beans±lactulose (55,56). However, lactulose has been shown to shorten oro-cecal transit time (57), and the test has been shown to have poor reproducibility with wide variation among healthy subjects (1). Inulin is less osmotically active and has been proposed as an alternative substrate to lactulose (58). Until further

data is available, use of breath testing to assess oro-cecal transit time is not recommended.

Breath tests are also used in the diagnosis of a variety of carbohydrate maldigestion syndromes. The lactose breath test is performed to diagnose lactose maldigestion/intolerance, which may result from lactase deficiency. Lactose, which is exclusively found in dairy products, is a disaccharide composed of galactose and glucose, and the brush border enzyme lactase phlorizin hydrolase is required for its digestion. Lactase deficiency can be primary or secondary (i.e., acquired lactase deficiency due to damage to, or absence, of the brush border). There is no gold standard test to diagnose lactose maldigestion and large scale data are lacking to compare the degree of agreement of the currently available tests (59). Jejunal biopsies followed by a lactase activity assay is invasive and unreliable due to patchy lactase distribution in the brush border (60). Genetic testing only detects primary lactase deficiency (61), and tests of blood glucose levels following lactose ingestion depend on glucose metabolism and are not reliable (61,62). One other limitation of the lactose breath test is false negative results in individuals with fixed hydrogen and non-methane production; however, these only constitute 3.4% of breath tests (63). As mentioned above, false positive results can also occur (64,65).

The fructose breath test is performed to diagnose fructose maldigestion/intolerance. In fructose intolerant subjects, unabsorbed fructose is fermented by the colonic bacteria causing IBS-like symptoms (66). A well-conducted study has shown that ingesting 25 g of fructose is the appropriate dose for fructose breath testing and symptom correlation (67). Again, limitations of the test include false negatives, which can occur in individuals who are fixed hydrogen non-methane producers; and false positives, which can occur in individuals with SIBO. Nucera *et al.* (64) showed that the number of positive fructose breath tests in IBS patients with positive lactulose tests dropped from 62 to 3% after 1 week of antibiotic treatment (64). Further studies are need to clarify the role of breath testing in sorbitol (68) and fructan (66) intolerance.

IBS-like symptoms are commonly seen in patients with SIBO and carbohydrate maldigestion (38,69). However, symptom profiles of these patients are non-specific and clinical history alone cannot differentiate the underlying cause. In assessment of 144 subjects with IBS-like symptoms, Jacobs *et al.* (42) found no difference in the intensity, frequency and duration of abdominal pain, bloating, fullness, belching, indigestion, nausea, vomiting, diarrhea and gas among patients with or without SIBO proven by aerobic/anaerobic/fungal small bowel cultures. Meta-analyses (70,71) have documented a wide variety of types of breath tests (i.e., different substrates) as well as considerable variability in the performance and interpretation of these breath tests in IBS patients vs. controls, but found that overall, IBS patients were 3 times more likely (odds ratio=3.3; 95% confidence interval 2.4–4.6) to have an abnormal breath test than controls. Breath testing remains a useful diagnostic tool for patients with unexplained IBS-like symptoms including gas and bloating, as well as diarrhea or constipation (70).

Performance of breath tests

1. We suggest that the correct dose of lactulose for breath testing is 10 g with or followed by one cup of water.
2. We suggest that the correct dose of glucose for breath testing is 75 g mixed with or followed by one cup of water.
3. We suggest that the correct dose of lactose for breath testing is 25 g mixed with or followed by one cup of water.
4. We suggest that the correct dose of fructose for breath testing is 25 g mixed with or followed by one cup of water.
5. We suggest that fructose and lactose breath testing should be performed for at least 3 h.
6. We suggest that the presence of bacterial overgrowth should be ruled out before performing lactose or fructose breath testing.
7. We recommend that hydrogen, methane and carbon dioxide should all be measured simultaneously during breath testing.

When performing lactulose breath tests for the determination of SIBO, the majority of studies have used a dose of 10 g (11,12,72). Higher doses incur the risk of speeding intestinal transit time, which could affect test data (57).

With regards to the glucose breath testing, variable doses of 50 g (10,73,74) and 75–100 g (11,15,41,75) have been used. Comparative studies using 50 g, 75 g or 100 g have not been performed. A 75 g dose is used for oral glucose testing in diabetics, as recommended by the World Health Organization (76); hence, from the practical standpoint stocking 75 g doses is less-expensive, easier to obtain for breath test centers and could provide an adequate amount of carbohydrate substrate.

