Understanding Our Tests: Hydrogen-Methane Breath Testing to Diagnose Small Intestinal Bacterial Overgrowth

Aylin Tansel, MD, MPH[1] and David J. Levinthal, MD, PhD[1]

There is increasing appreciation that small intestinal bacterial overgrowth (SIBO) drives many common gastrointestinal symptoms, including diarrhea, bloating, and abdominal pain. Breath testing *via* measurement of exhaled hydrogen and methane gases following ingestion of a readily metabolized carbohydrate has become an important noninvasive testing paradigm to help diagnose SIBO. However, because of a number of physiological and technical considerations, how and when to use breath testing in the diagnosis of SIBO remains a nuanced clinical decision. This narrative review provides a comprehensive overview of breath testing paradigms including the indications for testing, how to administer the test, and how patient factors influence breath testing results. We also explore the performance characteristics of breath testing (sensitivity, specificity, positive and negative predictive values, likelihood ratios, and diagnostic odds ratio). Additionally, we describe complementary and alternative tests for diagnosing SIBO. We discuss applications of breath testing for research. Current estimates of SIBO prevalence among commonly encountered high-risk populations are reviewed to provide pretest probability estimates under a variety of clinical situations. Finally, we discuss how to integrate breath test performance characteristics into clinical care decisions using clinical predictors and the Fagan nomogram.

KEYWORDS: small intestinal bacterial overgrowth; hydrogen-methane breath test; breath testing; diagnostic accuracy; prevalence; likelihood ratio; sensitivity; specificity; narrative review

SUPPLEMENTARY MATERIAL accompanies this paper at http://links.lww.com/CTG/A909

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INTRODUCTION

Small intestinal bacterial overgrowth (SIBO) is increasingly recognized as a pathophysiological driver of a wide range of gastrointestinal (GI) symptoms, including those that meet the symptom criteria for irritable bowel syndrome (IBS) (1). SIBO is characterized by abnormal bacterial colonization in the small intestine and is associated with GI symptoms such as bloating, distension, flatulence, abdominal discomfort, diarrhea, and, in severe cases, even weight loss and significant micronutrient deficiencies. Certain factors, such as the presence of underlying gut dysmotility or a history of GI surgery, increase the risk that an individual will develop SIBO. Recently, increased methane production *via* methanogens (microbes that produce methane with carbohydrate fermentation) has been recognized as a potential cause of constipation (and associated symptoms of bloating, gas, and/or abdominal pain) (2–4). Clearly, making a diagnosis of SIBO and/or methanogen overgrowth is critical for the management of patients suffering from a wide range of chronic GI symptoms.

The gold standard test for SIBO—direct culture of small intestinal contents—is technically cumbersome, invasive, costly, and subject to some contention regarding diagnostic thresholds of bacterial counts. Previously, diagnostic criteria for SIBO using aspirates were defined using a threshold of $\geq 10^5$ cfu/mL, but the North American consensus guidelines and the American College of Gastroenterology (ACG) guidelines recommend a threshold of $>10^3$ cfu/mL to define SIBO given evidence that asymptomatic controls rarely exceed 10^3 cfu/mL, values $>10^3$ cfu/mL have been shown to be clinically relevant, and $\geq 10^5$ cfu/mL was generally seen in patients with altered anatomy (3,5). This threshold is supported in a 16S ribosomal RNA gene sequencing–based study, which found that a culture-based cutoff of $>10^3$ cfu/mL correlated well with clinical symptoms, breath test results, and sequencing (6). Debate on the appropriate diagnostic threshold remains, for instance, the Asian-Pacific consensus guidelines support the use of both diagnostic thresholds (7). Variable sampling and processing techniques have been an additional limitation with the use of small bowel aspirates. However, several strides have been made to develop robust methods for small bowel sampling under aseptic, or near aseptic, conditions (3). Despite improved methods to collect and analyze small bowel aspirates, there are limitations to such testing and barriers to widespread implementation. Because breath testing provides an alternative, noninvasive, inexpensive, and relatively straightforward mode of testing, it has been rapidly and widely adopted to aid in the diagnosis of SIBO (8). Yet, the clinical

[1]Department of Medicine, Division of Gastroenterology, Hepatology, and Nutrition, University of Pittsburgh Medical Center, Pittsburgh, USA.
Correspondence: Aylin Tansel, MD, MPH. E-mail: tansela@upmc.edu.
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decisions surrounding the use of these breath tests remain poorly characterized. It is critical for clinicians to recognize the strengths and limitations of hydrogen-methane breath testing, how and when to conduct such testing, and the factors that influence the results and the study interpretation.

In this article, we present a comprehensive review the role of hydrogen-methane breath testing to diagnose SIBO. We will cover indications for testing, an overview of the test paradigm, strategies to optimize test performance, and a review of factors that may influence the interpretation of test results. We will then explore the test performance characteristics (sensitivity, specificity, positive and negative predictive values, likelihood ratios, and diagnostic odds ratio [DOR]) that influence clinical decisions on diagnosis and treatment. We will explore testing alternatives and complementary approaches for the diagnosis of SIBO and potential research applications of breath testing. Finally, we report the prevalence of SIBO in various clinical populations and discuss the application of breath testing under different clinical scenarios.

METHODS

We searched PubMed from its inception through January 20, 2023, using a combination of keywords and MeSH terms (see Supplementary Table 1, http://links.lww.com/CTG/A909). We did not apply any search restrictions. Articles were screened first by title and then by abstract for possible relevance to this review. In addition, we hand searched references of relevant articles.

Full-text references were retrieved if articles appeared to be applicable. We extracted information on study protocols used for breath testing preparation.

To determine the diagnostic accuracy (i.e., sensitivity and specificity) of breath testing, we specifically included studies that used jejunal aspirates as a gold standard reference. To determine the prevalence of SIBO in higher-risk populations, we included English-only studies (systematic reviews, randomized clinical trials, cohort, case-control, or cross-sectional studies) that evaluated the prevalence of SIBO in adults (those aged ≥18 years) using breath tests (with lactulose or glucose as the substrate) and/or small bowel aspirates (duodenal or jejunal). As additional *a priori* study inclusion criteria, the patient population had to include a minimum of 100 patients and sufficient information to determine the prevalence of SIBO in this patient population. We focused on clinical populations that are routinely encountered by gastroenterologists. Furthermore, studies also had to include a control group of patients (either healthy asymptomatic subjects and/or symptomatic patient controls). For prevalence information, we extracted data from all included studies including title, journal, year, study design, number of participants included (if the article was a systematic review, the number and types of studies were also extracted), reference standard test used and definitions of positive tests, prevalence data, and additional information on patient population and control population where available. Because the primary goal was to formulate reliable prevalence numbers to inform clinical applications, we favored the inclusion of systematic reviews rather than individual articles. However, if a large population study was performed and not included in available systematic reviews, those individual studies were additionally included in our analysis. For multiple systematic reviews on the same population, we favored studies with more updated and/or rigorous methodology but considered inclusion of multiple studies if additional prevalence information was provided on the population of interest. For systematic reviews, we assessed the quality of the reported data and rejected meta-analyses in which the between-study heterogeneity (I^2 statistic) was reported to be ≥ 90%. Both authors independently extracted data for all included studies and agreed on included studies with excellent Cohen kappa (>0.80). Where statistical calculations were needed, we used Stata 13.1 (College Station, TX).

Indications for breath testing

Hydrogen-methane breath testing is indicated for the following (1,3).

- Diagnosis of SIBO in symptomatic at-risk populations:
  - Symptoms generally attributed to SIBO include steatorrhea, weight loss/inability to gain weight, abdominal pain, gas, bloating, distension, diarrhea, constipation, and anemia/neuropathy. In addition, a syndrome of brain fog, gas, and bloating has been described with resolution after discontinuation of probiotics and a course of antibiotics (9). Symptoms of anxiety and depression have also been linked to SIBO, although unclear whether this is directly related to SIBO, in response to symptoms from SIBO, or another mechanism (10). See Table 1 for a list of symptoms compatible with SIBO and their proposed pathophysiological mechanisms.
  - At-risk populations include patients with motility disorders or who use medications that impact gut motility, those with surgically altered GI anatomy, patients with immunodeficiencies, or altered GI mucosal secretion or gut barrier function. See Table 2 for a list of mechanisms and associated disorders that increase the risk of SIBO.
- Evaluation for excessive methane excretion (methane-producing microbial overgrowth)
- Detection of specific disaccharidase deficiencies or impaired sugar absorption
- Assessment of the responsiveness of GI tract microbial colonization to antibiotic therapy

We focus our discussion on the diagnosis of SIBO in symptomatic at-risk populations and briefly detail the other indications.

Overview of the test

Hydrogen-methane breath testing refers to the measurement of components of exhaled gases and can be performed in the office or at home. Hydrogen-methane breath testing relies on an administered oral substrate (generally a solution containing a readily metabolized carbohydrate) that is then metabolized by gut microbiota. Glucose and lactulose are the most commonly used substrates. Glucose is readily absorbed in the proximal small intestine, with much less reaching the colon. Lactulose passes unabsorbed through the small intestine and readily enters the colon. Thus, glucose and lactulose substrates differ in their spatial and temporal interaction with gut microbiota. Lactose and fructose are other substrates used in breath testing, but these substrates are used to diagnose specific malabsorption states, rather than SIBO, and will be briefly discussed separately.