In subjects with lactose and fructose intolerance, unabsorbed substrate is fermented by the colonic bacteria (62) leading to symptoms such as gas, bloating and abdominal pain. Symptoms of lactose malabsorption generally occur after ingestion of 6–12 g lactose (120–240 ml of milk) (77–79). Although high doses of lactose (≥50 g) have been used for lactose breath test (80), 25 g (equivalent of 500 ml of milk) is within the normal range of consumption and is the recommended dose.

While absorption of lactose is dependent on the lactase enzyme, fructose is absorbed via facilitative diffusion mediated by the glucose/fructose transporter member 5 (GLUT-5) transporter (81,82). However, this passive absorptive system can be easily overwhelmed by excess amount of fructose. Rao *et al.* (67) have shown that healthy individuals have the capacity to absorb up to 25 g of fructose while 80% of these subjects had evidence of fructose malabsorption without symptoms when tested using 50 g of fructose. Therefore, the recommended dose for fructose breath testing is 25 g.

Significant variation exists in the literature in terms of the required length of study for assessment of carbohydrate malabsorption, ranging from 2 to 5 h (83). As the principle behind breath testing in carbohydrate malabsorption is fermentation of unabsorbed substrate by the colonic bacterial flora, it is crucial to allow enough time for the substrate to reach

the colon and be metabolized. Rao *et al.* (67) reported that the average time for reaching the gas peak concentration was 77 min (range=30–180 min) for an abnormal fructose breath test, thus suggesting that 180 min is sufficient to detect colonic fermentation. Length of glucose and lactulose breath testing for assessment of SIBO can be limited to two hours, therefore the committee considered a rise of ≥20 p.p.m. of hydrogen by 90 min as the ideal criterion for a positive test to *suggest* the presence of SIBO.

It should be noted that in the presence of SIBO, fructose and lactose are prematurely exposed to excessive small intestinal bacterial composition that will lead to early fermentation and elevation of exhaled gases (36,64,84,85). Therefore, performance of a lactulose or glucose breath test to rule-out SIBO should be considered prior to carbohydrate malabsorption breath testing to minimize false positive results.

All breath testing should incorporate measurement of CO_2 (or O_2) to adjust the breath sample for non-alveolar dilution of exhaled air (86). Concomitant measurement of CH_4 is also required. The majority of the methanogenic archaea in the human gut (including *M. smithii*) utilize H_2 in the generation of CH_4, potentially impacting H_2 measurements (87). Hydrogenotrophic methanogens in the gut (including *M. smithii*) utilize 4 mol of H_2 and 1 mol of CO_2 to produce each mol of CH_4 (refs 88,89). It has been suggested that by scavenging H_2 produced by neighboring microbes in this manner (the "sink effect") (88), *M. smithii* and other hydrogen-utilizing methanogens allow increased polysaccharide fermentation by neighboring microbes. Detection rate of an early rise in H_2 production significantly deceases in excess methane producers (87). Overall, given the importance of methane in association with GI symptoms and the interaction of methane with hydrogen production, measurement of methane should be integrated in all breath tests. It should be noted that measurement of CH_4 could increase the cost of breath testing, as portable gas chromatographs do not measure CH_4.

Interpretation of breath testing results

1. We suggest that a rise of ≥20 p.p.m. from baseline in hydrogen during the test should be considered positive for fructose and lactose breath testing.
2. We suggest that until better data are available, for clinical and research purposes, a rise of ≥20 p.p.m. from baseline in hydrogen by 90 min should be considered a positive test for SIBO.
3. We suggest that two peaks on breath test are not required for the diagnosis of SIBO.
4. Until further data is available, we suggest that a level of ≥10 p.p.m. be considered positive for methane on a breath test.

Similar to the European consensus statements (1,18) and based on the current evidence, we suggest using a cutoff value of ≥20 p.p.m. rise above baseline as positive for the lactose breath test (1). The test should be performed for at least 3 h to ensure the presence of colonic fermentation. We suggest the same criteria for interpretation of fructose breath testing based on the results of the study by Rao *et al.* (67) which showed that rise of hydrogen always occurs within 3 h of testing. The value of development of symptoms during fructose breath testing is unclear and needs further evaluation (67,90).