When the patient consumes the carbohydrate substrate, bacterial contact with the substrate results in the production of gases (hydrogen, methane, and hydrogen sulfide) *via* fermentation (1). Prior tests relied on measuring hydrogen gas only, but overall, there has been a strong shift to support measuring both hydrogen and methane gas (3,11). Hydrogen and methane gases are

Table 1. Symptoms implicated with small intestinal bacterial overgrowth and possible mechanisms (116,117)

Symptom	Possible mechanisms
Bloating	Multifactorial: increased gas production from bacterial fermentation, visceral hypersensitivity, and decreased small intestinal elasticity and/or transit
Diarrhea	Multifactorial: bacterial digestion produces gas and osmotically active byproducts, bacteria and byproducts injure mucosa resulting in increased water output, resulting in lactase deficiencies, and bacterial deconjugation of bile salts interferes with fat absorption
Abdominal pain	Multifactorial: brain-gut, visceral hypersensitivity, and decreased small intestinal elasticity and/or transit
Constipation	Methane production slows intestinal transit; increased gas distension interferes with intestinal contractions
Anemia/neuropathy	Bacterial consumption of vitamin B12
Inability to gain weight and weight loss	Reduced availability of nutrients due to bacterial digestion
Steatorrhea/fat-soluble vitamin deficiencies	Bacterial deconjugation of bile acids resulting in insufficient absorption of fats and fat-soluble vitamins
Systemic reactions (i.e., brain fog, anxiety/depression, and dermatologic conditions)	Multifactorial: increased bacterial counts and intestinal barrier destruction can result in hypersensitivity reactions/immune response; vitamin deficiencies; bacteria and byproducts may traverse the disrupted intestinal barrier
Distension	Multifactorial: increased gas production from bacterial fermentation; decreased small intestinal elasticity and/or transit

exclusively produced by intestinal bacteria, and these gases diffuse across the gut mucosa into the portal circulation where they undergo gas transfer in the alveolar spaces and are subsequently exhaled. In healthy individuals, this process is initiated predominantly in the large intestine, where most gut bacteria reside. However, in patients with SIBO, bacterial fermentation of the substrate occurs more proximally within the small intestine. These gases are exhaled with normal tidal breathing and collected in bags at regular intervals throughout the breath testing period (1,3). Inferences about the anatomic location within the gut in which these fermentation reactions occur are based on the temporal pattern of gas production and exhalation following the ingestion of the substrate (3). In general, commercially available gas analyzers detect hydrogen somewhat more accurately

Table 2. Mechanisms that link the presence of clinical conditions, patterns of medication use, and history of surgical manipulations to higher risks of small intestinal bacterial overgrowth (1,79,80,118)

Mechanism	Cause
Gastric achlorhydria	Proton pump inhibitor use, autoimmune gastritis, and vagotomy
Anatomic abnormality	Small intestinal diverticulosis, obstruction, radiation enteritis, ileocecectomy, altered surgical anatomy (particularly surgeries with blind limb: Roux-en-Y, Biliroth II; lack of ileocecal valve: ileocecectomy), small bowel stricture, small bowel adhesion, and enterocolic fistula
Small intestinal motility disorder	Diabetes, scleroderma, chronic intestinal pseudo-obstruction, diverticulosis, irritable bowel syndrome, Crohn's disease, amyloidosis, visceral myopathies, collagen vascular disease (hypermobility joint syndrome, systemic sclerosis/scleroderma, and SLE), amyloidosis, neurologic disorders (i.e., Parkinson), vagotomy, chronic intestinal pseudo-obstruction, hypothyroid, and decreased motility with aging
Gastrocolic or coloenteric fistula	Crohn's disease, malignancy, and prior surgery
Abnormal mucosa	Celiac disease and Crohn's disease
Immunity defense	AIDS, combined variable immunodeficiency, immunosuppressive medications, and immunoglobulin A deficiency
Altered pancreaticobiliary secretions	Chronic pancreatitis, exocrine pancreatic insufficiency, and cirrhosis
Medications that slow transit	Opioids, anticholinergics (i.e., dicyclomine, hyoscyamine, and tricyclic antidepressants), dopamine agonists, calcium channel blockers, clonidine, GLP analogs, and antidiarrheals
Miscellaneous/unknown	Fibromyalgia

GLP, glucagon-like peptide.

than methane, but the measurement of both gases substantially improves the test's overall diagnostic accuracy and is recommended as a standard approach (3). There is increasing interest in measuring hydrogen sulfide gas, but hydrogen sulfide detection capability is not currently incorporated into most gas analyzers used in routine clinical practice (1). In addition, measuring carbon dioxide concentration if available aids not only to identify substandard samples but also allows for normalization of results to potentially improve diagnostic accuracy *via* correction factors that are applied to measured gas concentrations (12,13). Another less common alternative to measuring carbon dioxide is the measurement of oxygen concentration, which is similarly applied to confirm high-quality sample collection and properly account for sample dilution (3,14,15).

How is the hydrogen-methane breath test performed?

Preparations for the test. Because breath testing is so reliant on gas production related to bacterial fermentation of the substrate in the small intestine, preparations before the test are necessary to decrease the presence of hydrogen and methane gases related to bacterial metabolism of substrates from other dietary sources (i.e., colonic bacterial fermentation from previously ingested food) (15). Preparation additionally involves minimizing the effect of medications and lifestyle factors that may alter the results.

Diet. The day before the test, patients should follow a low-residue diet consisting of white rice, fish, chicken, eggs, white (dairy-free) bread, clear broths, and plain black tea or coffee (3,8,15,16). During the restricted diet period, salt, pepper, powdered spices, and herbs are allowed (16). Cooking oil may be used in small amounts (17). A minimum of an 8-hour (ideally 12-hour) overnight fast (water only) is recommended before the test (3,18). Gum chewing and candy should be avoided during the test preparation and testing periods to avoid inadvertently driving bacterial fermentation of sugars (16,19).

Medications. Multiple medications can influence intestinal transit time, thereby affecting breath test interpretation, and if able to be tolerated by the patient, they should be held before testing. For instance, promotility drugs and laxatives should ideally be withheld at least 1 week before breath testing (3). The recent European H_2-CH_4-breath test group consensus statement recommends that fermentable carbohydrates (e.g., lactulose or lactose in gram doses), prokinetics, and laxatives should be stopped at least 24 hours before breath testing (15). Opioids have a well-established effect in delaying intestinal transit, and thus, opioids should be stopped the day before and during the test (8). If any of these medications are unable to be stopped due to patient intolerance or other clinical factors, this should be documented, and the test should be interpreted with some caution (3,15). Antibiotics can significantly alter the hydrogen and methane composition of exhaled breath by reducing the bacterial loads within the gut (20,21). Therefore, it is recommended that antibiotics should be stopped for 4 weeks before the test (15). There is limited evidence regarding the potential interference of probiotics and prebiotics with breath testing results (22,23). Although the North American Consensus guidelines for breath testing do not specify whether these agents should be held, the recent European H_2-CH_4-breath test group consensus statement recommends holding probiotic use for 24 hours before testing (15). Evidence suggests that proton pump inhibitors (PPIs) and histamine H_2-receptor antagonists (H_2 blockers) can influence the gut microbiome, but the need to hold these medications before breath testing is controversial (3,15,24,25). Currently, the North American consensus guidelines recommend that PPIs and hydrogen blockers do not need to be held (3). The recent European H_2-CH_4-breath test group consensus statement recommends that breath testing should be delayed at least 2 weeks after a colonoscopy due to known influences of bowel preps on gut microbial loads and community structure with persistent effects observed 2 weeks later (15,26). Some centers suggest delaying the breath test at least 4 weeks to minimize the influence of the colonoscopy on test results (15,18). Patients with diabetes should have directed instructions on how to adjust their medications before testing.

Lifestyle factors. The combustion of tobacco creates a potent, non-GI tract source of hydrogen gas that directly influences breath testing results (27–29). Increased hydrogen in exhaled breath increases markedly during active smoking. Although these levels do decline after smoking, breath hydrogen levels can remain above typical basal values for at least several hours after smoking (27–29). Thus, patients should be encouraged to refrain from smoking later in the evening before the test and throughout the testing period (3,15). Given that gas exchange rates are proportional to ventilation volumes, physical exercise should not be performed during the breath testing period, as increased ventilation could falsely reduce measured breath hydrogen concentrations *via* increased diffusion rates (3,15).

Choice of substrate. The North American Consensus guidelines recommend a substrate dose of 75 g glucose (preferred over 50 g) or 10 g lactulose (30). However, European guidelines favor 50 g for glucose or 10–20 g of lactulose (15). The chosen substrate is mixed with or followed by 1 cup of water (3). Some patients may not be able to rapidly ingest the test substrate due to the volume; if this is an issue, potentially smaller volumes or reduced substrate loads (i.e., 50 g glucose rather than 75 g) can be substituted as an alternative approach and recorded. Glucose is regarded as a more specific substrate and more commonly performed (31). Despite the increased sensitivity, some centers continue to prefer using lactulose as their primary substrate for breath testing (32). One way in which lactulose breath tests differ from glucose breath tests is that lactulose breath tests that are positive often have a double peak with an initial peak in the small intestine and then a second peak when lactulose reaches the colon, but this feature may not always be present (1). In the past, lactulose breath tests had also been used to determine transit times, but this approach is not considered reliable and is no longer recommended (1,15). Because lactulose is not directly absorbed by the small intestinal mucosa, lactulose breath testing results are more sensitive to alterations in small intestinal motility patterns than are glucose breath tests. Thus, lactulose may lead to higher false-positive rates than glucose substrates in patients with particularly rapid transit and in diarrheal states. There has been a gradual shift toward using glucose testing as a default substrate to improve the diagnostic accuracy of breath testing for the purposes of diagnosing SIBO (15,31). Yet, lactulose breath tests appear to still have some advantages over glucose substrates. For example, lactulose is a useful alternative substrate in patients with diabetes, as glucose can result in hyperglycemia that secondarily impacts test results (1). Lactulose may also be preferred in patients with slower GI transit who have a higher risk for false-negative test results with glucose testing, although this point remains unproven (33). In

addition, lactulose breath testing results may predict response to therapy. In a study of patients with diarrhea-predominant IBS undergoing treatment with rifaximin, patients with a positive baseline lactulose breath test were more likely to have global response to rifaximin therapy, with improvement in abdominal pain and frequency of loose stools (59.7% vs 25.8%) (32).

Breath test administration. After a baseline breath sample is collected, the test substrate (glucose or lactulose) is administered in a single bolus over a brief amount of time. Bacterial contact with the test substrate leads to fermentation and production of hydrogen, methane, and hydrogen sulfide gases. These gases are absorbed into the circulation and released into the alveolar sacs and subsequently exhaled. Exhaled breath samples are collected in regular intervals, generally every 15 or 20 minutes over a period of 180 minutes. Symptoms reported by patients during the study period should be recorded in the study log. This can help correlate symptoms to specific gas levels and provide insight into the patient's symptom pattern. To collect breath samples, patients are advised to breathe normally into an alveolar collection device equipped with a syringe port to withdraw gas samples. Adult patients typically exhale a ~100 cc volume into the bag, whereas pediatric patients generally exhale ~50 cc volumes. A 20–30 cc volume of end-expiratory air is immediately collected and submitted for analysis using a gas chromatograph to detect hydrogen, methane, and, if available, carbon dioxide. Gas concentration data are reported in parts per million (ppm). The accuracy of most commercial gas analyzers is generally quite precise. For example, the Model SC (QuinTron Instrument, Milwaukee, WI) has a margin of error of ±3 ppm (17). Breath hydrogen samples are traditionally stable for 6 hours at room temperature, and if measurements are delayed beyond this, storage at −20 °C is needed (34,35). However, different storage approaches can help keep samples stable for longer periods. It is recommended to follow manufacturing recommendations regarding sample transportation and storage (15). Recent research into prolonged storage of samples from at-home testing suggests that methane gas levels may be more affected over longer storage times compared with hydrogen gas levels (36).

Conditions that influence breath test results and how to manage them. Higher levels of hydrogen and/or methane gas measured at baseline (before test substrate administration) suggest ongoing fermentation of carbohydrates. This is generally thought to be most commonly due to poor compliance with the dietary preparations before the test (and thereby measurement of fermentation from the colon), but higher baseline hydrogen levels may reflect other factors such as poor oral hygiene or the presence of foregut dysmotility (3). If high baseline hydrogen levels are observed, the test can be considered indeterminate, or at some centers, the test is aborted and rescheduled for another time (37). Because of these influential factors, it is critical to emphasize dietary prep before testing. The definition of an increased baseline level has not been defined, and interpretation of this point varies among centers. Elevated baseline levels of hydrogen defined with either a cutoff ≥10 or ≥ 20 ppm or methane ≥10 ppm have been reported in approximately a quarter of patients undergoing breath testing (17,38).

Rome guidelines had previously advocated for the use of chlorhexidine mouthwash before substrate administration to diminish fermentation by bacterial flora from the oral cavity (18,39). One method for administering chlorhexidine is to administer 10 mL of chlorhexidine (1.2 mg/mL) mouthwash around the mouth for 20–30 seconds, forcing it between teeth and gargling before spitting it out and rinsing the mouth with water (17). In 1 study of 388 consecutive hydrogen-methane breath tests, chlorhexidine mouthwash significantly reduced hydrogen and methane gas in patients with higher baseline values (defined as ≥ 10 ppm of either hydrogen or methane), reducing breath hydrogen in 67% of patients and/or methane gas in 93% (17). This study highlights the ability of oral dysbiosis/oral hygiene to impact measured gas levels, in some cases to a degree that impacts the interpretation of the test. The study also found that a single mouthwash immediately before breath testing resulted in an apparent early increase in expired gases in the first postsubstrate samples in 30 of 43 (69.7%), suggesting that the first single mouthwash may not be adequate to eliminate the role of oral flora, and mouthwashes before every sample may be more effective (17). However, further studies are needed to determine how frequently and how impactfully oral dysbiosis confounds breath testing in routine clinical practice. The North American consensus and ACG guidelines on breath testing do not specify whether oral antiseptic solutions are necessary, but the recent European guidelines do advocate for oral antiseptic use before testing (1,3,15). A recent study found that light walking for an hour, being careful to avoid hyperventilation, may reduce high baseline hydrogen and methane levels and allow for a meaningful examination while reducing diagnostic delays from rescheduling the test, but more research is needed to support this approach (38,40).

Increased baseline hydrogen levels could potentially also reflect the presence of significant foregut dysmotility, such as in patients with severe esophageal dysmotility (i.e., achalasia) or significantly impaired gastric motility (i.e., gastroparesis) (41). The concern with these conditions is that the ingested substrate will be metabolized by bacteria residing in the mouth, esophagus, or stomach and may not reliably reach the small intestine within the time windows typically used for interpreting breath test data. These conditions should be considered when determining whether the patient would be best served with adapted testing protocols (i.e., assessment of the orocecal transit time [OCTT]) or an alternative testing approach (i.e., small bowel aspirates).

Test results and interpretation.

Hydrogen-methane breath tests are generally easy to record and straightforward to interpret. Hydrogen and methane gas levels recorded from samples collected over time are presented in a report and plotted graphically (sample test in Figure 1a,b). Figure 2 demonstrates various commonly observed patterns of glucose breath test results and their interpretation. There is consensus among all current guidelines to define a hydrogen gas rise of ≥20 ppm from baseline by 90 minutes as a positive test result for SIBO in both clinical and research settings (1,3). A methane gas rise ≥10 ppm at any time during the study period is also defined as positive breath test (1,3). Former protocols used different substrate dosages and cutoff levels. For example, the modified Rome protocol used a 50 g glucose load and a >12 ppm hydrogen and methane cutoff (18). Comparison of the North American and modified Rome protocols (North American 3,102 patients; modified Rome 3,193 patients) found that positive glucose breath tests were more common with the North American protocol (39.5% vs 29.7%, $P < 0.001$) (30). This result appeared to be due to generally higher peak methane levels with the North American protocol ($P < 0.001$) (30). Average times to achieve peak hydrogen and methane production levels were not found to differ between protocols. In addition, GI and extraintestinal symptoms during breath testing were more prevalent with the North

a

	Sample	Ppm H_2	Ppm CH_4	Concentration CO_2 (%)	(f) CO_2
Baseline	1	2	0	4.0	1.38
15 min	2	3	0	3.7	1.49
30 min	3	5	0	3.8	1.45
45 min	4	32	0	3.8	1.45
60 min	5	46	0	3.9	1.41
75 min	6	84	0	4.0	1.38
90 min	7	122	0	4.3	1.28
105 min	8	162	0	3.9	1.41
120 min	9	153	0	4.1	1.34
135 min	10	142	0	4.1	1.34
150 min	11	146	0	4.1	1.34
165 min	12	135	0	3.9	1.41
190 min	13	149	0	4.2	1.31

b

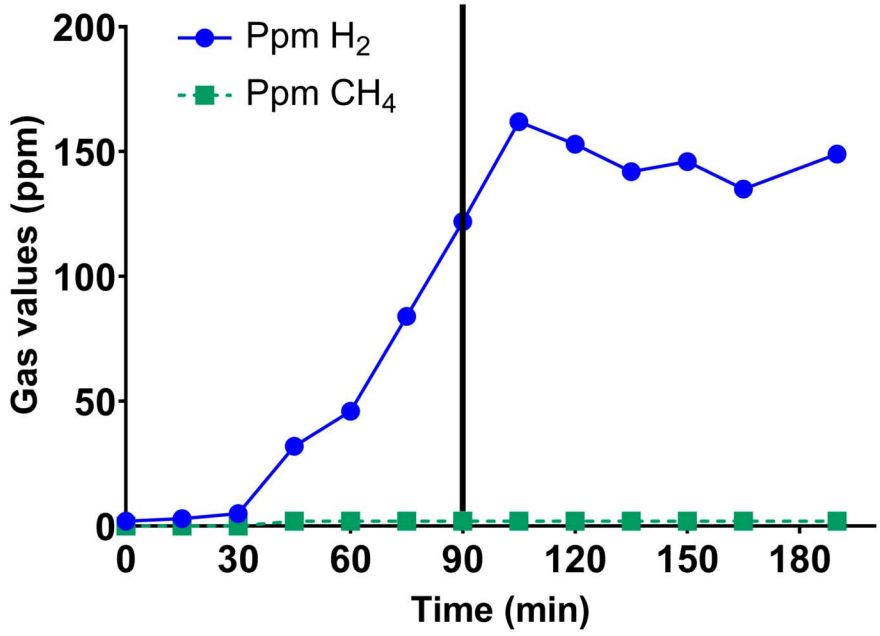

Figure 1. (**a** and **b**) Sample glucose breath test reports generally consist of a table (**a**) with individual breath test values and plot of the data (**b**). The test is generally measured in 10–30-minute intervals and commonly performed in 2–3 hours. Breath test values at 90 minutes are used for interpretation (highlighted box), but all numbers are provided given variable individual factors that can influence values (i.e., orocecal time). Test interpretation uses the values of the first 90-minute results: peak hydrogen production: normal <20 ppm, increased methane production at any time: normal <10 ppm, f(CO_2) closer to 1.00 is ideal. Because the corrected gas levels do not alter the interpretation, observed hydrogen and methane are plotted in Figure 1b. ppm, parts per million.

American protocol (30). As such, there is a higher test positivity rate with the North American Consensus protocol.

Hydrogen. The patterns of hydrogen peaks in positive tests will vary based on the substrate given. In positive glucose breath tests, there is a peak in hydrogen production when the substrate enters the jejunum. In positive lactulose breath tests, there is generally a double peak, with the second peak occurring when the lactulose enters the colon (1). Lactulose breath tests have been previously considered a method to determine OCTT; however, for a variety of factors, this is not considered a completely reliable approach (1). As such, there is no current role in using lactulose to measure OCTT, and the presence of 2 separate peaks on lactulose breath testing is not required for a diagnosis of SIBO (1).