Erdogan *et al.* (91) assessed the optimal cut-off of glucose breath testing (75 g glucose) for detection of SIBO and correlated this with duodenal aspirates and culture (both at $\geq 10^3$ and $\geq 10^5$ c.f.u./ml) in 150 patients. Sensitivity of breath test was lower at 20 p.p.m. vs. 12 p.p.m. rise of H_2, at both $\geq 10^3$ (32 vs. 42%) and $\geq 10^5$ c.f.u./ml (48 vs. 64%) culture growth levels. Specificity was higher at both $\geq 10^3$ (90 vs. 81%) and $\geq 10^5$ c.f.u./ml (86% vs. 78%) culture growth. George *et al.* (92) compared the utility of hydrogen rise of 20 p.p.m. above baseline by 90 min after ingestion of lactulose vs. dual hydrogen peaks (first peak of 10 p.p.m. above baseline before second peak of 20 p.p.m.) in 740 patients with gastroparesis. Only the hydrogen rise of 20 p.p.m. above baseline was correlated with severity of bloating, postprandial fullness and early satiety. Recently, assessment of over 15,000 lactulose breath tests shows that median and mean gas production levels do not elicit a double peak (93). We suggest using a rise of ≥20 p.p.m. from baseline in hydrogen to diagnose SIBO. On the basis of current evidence, a double peak should not be used to diagnose SIBO and has no validity.

The interpretation of breath tests with elevated baseline H_2 >20 p.p.m. is not clear. Further studies are needed to clarify whether this is the result of lack of adherence to diet and fasting or in fact representative of a SIBO variant. The interpretation of breath tests with no CH_4 and low fixed H_2 production remains unclear. Such breath tests have been suggested to be due to abundance of H_2S-producing bacteria, which cannot be detected using currently available gas chromatography devices (94). Further studies are needed to clarify the significance of such a pattern.

The optimal criterion to define excessive methane production is not clear. The production pattern of methane and hydrogen are different on breath testing. Unlike hydrogen, subjects with excessive methane production elicit an elevated methane level at baseline and the rise of methane during breath testing is not as sharp as hydrogen gas (63,93). Hence, using the same cutoff as hydrogen for methane is not advised. Recently it has been shown a fasting level of ≥5 and ≥10 p.p.m. can respectively predict excessive methane production with specificity of 99.7 and 100% while sensitivity was of 96.1% and 86.4% (63). The sensitivity threshold of many commercially available gas chromatography instruments is 3 p.p.m., and as such this is the lowest value that could be considered positive (95). Using a combination of rise in hydrogen >20 p.p.m. and >5 p.p.m. of methane, the sensitivity of breath test was lower at $\geq 10^3$ (42%) compared to $\geq 10^5$ c.f.u./ml (56%) whereas specificity was higher at $\geq 10^3$ (85%) compared to $\geq 10^5$ c.f.u./ml (78%) (91). Until further data is available we suggest using a cut of ≥10 p.p.m. for methane positivity.

Gaps in knowledge

In addition to the aforementioned consensus statements, the committee also identified the following gaps in knowledge and technology in breath testing:

- The lack of a validated gold standard test for diagnosing SIBO.
- The lack of techniques capable of acquiring sterile and anaerobic aspirates from the various segments of small bowel.
- Integration of deep sequencing techniques for further assessment of bacterial diversity in SIBO patients.
- The optimal doses of lactose for breath testing, as well as the optimal test duration.
- Determination of various substrate doses in the pediatric population.
- Assessment of breath testing results in relation to race, ethnicity, age and gender.
- Determination of the effect of pre/probiotics on hydrogen and methane production.
- Determination of the extent of effect of absorbable and non-absorbable antibiotics on breath testing results.
- Determining the role of breath testing in assessment of SIBO after therapy.
- Determination of the cause of non-methane fixed-hydrogen pattern (i.e., flatline).
- Determination of the significance of elevated baseline hydrogen levels despite fasting and adherence to pre-test diet.
- Determination of the optimal time interval for breath test sampling during breath testing.
- Studies to determine whether different diets in different parts of the world can affect the results of the different breath tests, and as such if a universal standardized pre-breath test diet would need to be established.
- Development of gas chromatographers capable of detecting H_2S in exhaled air as another potentially important gas produced by gut microbiome.
- The importance of maldigestion of other substrates and their use in breath testing such as inulin and sucrose.
- Future assessment of exhaled breath volatile organic compounds (VOCs) in association with gut microbiome and H_2/CH_4 levels.

DISCUSSION

Breath testing remains a useful, inexpensive, simple, and safe diagnostic tool in gastroenterology. Using an evidence-based approach, consensus was reached on 26 statements providing practical guidance regarding the indications, preparation, performance and interpretation of BT in clinical practice and research. Furthermore, current gaps of knowledge are identified for future research directives. As compared to previous guidelines, these consensus statements are updated based on the most recent literature and provide detailed recommendations and clarity on drug regimens prior to BT, dose of substrates, established indications, limitations and interpretation of the results based on both hydrogen and methane gases.