Methane. There is increasing recognition that methane gas production directly impacts GI function. Recent ACG guidelines proposed a new term to describe levels of increased methane gas production—intestinal methanogen overgrowth (IMO) (1). Distinguishing IMO from SIBO acknowledges that a different clinical phenotype is associated with higher levels of methane production (constipation) than hydrogen production (typically

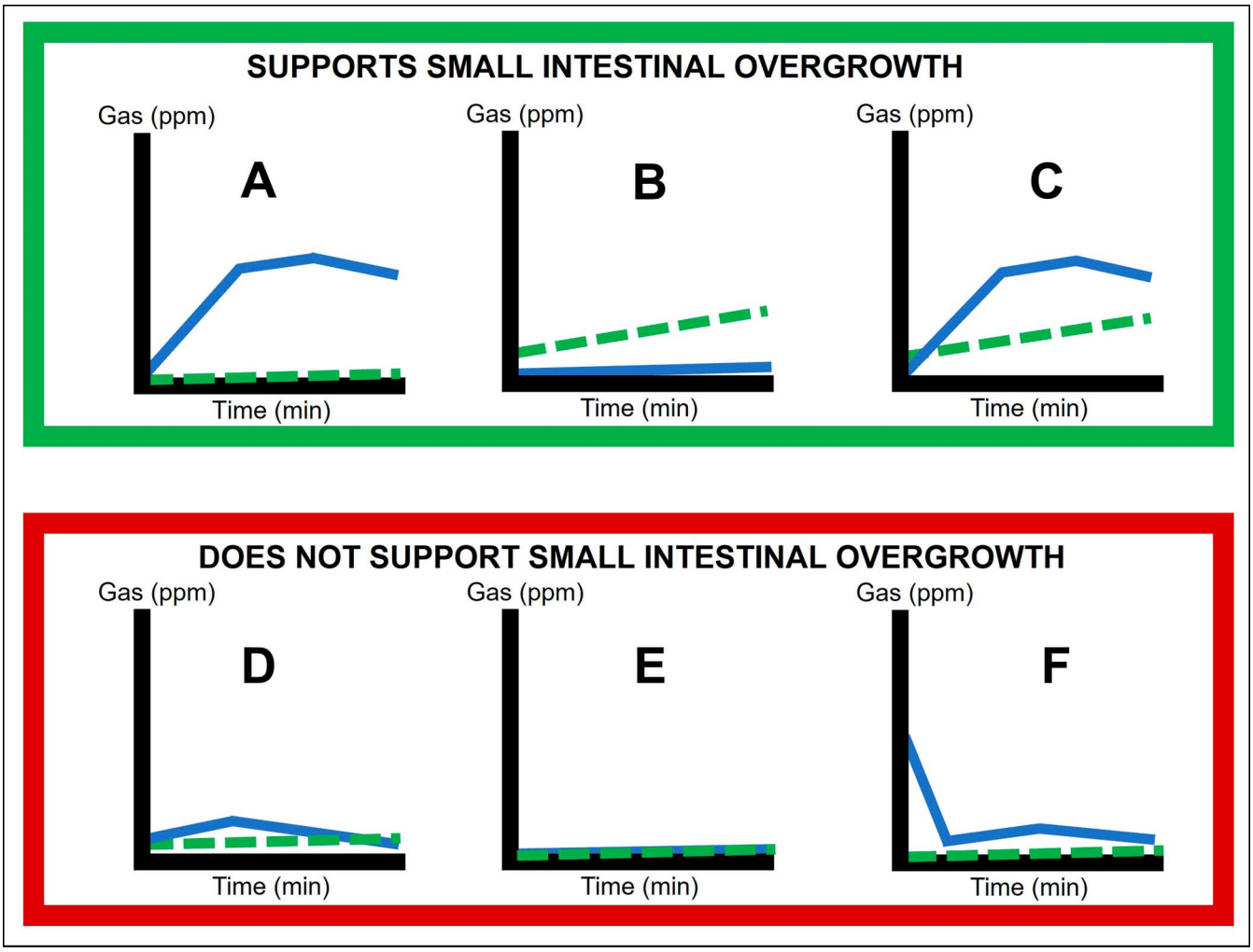

Figure 2. Possible patterns of glucose breath test results and their interpretation. x axis is time (minutes), y axis is gas (ppm), solid line is hydrogen values, and the dashed line represents methane values. (**a**–**c**) Small intestinal bacterial overgrowth (SIBO) supported: A. high hydrogen, no methane; (**b**) no hydrogen, high methane; (**c**) high hydrogen, high methane. (**d**–**f**) SIBO not supported: (**d**) low hydrogen, low methane; (**e**) no hydrogen, no methane (flat line); (**f**) high hydrogen baseline—consider retesting. ppm, parts per million.

diarrhea). The IMO terminology also more accurately reflects the fact that methanogens are not actually bacteria but belong to the Archaea kingdom (1). Furthermore, methanogens may overpopulate either the colon or the small intestine (1). Finally, methanogens may not be adequately treated by single antibiotic treatments used in SIBO management and may require unique antibiotic treatment combinations (i.e., rifaximin and neomycin) (42). A level of methane gas level ≥10 ppm observed at any time during the breath testing (including at baseline in a fasting patient) is considered a positive IMO test result (43).

Multiple studies have identified that higher methane levels are positively associated with constipation and are inversely associated with diarrheal disorders (44,45) Methane gas has been shown to directly inhibit intestinal transit in dogs by 59% compared with insufflated room air (46), and methane levels correlate with the slower intestinal transit times (2,46–49). Methane-predominant SIBO/IMO is more prevalent in patients with constipation-predominant IBS (47–50). It is possible that future testing protocols may be modified if the intent of the breath test is to diagnose IMO. For example, lactulose has advantages over glucose substrates to determine IMO, given that lactulose accesses more distal regions of the GI tract and archaea overgrowth may occur in either the small intestine or the colon (1). Different testing protocols specifically for IMO could be considered in the future. A recent study evaluating a fasting single methane measurement (SMM) ≥10 ppm compared against standardized 2-hour breath tests demonstrated high test performance (compared with 2-hour glucose breath test: sensitivity 86.4%, specificity 100%, positive predictive value 100%, and negative predictive value 97.0%; compared with 2-hour lactulose breath test: sensitivity 86.4%, specificity 100%, positive predictive value 100%, and negative predictive value 97.6%) (43). The study also demonstrated that the SMM value is associated with constipation, is associated with *Methanobrevibacter smithii* colonization (a known intestinal methanogen), and that SMM decreases after antibiotics (43). In addition, studies assessing patients with a positive breath test for IMO have found that over 75% of patients had $CH_4 \geq 10$ ppm at baseline (51,52). As such, SMM testing could prove a useful surrogate for assessing treatment response. Future research studies are needed to identify additional clinical characteristics of patients with IMO and

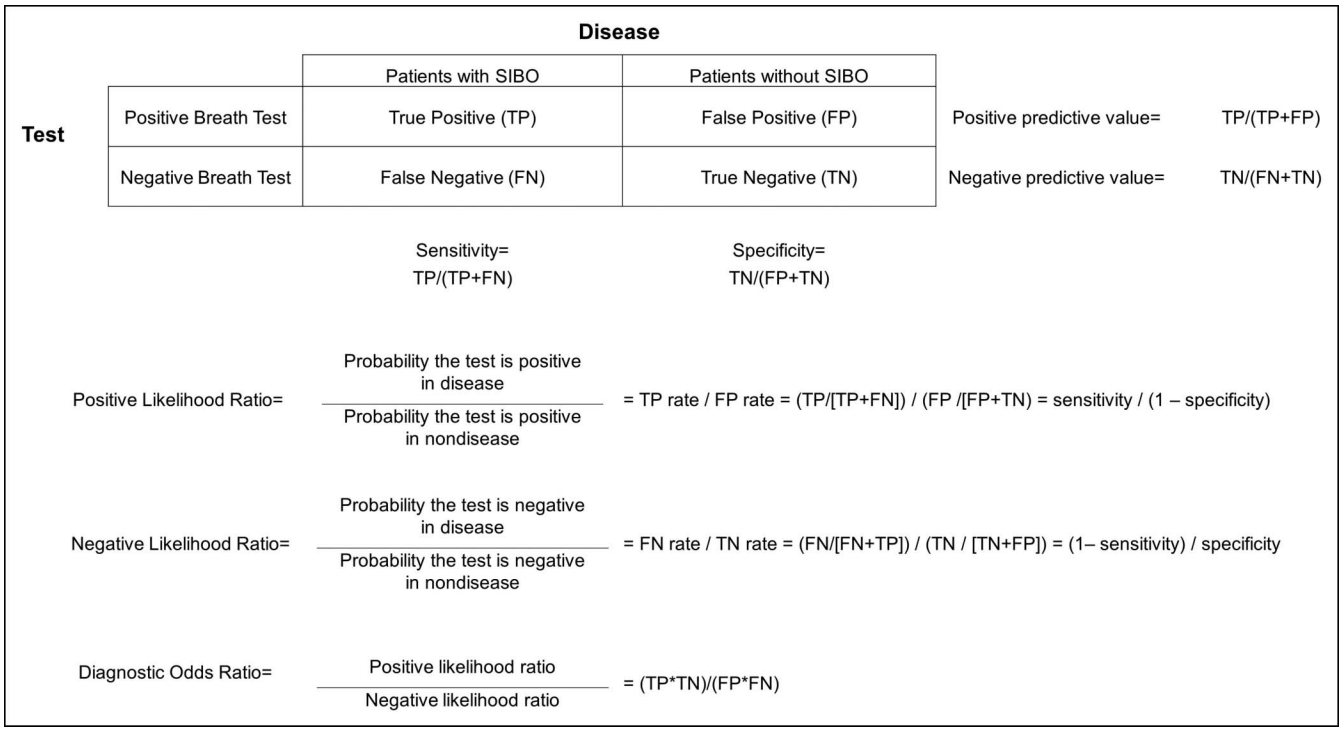

Figure 3. Diagnostic test parameters and formulas. SIBO, small intestinal bacterial overgrowth; ppm, parts per million.

whether protocol adaptations may be helpful to detect patients with IMO.

Carbon dioxide. If available, measurement of CO_2 concentration (%) is used to derive a correction factor, f(CO_2), that accounts for dilution of the alveolar sample by ambient air or dead space, thereby allowing for correction of observed gas levels and also identifying significant sampling errors (3,15). To correct the sample concentrations of hydrogen and methane for possible dilution because of incorrect gas sampling, the observed H_2 and CH_4 values are multiplied by the correction factor calculated from f(CO_2) = alveolar CO_2 concentration/sample CO_2 concentration, yielding normalized H_2 and CH_4 values (13). At our center, we assume an alveolar CO_2 concentration of 5.5%, but some centers use 5% (13,36,37,53). Correction factor values close to 1.00 indicate excellent sample collection with minimal to no dilution, whereas high values indicate poor sample collection. Guidelines discuss the measurement of CO_2 but do not formally discuss how it should be implemented in test interpretation (3). Our approach is to consider samples valid when CO_2 correction factor $\leq$ 2.5, similar to the published literature (36). Regardless, carbon dioxide corrections generally only impact test interpretation for a minority of patients. One study that evaluated differences in results after correction found high agreement of positive breath test results with and without CO_2 correction (Cohen kappa 0.92) (13).