While unanswered questions remain and there are challenges that need further clarification, it is hoped that these consensus statements create uniformity in the use of BT and thus improve and optimize patient care.

ACKNOWLEDGMENTS

We sincerely thank Dr Gillian Barlow and Ms Kathleen S. Chua for their important contributions.

CONFLICT OF INTEREST

Guarantor of the article: Ali Rezaie, MD, FRCPC.

Specific author contributions: Conceived and planned the consensus group and designed the pre-meeting survey questions: A.R. and M.P.; responded to the pre-meeting survey questions, and all authors participated in the consensus group meeting: M.B., A.L., H.L., R.M., S.R., M.S., M.V., and S.Z.; voted on the consensus statements: M.B., A.L., H.L., R.M., S.R., M.S., M.V., M.P. and S.Z.; drafted the manuscript: A.R. and M.P.; and reviewed and edited the manuscript: M.B., A.L, H.L., R.M., S.R., M.S., M.V., and S.Z.; all the authors approved the final draft submitted.

Financial support: The consensus group meeting was supported in part by Commonwealth Laboratories (Commonwealth Laboratories, Boston, MA). Commonwealth Laboratories had no role in the election of committee members, determination of the topics of presentations/discussion, drafting or approving these consensus statements.

Potential competing interests: Ali Rezaie reports support from the following sources for teaching, research, and consultation, outside the submitted work: Commonwealth Laboratories, Actavis, and Salix Pharmaceuticals. Henry Lin reports related intellectual property rights. Michelle Buresi, Richard McCallum, Salam Zakko and Miguel Valdovinos report no conflicts of interest. Anthony Lembo reports receiving fees for serving on advisory boards from Allergen, Furiex Pharmaceuticals, Prometheus Laboratories, Salix Pharmaceuticals, Valeant Pharmaceuticals, Forest Laboratories, Alkermes, AstraZeneca, and Ironwood Pharmaceuticals. Satish Rao reports support from the following sources for teaching, research, and consultation, outside the submitted work: Forest Laboratories, Hollister, Ironwood Pharmaceuticals, Sucampo Pharmaceuticals, In Control Medical, Vibrant, American Medical Systems, Sun Sweet Corporation, Synergy Pharmaceuticals, Salix Pharmaceuticals, and Ventrus Laboratories. Max Schmulson has received grant supports from Alfa Wasserman and Nestle. He has served on the Advisory Board of Alfa Wasserman, is currently a consultant for Commonwealth Laboratories Inc and Commonwealth Diagnostics International Inc and in the past has been a consultant for Almirall, Janssen, Nestle, Novartis, Procter and Gamble, Senosiain and Takeda Mexico. Has been a speaker for Alfa Wasserman, Commonwealth Diagnostics International Inc, Janssen, Mayoly-Spindler and Takeda Mexico. Mark Pimentel is a consultant for Valeant Pharmaceuticals, Commonwealth Laboratories, Synthetic Biologics, Micropharma, and Naia Pharmaceuticals, and is on the advisory boards for Valeant Pharmaceuticals and Commonwealth Laboratories. Cedars-Sinai has a licensing agreement with Valeant Pharmaceuticals International Inc., Commonwealth Laboratories, and Synthetic Biologics.

Study Highlights

WHAT IS CURRENT KNOWLEDGE

- ✓ Breath testing represents an important, simple and safe test to diagnose carbohydrate maldigestion syndromes and small intestinal bacterial overgrowth (SBIO).
- ✓ There is significant heterogeneity in test performance/preparation, the indications for breath testing and the interpretation of results.

WHAT IS NEW HERE

- ✓ Consensus doses for lactulose, glucose, fructose and lactose breath tests are 10, 75, 25 and 25 g, respectively.
- ✓ Breath testing is useful in the diagnosis of carbohydrate maldigestion, methane-associated constipation but not in the assessment of oro-cecal transit.
- ✓ For glucose or lactulose breath tests for SIBO, a ≥20 p.p.m. rise in hydrogen by 90 min is considered positive.
- ✓ Methane levels ≥10 p.p.m. are considered methane-positive.
- ✓ For assessment of carbohydrate maldigestion, a rise in hydrogen of ≥20 p.p.m. above baseline during breath testing is considered positive.

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