Test performance characteristics

Several characteristics are used to evaluate the utility of a diagnostic test in discriminating the presence or absence of a disease. These test characteristics are sensitivity, specificity, positive predictive value, negative predictive value, likelihood ratios, and DOR (Figure 3). Here, sensitivity is defined as the ability of a positive breath test to accurately predict the presence of SIBO, expressed as the percentage of persons with SIBO who test positive. Specificity is defined as the ability of a negative breath test to accurately predict the absence of SIBO, expressed as the percentage of persons without SIBO who test negative. Positive predictive value is the ability of breath tests to separate true-positive results from false-positive results in a given population, expressed as the percentage of persons with a positive breath test who indeed have SIBO. Negative predictive value is the ability of breath tests to separate true-negative results from false-negative results in a given population, expressed as the percentage of persons with a negative test result who do not have SIBO. Finally, likelihood ratios are perhaps the most useful tool for clinical management and decision making. Positive likelihood ratios (PLRs) are defined as the probability of a positive breath test in a patient with SIBO, divided by the probability of a positive breath test in a person without SIBO. Negative likelihood ratios (NLRs) are defined as the probability of a negative breath test in a patient with SIBO, divided by the probability of a negative test in a person without SIBO. Likelihood ratios are always a positive number (ranging from zero to infinity). Likelihood ratios greater than 1 argue for the diagnosis of interest, and the larger the number, the greater the posttest odds that the patient truly has the SIBO. Findings between 0 and 1 argue against the diagnosis of interest. Values close to 0 indicate that SIBO is less likely and values close to 1 are equivocal. The DOR is a measure of the effectiveness of a diagnostic test. Here, it is defined as the ratio of the odds of the breath test being positive if the subject has SIBO, relative to the odds of the breath test being positive if the subject does not have SIBO. DORs for useful tests are greater than 1, with greater DORs indicating better test performance (e.g., a DOR of close to 10 indicates a very good test). Finally, the area under the curve (AUC) of receiver operator curves plots sensitivity and specificity, where 1 − specificity is on the x axis, and sensitivity is on the y axis. The AUC helps estimate the discriminative power of a test, where 1 indicates a perfect test, and lower values suggest a less

Table 3. Test characteristics of glucose and lactulose breath tests based on published data using jejunal aspirates as the gold standard (54)

Test characteristics	Glucose breath test (668 patients, 14 studies)	Lactulose breath test (214 patients, 4 studies)
Pooled sensitivity	54% (48%–61%)	42% (32%–53%)
Pooled specificity	83% (79%–87%)	71% (62%–78%)
Pooled positive likelihood ratio	2.45 (1.51–3.97)	1.30 (0.77–2.22)
Pooled negative likelihood ratio	0.60 (0.45–0.80)	0.79 (0.57–1.08)
Pooled diagnostic odds ratio	5.17 (2.42–11.05)	1.77 (0.72–4.37)
Area under the curve of summary receiver operating characteristic (SROC) curve	0.7418	0.5582

discriminating test (i.e., a completely nondiscriminating test has an AUC of 0.5) (Table 3).

A recent systematic review with meta-analysis determined the diagnostic performance of lactulose and glucose breath tests for detecting SIBO (54). Using a total of 14 studies in which jejunal aspirate cultures were obtained as the gold standard comparator to breath tests in the same group of patients, and using a random effects model, Losurdo et al calculated the pooled weighted sensitivity, specificity, positive and NLR, and DOR of breath tests (54). As can be seen below, glucose substrates yielded generally improved breath test performance characteristics compared with breath tests that used lactulose substrates. Additional sensitivity analysis using different jejunal aspirate thresholds found that glucose breath tests performed similarly with either definition: sensitivity 40.7% and specificity 84.0%, with diagnostic cutoff $>10^3$ CFU/mL, and sensitivity 55.3% and specificity 83.9%, with diagnostic cutoff $>10^5$ CFU/mL (54). There were not sufficient data on lactulose breath tests to determine test performance with different jejunal aspirate thresholds (54). Overall, breath testing in the current era would appear to have characteristics of a moderately good test for diagnosing SIBO.

Complementary and alternative tests used to diagnose SIBO

Complementary test: measurement of hydrogen sulfide gas. A wide range of microbiota can cause SIBO. These bacteria can be grouped broadly into hydrogen producers and hydrogen consumers, which consist of methanogens (archaea) and sulfate-reducing bacteria (55,56). Gas exchange occurs between hydrogen producers and hydrogen consumers (methanogens and sulfate-reducing bacteria), and this interaction within the gut lumen influences the amount of hydrogen gas that is ultimately absorbed into the circulation and measured in the exhaled breath. Measurement of hydrogen sulfide gas allows for an assessment of the presence of sulfate-reducing bacteria (55,57). This additional measurement is still in development and is not yet widely available in all commercially available breath tests. Current issues unique to H_2S measurements include sample transportation and stability concerns, and importantly, standardized thresholds cutoffs for H_2S are lacking. Despite these limitations, addition of hydrogen sulfide gas measurement has potential to further enhance test reliability to diagnose SIBO. H_2S levels have been linked to a diarrhea phenotype (55–57). Furthermore, a recent study showed an association of H_2S levels with increased predominance of H_2S producers, including *Fusobacterium* and *Desulfovibrio* species (56).

Complementary testing: transit time. The 90-minute cutoff period used in standard breath testing may not reliably account for patients with altered OCTTs. OCTT can be measured using a barium study with small intestinal follow through or potentially *via* a wireless motility capsule examination. In a study of 60 normal individuals, the average OCTT was 105 minutes (25%–75% range: 90–135 minutes) (58). Thus, the use of 90 minutes as the cutoff period to detect SIBO seems appropriate in most normal individuals. However, the interpretation of an elevated, later peak at times >60 minutes but <90 minutes could indicate a faster transit time, rather than distal SIBO (15). For instance, patients with Roux-en-Y gastric bypass (RYGB) have widely ranging OCTTs. A recent study of RYGB patients found that the median OCTT was 60 minutes (range 10–345 minutes) (59). When lactulose breath tests were combined with the patient's specific OCTT, 26 of the 36 RYGB patients had increases in hydrogen levels ≥ 20 ppm or methane levels ≥ 10 ppm that occurred within their OCTT, suggesting a true-positive rate of 72.2% if lactulose breath test were used as a stand-alone test with a 90-minute cutoff. Antibiotic response rates further confirmed these findings, with a 78.3% antibiotic treatment response in the true-positive breath test group, compared with 33.3% response rates in the false-positive breath test group ($P = 0.03$) (59). These observations suggest that adjunctive measurement of OCTT is an important consideration when performing breath testing in RYGB patients, but measuring the OCTT is cumbersome (15). Given that methods of direct jejunal culture in RYGB patients are not well defined (i.e., it is unclear whether one should obtain aspirate from the pancreaticobiliary limb, Roux limb, or common channel) and are invasive, symptomatic patients with RYGB may be better served with empiric antibiotic treatment, especially given the high prevalence of SIBO in this population (Table 4).

Patients with markedly delayed OCTTs are at higher risk of false-negative breath test results when using the standard 90-minute cutoff value (15). This is a particularly problematic issue in patients with scleroderma. In a study of 55 patients with scleroderma, the mean OCTT was 150 minutes (25%–75% 142.5–165 minutes) (58). However, patients with scleroderma also have slower esophageal and gastric motility that make the transit of the oral substrate very difficult to predict. Symptom scores may be a more useful approach to diagnose this population. Logistic regression of patients with scleroderma with a positive glucose breath test found that the significant risk factors for SIBO were diarrhea (odds ratio: 11.043, $P = 0.0009$) and constipation (odds ratio: 48.537, $P = 0.006$) (60). Another study confirmed that diarrhea is a strong predictor of SIBO in patients with scleroderma with an OR 8.8 (95% confidence interval 4.1–19, $P < 0.0001$) (61) Of interest, the use of a symptom score could be a useful screening approach for this population. A global symptomatic score (GSS) of digestive symptoms ≥5 was found to have sensitivity

Table 4. Prevalence of small intestinal bacterial overgrowth in different populations

Population	SIBO prevalence % (95% CI)	Method of diagnosis	Source	No. of patients (number of studies)	Ref.
IBS	MBT: 35.5% (33.6%–37.4%) (controls 29.7% [27.6%–31.8%]) MAC: 13.9% (11.5%–6.4%) (controls 5.0% [3.9–6.2%]) LBT: 62.3% (58.7%–65.9%) (controls 33.5% [27.5%–39.5%]) GBT: 20.7% (18.6%–22.8%) (controls 4.4% [3.0%–5.9%]) Bacterial count 10^5 CFU/mL: 13.9% (11.5%–16.4%) (controls: 5.0% [3.9%–6.2%]) Bacterial count 10^3 CFU/mL: 33.5% (30.1%–36.9%) (controls 8.2% [6.8%–9.6%]) Subgroups: IBS-D: 35.5% (32.7%–40.3%) IBS-C: 22.5% (18.1%–26.9%) IBS-mixed: 25.2% (22.2%–28.4%)	GBT, LBT, JAC, and DAC	Systematic review	3,192 patients with IBS and 3,320 mixed controls (25 studies)	Shah 2020 (119)
IBS	GBT: 31% (24%–38%) LBT: 47% (39%–56%) MAC: 19% (8%–30%) Bacterial count 10^5 CFU/mL: 13% (2%–24%) Bacterial count 10^3 CFU/mL: 28% (14%–43%) Different Rome criteria: Rome I: 72% (44%–91%) Rome II: 40% (27%–54%) Rome III: 35% (28%–43%)	GBT, LBT, JAC, and DAC	Systematic review	8,398 patients with IBS and 1,432 mixed controls (50 studies)	Chen 2018 (90)
Roux-en-y	GBT: 73.4% (symptomatic controls 36%)	GBT only	Matched cohort study	17,973 patients (271 RYGB matched to 573 symptomatic patients with native anatomy)	Dolan 2021 (120)
Parkinson's	MBT: 46% (36%–56%) GBT: 35% (20%–50%) LBT: 51% (37%–65%) Western countries: 52% (40%–64%) Eastern countries: 33% (22%–43%) Largest case-control GBT: 30.2% (23.5%–36.9%) (controls: 9.5% [5.4%–13.6%])	GBT and LBT Largest case-control (121): GBT	Systematic review	973 patients (11 studies) 182 patients and 200 healthy age/sex/BMI-matched mixed controls (121)	Li 2021 (122)
Liver disease	MBT: 35.8% (32.6%–39.1%) (controls: 8.0% [5.7%–11.0%]) GBT: 32.1% (28.6%–35.7%) (controls: 5.1% [2.9%–8.6%]) LBT: 50.0% (41.9%–58.1%) (controls 18.8% [13.2%–26.2%]) JAC: 68.3% (59.6%–76.0%) (controls 7.9% [3.4%–12.7%]) Subgroups: Cirrhosis: 40.1% (36.6%–43.8%) (controls 7.3% [4.9%–10.8%]) Nonalcoholic fatty liver disease: 33.5% (27.4%–40.2%) (controls: 7.3% [4.9%–10.8%])	GBT, LBT, and JAC	Systematic review	1,000 patients with CLD and 488 healthy mixed controls (19 studies)	Shah 2017 (123)

Table 4. (*continued*)

Population	SIBO prevalence % (95% CI)	Method of diagnosis	Source	No. of patients (number of studies)	Ref.
Systemic sclerosis (Scl)	BAT: 34% (27%–42%) GBT: 27% (20%–35%) LBT: 56% (46%–67%) JAC: 35% (25%–51%) Western countries 38% (31%–47%) Asian countries 15% (10%–23%) Studies also reporting the prevalence of the healthy control population GBT (64): 18% (healthy controls 5%) LBT (58,98): 50% (healthy controls 5.8%)	GBT, LBT, and JAC	Systematic review	700 Scl and 217 controls (14 studies) 204 Scl and 180 healthy asymptomatic controls (58,64,98)	Feng 2021 (61)
IBD	MBT: 22.3% (19.9%–24.7%) Subgroups: Crohn's disease: 25.4% (22.5%–28.3%) Ulcerative colitis: 14.3% (10.5–18.1) Only case-controls: IBD 23.2% (19.55%–26.85%) Crohn's 32.1% (26.1%–38.0%) UC 15.8% (11.5%–20.1%) (mixed controls 4.2% [2.2%–6.1%])	GBT and LBT	Systematic review	1175 patients with IBD and 407 controls (11 studies) 5 case-control studies (124–128): 513 cases, 407 controls (310 healthy asymptomatic; 97 nonspecific GI symptoms)	Shah 2019 (129)
IBD	57% in patients with IBD (n = 264) Subgroups: Crohn's disease 56% (n = 163) Ulcerative colitis 58% (n = 101) CH_4 predominant: Crohn's 3.8% UC 6.9% Non-IBD 16.0% H_2 predominant: Crohn's 46.2% UC 45.1% Non-IBD 46.8%	LBT only	Retrospective cohort	14,847 consecutive breath tests of patients with IBS-like symptoms referred for breath testing (486 IBD and 10,505 non-IBD symptomatic controls with at least 1 BT)	Gu 2020 (16)
Chronic pancreatitis	MBT: 38.6% (25.5–53.5%) (controls 9.9% [4.9%–19%]) GBT: 26.7% (18.0%–37.7%) LBT: 65.3% (38.1%–85.1%)	GBT and LBT	Systematic review	518 patients with CP and 372 controls (13 studies)	Kurdi 2019 (130)
CD	BAT: 18.3% (11.4%–28.1%) MBT 20.8% (11.9%–33.7%) MAC 12.6% (5.1%–28.0%) Patients with nonresponsive celiac disease while on a GFD were 17.1% (9.5%–28.7%)	GBT, LBT, JAC, and DAC	Systematic review	742 CD (14 studies) 4 case-control with 178 healthy controls and 125 symptomatic controls	Shah 2022 (131)
CD	BAT: 20% (10%–30%) (healthy controls: 1.9%) (symptomatic controls: 31.2%) MBT: 23% (10%–37%) GBT: 19% (7%–31%) LBT: 28% (7%–49%) JAC: 11% (3%–19%) Subgroups: Symptomatic CD despite gluten-free diet: 28% (10%–47%) Patients with asymptomatic CD: 10% (3%–16%)	GBT, LBT, JAC, and DAC	Systematic review	614 patients with CD (11 studies) 3 studies with controls (102 healthy controls; 125 symptomatic controls)	Losurdo 2017 (132)

Table 4. (*continued*)

Population	SIBO prevalence % (95% CI)	Method of diagnosis	Source	No. of patients (number of studies)	Ref.
FD	DAC: 19.4% (>10^3 cfu/mL) 14.5% (>10^4 cfu/mL) 8.3% (>10^5 cfu/mL) FD subtypes: PDS 20.8% EPS 12.5% PDS-EPS 31.6% (GER controls [>10^3 cfu/mL]: 3.3%) (IBS controls [>10^3 cfu/mL]: 16.7%)	DAC	Prospective cohort	227 patients with FD, 30 controls with upper endoscopy for GER, and 90 patients with IBS fulfilling Rome IV Functional dyspepsia subtypes among 227 patients: 63.4% PDS, 28.2% EPS, and 8.4% overlapping PDS-EPS	Tziatzios 2021 (133)
Older community population (>61 yr)	GBT: 15.6% (controls: 5.9%)	GBT	Prospective cross-sectional	People living in the community. 294 people with age >61 yr; controls 34 people aged 24–59	Parlesak 2003 (99)

For several studies, estimates for the approximate prevalence of SIBO are for the entire population of interest, including asymptomatic patients within the population. If available, further characterization of the control population (healthy asymptomatic and symptomatic), specific patient population (IBS and GER), mixed (healthy asymptomatic and symptomatic), and prevalence of SIBO in these control populations is provided.
BAT, breath test and aspirate culture results combined; BT, breath test; CI, confidence interval; DAC, duodenal aspirate culture (defined as ≥10^3 colony-forming units/mL of duodenal aspirate and/or the presence of colonic type bacteria); EPS, epigastric pain syndrome; FD, functional dyspepsia; GBT, glucose breath test only; GER, gastroesophageal reflux; JAC, jejunal aspirate culture; IBS, irritable bowel syndrome; LBT, lactulose breath test only; MAC, mixed aspirate culture (duodenal or jejunal aspirate data combined with cutoff 10^5 cfu/mL); MBT, mixed breath test (lactulose and glucose breath test data combined); PDS, postprandial distress syndrome; PDS-EPS = meets both PDS and EPS criteria.

and specificity to predict SIBO in patients with scleroderma of 82% and 86%, respectively, and a predictive positive and negative value of 0.868 and 0.905, respectively (60). This is further supported with another study, which found that patients with scleroderma with SIBO had a median GSS score of 8 (25th–75th percentile 3.25–10.75) (58). Eradication of SIBO was achieved in 73.3% of these patients, resulting in a significant reduction of symptoms in 72.7% of these patients (GSS score 2, 25th–75th percentile 1–3, $P < 0.05$) (58). Given the high pretest probability that a patient with scleroderma with compatible GI symptoms has SIBO, it is reasonable to forego OCTT measurement and breath testing and pursue empiric SIBO treatment. A meta-analysis of treatment with rifaximin in patients with scleroderma documented high clinical response rates (>70%) with cessation of diarrhea, abdominal symptoms, and normalization of lactulose hydrogen breath tests (58). This meta-analysis supports empiric antibiotic treatment for in patients with scleroderma with symptoms supportive of SIBO, particularly diarrhea. Current antibiotic guidelines for recurrent bacterial overgrowth in patients with scleroderma per European Alliance of Associations for Rheumatology recommendations involve the oral administration of amoxicillin during the first month (500 mg 3×/24 hours), ciprofloxacin during the second month (500 mg 2×/24 hours), and metronidazole during the third month (500 mg 3×/24 hours) (62,63). Yet, there may still be value in breath testing in this population if there is lack of clinical response to antibiotics; if so, the testing should be coupled with an assessment of OCTT (64).

Complementary test: fructose and lactose breath testing provide information regarding carbohydrate malabsorption and intolerance. Breath tests that use fructose and lactose substrates provide information on specific carbohydrate malabsorption states that account for dietary triggers of GI symptoms (65). These tests are generally performed at specialized tertiary academic motility centers. Testing to rule out SIBO before using these tests is important, given that SIBO itself confounds the interpretation of fructose- and lactose-based testing (66,67). Fructose and lactose substrates are each dosed at 25 g and mixed with or followed by 1 cup of water (3). Fructose and lactose breath testing should be performed for at least 3 hours (3). A rise of ≥20 ppm from baseline in hydrogen during the test is generally considered positive for both fructose and lactose breath tests (3), and it is particularly important to document the occurrence of symptoms that occur during the testing. A combination of objective hydrogen gas measures and symptom reporting is important for test interpretation. One method to interpret fructose and lactose intolerance classifies breath testing results into the malabsorption, intolerance, normal, or hypersensitivity categories (see Supplementary Table 2, http://links.lww.com/CTG/A909) (68). However, fructose absorption is influenced by the presence of other sugars, such as sorbitol and glucose. Thus, the isolated fructose substrate may not reliably reflect normal patterns of absorption and can lead to false-positive results during breath testing. As an alternative to breath testing for lactose and fructose malabsorption, a temporary diagnostic and therapeutic trial of diet elimination that includes eliminating dairy, fructose, and artificial sweeteners could be considered (65).

Alternative test: small intestinal aspirates. Direct culture of small intestinal fluid aspirate is the current gold standard modality to diagnose SIBO (3,15). Yet, there are several issues with this approach. First, small intestinal aspirates require an inherently invasive procedure (endoscopy) that is time consuming and expensive, and standardized techniques and tools for aseptic collection of small intestinal aspirates are lacking (3,15). Second, there is no consensus on sample handling and the precise microbiological techniques required for optimal culture of aspirate

samples (3,15). Oral bacteria may contaminate aspirates, and obligate anaerobes may be harder to culture. In a retrospective cohort of patients who underwent both duodenal aspirate and lactulose breath tests, 20% had gram-positive flora contamination of duodenal aspirate (defined as levels $>10^5$ cfu/mL) from presumptive oral or skin flora, and there were significant discrepancies between the 2 tests (disagreement in 37.6% of cases, κ statistic $= -0.02$) (69). Third, due to bias in the region sampled (only proximal locations within the small bowel are directly accessed), patchy distribution of bacterial overgrowth or distal SIBO may be missed (1,3).

Despite these limitations, small intestinal aspirates performed under ideal conditions remain the gold standard for detecting SIBO and have an additional benefit of providing a mechanism for determining the identity of organisms and their antibiotic sensitivities (70). For example, small intestinal fungal overgrowth can be distinguished from bacterial overgrowth only *via* the use of direct cultures (71). Technical modifications to aspirate procedures have helped to minimize contamination and optimize the ability to culture collected samples (72,73). The general aseptic technique required to obtain small bowel aspirate fluid involves deep duodenal intubation, minimizing suction during scope insertion through the mouth, esophagus, and stomach. One approach to obtain this is to advance a sterile catheter with side holes through the biopsy channel when the endoscope is positioned in the third or fourth portion of the duodenum (70,74). Then, with gentle suction, a minimum of 3–5 mL of fluid is aspirated and sent for aerobic and anerobic cultures (70). A sterile technique is used, and the sample is rapidly transported to the laboratory for seeding appropriate media to yield more precise colony counts. Cultures with colony counts $\geq 10^3$ are considered diagnostic of SIBO (1,3), but the normal ranges of colony counts vary greatly along the length of the gut, increasing in the more distal regions of the GI tract, and there remains some controversy about the exact colony counts that define SIBO (7,75).

Alternative test: empiric therapeutic trials of antibiotics. If the pretest likelihood of SIBO is high enough, many physicians choose to prescribe antibiotics as a diagnostic and therapeutic trial, rather than conduct breath testing. Because symptoms are generally poor predictors for SIBO and antibiotic use can worsen gut dysbiosis or even result in *Clostridium difficile*, it is generally advisable to pursue diagnostic workup before antibiotic therapy (1).

Rifaximin, nonabsorbable antibiotic (550 mg 3 times a day for 14 days), has the most data for use in treatment of SIBO (76,77). Significant benefits of rifaximin are that it is generally well tolerated and repeated administrations appear to have a low risk of microbial resistance (78). However, in the United States, costs and limited insurance coverage hinder rifaximin use (11). Systemic antibiotics can also be used to treat SIBO and include amoxicillin/clavulanic acid (875 mg twice a day), ciprofloxacin (250–500 mg twice a day), doxycycline (100 mg twice a day), metronidazole (250 mg 3 times a day), neomycin (500 mg twice a day), norfloxacin (400 mg twice a day), tetracycline (250 mg 4 times a day), or trimethoprim-sulfamethoxazole (160/800 mg twice a day) (11,79). Antibiotic courses for systemic antibiotics are variable, typically given for 10–14 days, but some have reported even longer durations (11,79). There are significant limitations for determining best usage of antibiotics due to multiple bacteria implicated in SIBO, bacterial populations with varying antibiotic susceptibilities, differing clinical populations, and significant study heterogeneity that have evaluated antibiotic treatment strategies (80). Because of the significant heterogeneity, meta-analyses of specific antibiotic responses should not be performed. Instead, future studies should concurrently report local antibiotic susceptibility patterns of implicated SIBO pathogens (i.e., *Escherichia coli* and *Klebsiella*, etc.) or ideally report SIBO pathogen susceptibilities if cultures are performed (reporting both pathogen prevalence and antibiotic susceptibility patterns of these pathogens) to significantly enhance our understanding and guide optimized antibiotic strategies.

In populations with a high risk of developing recurrent SIBO, the general recommendation is to rotate through different antibiotics in recurring treatments to reduce the development of bacterial resistance. A retrospective review found that rotating antibiotics (quinolone and azole, 1 after the other for 10 consecutive days per month over 3 months) resulted in improved long-term remission rates (defined as negative glucose breath test) compared with patients receiving repeated treatment with a single antibiotic (70% vs 51%, $P = 0.05$) (81). Remission in this study was also associated with a significant improvement in quality of life ($P = 0.035$) and in bloating symptoms ($P = 0.004$) (81). Concern for antibiotic resistance and alterations in the microbiome with long-term use of antibiotics should limit the usage of this treatment approach (82). For instance, rotating antibiotics was shown to have changes in the microbiome in pediatric patients with short bowel syndrome (82). Evaluating and minimizing factors resulting in recurrent SIBO should be pursued in patients with recurrent SIBO to decrease the need for repeated courses of antibiotics (11,79). Future understanding of organisms and antibiotic susceptibility profiles will lead to an improved treatment approach. Finally, symptomatic patients with methane-positive breath tests are best treated with combination rifaximin and neomycin therapy, with small studies showing both improved clinical response and methane elimination on follow-up breath tests (42).

Of interest, there is some evidence that herbal supplements with anti-microbial properties may effectively treat SIBO. One study demonstrated that herbal supplements had similar efficacy for SIBO treatment as rifaximin (83), but more studies are needed to confirm this observation. Other herbal remedies have been described, but more data are needed to support the approach (84–86). If true, herbal remedies could be potentially used as an alternative choice to antibiotics in a diagnostic and therapeutic trial paradigm for presumptive SIBO.

Implementation of an empiric therapeutic trial of antibiotics is currently an approach used for IBS with diarrhea (IBS-D) (87). SIBO is thought to be 1 mechanism that can contribute to IBS, although further understanding is needed to optimize diagnosis and management (87). Both ACG and American Gastroenterological Association guidelines encourage the use of rifaximin as an initial therapy for IBS-D (87,88). In large, double-blind placebo-controlled trials of patients with symptoms compatible with IBS-D, rifaximin resulted in significant improvement in both abdominal pain and stool consistency compared with placebo (40.8% vs 31.7%, $P = 0.001$) (89). The mechanism of action of rifaximin for IBS-D is likely partially related to SIBO treatment or at least some alteration in gut microbiome structure (87). More than one-third of individuals with IBS test positive for SIBO (90). Older age, female sex, and an IBS-D subtype, but not PPI use, were associated with SIBO among individuals with IBS symptoms (90). The efficacy of retreatment with rifaximin in patients with IBS who experienced a relapse of symptoms was assessed in a randomized clinical trial (91). Of patients who were initially treated

with rifaximin, 64% relapsed and were then randomized to receive rifaximin or placebo for 2 weeks. Rifaximin retreatment appeared to be superior to placebo at improving IBS-D symptoms in these circumstances (91). This observation led to the U.S. Food and Drug Administration approval for rifaximin for treatment of IBS-D, with up to 2 additional treatments for symptom recurrences. Meta-analysis supports the efficacy of this treatment approach with a number needed to treat of 9 (92). The safety profile of rifaximin is excellent, with modeling data suggesting rifaximin as an initial treatment for IBS-D with a number needed to harm of 8,971 (93). The predictive value of breath testing in determining clinical responses to a 14-day course of rifaximin has been evaluated. In 1 study, patients with IBS-D symptoms underwent breath testing before and after rifaximin therapy, and those patients with positive lactulose breath tests had higher rates of rifaximin response (59.7%; 37 of 62) compared with those patients with negative lactulose breath tests (25.8%; 8 of 31) (32). Furthermore, a higher proportion of patients with normalized lactulose breath tests following rifaximin treatment experienced symptom relief (76.5%, 13 of 17) (32). These findings confirm that rifaximin modulates the gut microbiome and that empiric trials of rifaximin could become a reasonable diagnostic test for patients with symptoms of IBS-D and suspected SIBO. In summary, rifaximin is a preferred initial treatment modality for IBS-D, but its use is not contingent on a positive breath test result. Rather, because of its excellent safety, tolerability, and efficacy profile, and the high pretest probability that patients with IBS-D positively respond to rifaximin, a therapeutic and diagnostic trial of rifaximin is a reasonable clinical practice (87).

How should hydrogen-methane breath tests be used in research?

Breath testing is an appealing, safe, noninvasive research tool to assess for SIBO in study populations and in populations of symptomatic individuals with poorly understood pathogenesis. Yet, the diagnosis and management SIBO have many outstanding questions: who to test, how to test, how to treat, and how to prevent? Breath testing is an objective test that will help to answer these questions. Importantly, breath testing provides a much-needed objective metric to assess the efficacy of pretreatment and posttreatment responses in symptomatic patients. This is crucial because symptom improvements alone do not appear to be reliable clinical trial end points. For instance, patients with IBS have high placebo responses that last for more than 6 weeks with response rates more than 15% for composite end points and more than 35% for abdominal pain responses (94). Therefore, breath testing helps to offer potential insight into the role of SIBO in symptom etiology and disease processes. Antibiotics, the cornerstone of SIBO treatment, have significant limitations given the range of implicated pathogens and antibiotic resistance profiles (1). Breath testing can provide a guide for patients who fail to respond to antibiotics. Furthermore, breath testing is safe and noninvasive and thus can be performed on anyone, including pregnant women and children (37). Breath tests may offer pretesting and posttesting for clinical trials with antibiotics to help further subgroup antibiotic responsiveness where symptom surveys may be less accurate. Further research is needed to determine ideal treatment protocol guidelines (i.e., baseline cutoffs) and the technique and interpretation of additional measurements (i.e., hydrogen sulfide and OCTT).

Research into improving sample detection has great promise for future diagnostic testing for SIBO. These efforts include the Smart Capsule Bacterial Detection System and the Gas Hydrogen Capsule (95–97). A capsule is swallowed that traverses the entire GI tract while measuring hydrogen, carbon dioxide, oxygen, and methane gases (95–97). By sampling concurrently at the direct source (*via* capsule) and *via* breath testing, we can understand variations of the small bowel that may affect intraluminal gas production and absorption (96). Studies combining breath testing with microbiome cultures will allow for new insights into signature patterns of bacterial overgrowth, linking gases measured and symptoms that could potentially enhance treatment strategies (56). Finally, future research that identifies specific bacteria implicated in SIBO and their antibiotic susceptibility patterns should lead to better, targeted therapies and better predictive models for clinical responses to treatment.

Given the variety in test accuracy using different diagnostic cutoffs, 1 potential area for research could be the evaluation of breath test results using gradations of results (i.e., strongly positive, moderately positive, weakly positive, weakly negative, moderately negative, and strongly negative) rather than dichotomous positive/negative assessments of the breath test results. Such nuances may improve the clinical utility of breath tests, enhancing diagnostic accuracy and potentially predicting clinical response given different degrees of test positivity. This approach also may predict which patients may require specific antibiotic treatment strategies (i.e., need for repeated courses or longer duration of antibiotics), but more research is needed.

How should hydrogen-methane breath tests be used in clinical care?

Breath testing provides useful information that guides clinical decision making and can be used to address multiple clinical and research questions. In this section, we discuss how to leverage knowledge of the performance characteristics of breath testing in making real-world clinical decisions about breath testing.

Clinical care uses. Test performance characteristics heavily influence the utility of a test in clinical practice. The prevalence of SIBO in a given patient population is perhaps the most important factor to consider in deciding whether to perform a breath test. Table 4 summarizes the prevalence of SIBO in various populations of patients who are commonly encountered by gastroenterologists in routine practice. The prevalence of SIBO in a reference patient population can be regarded as the pretest probability of SIBO. These pretest estimates may change as future studies are published and thus should be interpreted with caution as significant heterogeneity exists between study populations and testing protocols. For example, SIBO prevalence in patients with scleroderma may appear lower than other diseases as the population consisted of patients with scleroderma regardless of symptoms measured against healthy asymptomatic controls and testing did not properly account for OCTT (58,98). As testing protocols change, the prevalence in various patient populations will likely change. For instance, estimates for the older community population (aged >61 years) suggest a prevalence of 15.6% for the glucose breath test; however, a positive test was defined as only hydrogen gas ≥10 ppm, which is a lower threshold than currently supported by guidelines (99). Of interest, even given the lower threshold used and lack of methane, this study did demonstrate differences in rates of diarrhea between patients with a positive breath test and negative breath test. The older community population with a positive breath test were more likely to have diarrhea/loose stool compared with those with a negative breath test (21.7% vs 10.7%, P 0.024), with no significant differences for constipation (26.7% vs 21.4%, $P = 0.272$) or dyspeptic complaints (73.9% vs 67.3%, $P = 0.242$). (99) Finally, we

considered, but did not include, studies in patient populations rarely encountered in routine clinical practice, studies with significant heterogeneity, or studies where the reported association of SIBO was potentially confounded by additional variables (such as lifestyle, diet, and concurrent diseases) (100–109). Although a newer systematic review/meta-analysis on liver disease was available, the results of this analysis pooled all test modalities, and thus, we selected an older systematic review as the reference for SIBO prevalence for liver disease (110).

Clinical suspicion for SIBO is driven by the presence of compatible GI symptoms. However, individual GI symptoms differ in the strength of their relationship to the presence or absence of SIBO. Table 5 summarizes the breath test performance characteristics relative to several GI symptoms that were either the indication for breath testing or that occurred during breath testing (68). Interestingly, only diarrhea appeared to have a good PLR when present either at baseline or during breath testing. Other GI symptoms appeared to have poorer relationships with SIBO as detected by positive breath test results (Table 5). In another study, the prevalence of specific GI symptoms in those undergoing culture of small intestinal aspirates showed only a modest signal that diarrhea is associated with SIBO; however, the highest rates of symptom improvement with antibiotic treatment were observed in those with positive breath test results and symptoms of diarrhea and/or bloating (Table 6) (111). These results further support that the presence or absence of diarrhea, and potentially bloating, are important clinical signs that significantly influence the likelihood of a positive SIBO culture and, if positive, a greater likelihood of symptom responses to antibiotic treatment.

Clinical application of likelihood ratio. A PLR of 2 or greater increases the likelihood of SIBO, whereas intermediate likelihood ratios between 1 and 2 only modestly increase the posttest odds of SIBO. An NLR of 0.5 or lower decreases the likelihood of SIBO, whereas intermediate likelihood ratios between 0.5 and 1 only modestly decrease the posttest odds of SIBO. Using the pretest probability of SIBO (i.e., the prevalence of SIBO in a given patient population) and the likelihood ratios associated with breath testing, the posttest probability of SIBO can be calculated as shown below:

$$Pre-test\ odds = \frac{pre-test\ probability}{(1\ pre-test\ probability)}$$

$$Post-test\ odds = pre-test\ odds\ likelihood\ ratio$$

$$Post-test\ probability = \frac{post-test\ odds}{(post-test\ odds+1)}$$

The Fagan nomogram is an easier and more practical method to determine the posttest probability (Figure 4) (112,113). A line is drawn through the patient's pretest probability and the likelihood ratio of the breath test (PLR if the breath test is positive and NLR if the breath test is negative) to determine the associated posttest probability (the intersection of the line with the right axis).

A few illustrative examples demonstrate the application of these methods to clinical decisions with breath testing for diagnosing SIBO. For the purposes of simplicity and more reliable estimates of the test characteristics, we have used data from glucose breath testing studies.

Example 1. A patient with a diagnosis of celiac disease had relief of GI symptoms for a few years after initiating a gluten-free diet but recently has been experiencing symptoms of bloating and diarrhea in the past 4 months. The patient is confident in adherence to gluten avoidance, which is confirmed by negative celiac serology tests. Based on available data, the pretest probability of SIBO in this patient with celiac disease and active symptoms compatible with SIBO is 28% (Table 7). Breath testing has a positive predictive value of 2.45 and a negative predictive value of 0.60. Thus, using the Fagan nomogram, the posttest probability of SIBO with a positive glucose breath test is increased to 48.8%, and the posttest probability of SIBO with a negative glucose breath test is decreased to 18.9% (Figure 4). The breath test is ordered and comes back positive. The patient is offered treatment with a course of metronidazole, and the symptoms of diarrhea and bloating improve substantially.

Table 5. Diagnostic characteristics for both presenting symptoms and symptoms that occurred during the breath test

	Baseline symptom						Symptom occurred during the breath test					
	Sensitivity	Specificity	PPV	NPV	PLR	NLR	Sensitivity	Specificity	PPV	NPV	PLR	NLR
Diarrhea	3.0%	99.0%	88.1%	29.2%	**3.00**	0.98	12.0%	96.0%	88.1%	30.6%	**3.00**	0.92
Gas	7.0%	90.0%	63.4%	28.1%	0.70	1.03	16.0%	73.0%	59.5%	26.0%	0.59	1.15
Indigestion	5.0%	92.0%	60.7%	28.1%	0.63	1.03	11.0%	79.0%	56.5%	26.4%	0.52	1.13
Distension	5.0%	92.0%	60.7%	28.1%	0.63	1.03	8.0%	86.0%	58.6%	27.4%	0.57	1.07
Abdominal pain	9.0%	85.0%	59.8%	27.4%	0.60	1.07	18.0%	73.0%	62.3%	26.4%	0.67	1.12
Fullness	9.0%	85.0%	59.8%	27.4%	0.60	1.07	19.0%	66.0%	58.1%	24.8%	0.56	1.23
Bloating	10.0%	83.0%	59.3%	27.1%	0.59	1.08	18.0%	69.0%	59.0%	25.4%	0.58	1.19
Cramping	5.0%	91.0%	57.9%	27.9%	0.56	1.04	15.0%	76.0%	60.7%	26.5%	0.63	1.12
Nausea	7.0%	85.0%	53.6%	27.0%	0.47	1.09	20.0%	61.0%	55.9%	23.5%	0.51	1.31
Belching	6.0%	86.0%	51.5%	27.0%	0.43	1.09	21.0%	57.0%	54.7%	22.6%	0.49	1.39

Values shown are the sensitivity, specificity, positive predictive value, negative predictive value, positive likelihood ratio, and negative likelihood ratio. Values were calculated from published data from 883 patients who underwent glucose breath testing (68). Diarrhea has a high positive likelihood ratio for small intestinal bacterial overgrowth (SIBO). This means that when diarrhea is present—either as an indication or occurs during the breath test (see bolded values)—it increases the likelihood of the patient having SIBO.
NLR, negative likelihood ratio; NPV, negative predictive value; PLR, positive likelihood ratio; PPV, positive predictive value.

Table 6. Symptoms reported before upper endoscopy with duodenal aspirates among 1,263 patients

Symptom	Patients experiencing symptom	Frequency of positive culture by symptom (%)	Frequency of symptom improvement after antibiotics in patients with positive culture (%)
Diarrhea	480	126 (26.2%)	60 (47.6%)
Gas-related symptoms (gas, bloating, distention, eructation, and flatus)	419	140 (33.4%)	60 (42.8%)
Diffuse/upper abdominal pain	397	116 (29.2%)	39 (33.6%)
Dyspepsia/GERD	199	69 (34.6%)	3 (4.3%)
Nausea/vomiting	174	62 (35.6%)	12 (19.3%)

Patients could have multiple symptoms; therefore, there is an overlap in counts. The frequency of positive small intestinal culture by clinical symptom is provided. Finally, the frequency of improvement of symptoms in patients with positive small intestinal culture after treatment with antibiotics is provided. Adapted from Franco 2015 (111).
GERD, gastroesophageal reflux disease.

Example 2. A patient with a history of RYGB 8 years before presentation is evaluated for 5 months of persistent diarrhea with previous negative workup, which included stool studies and colonoscopy. Based on available data, the pretest probability of SIBO in this patient is 73.4% (Table 8). Given that diarrhea is the most prominent symptom, the PLR of SIBO being present is 3.0 (Table 5). Thus, using the Fagan nomogram, the probability of SIBO using the PLR is increased to 89.2%, which can be used as the adjusted pretest probability estimate for a positive test result if breath testing was performed. However, such a high pretest probability of SIBO in this population makes it reasonable to offer an empiric diagnostic and therapeutic trial of antibiotics, rather than to pursue breathing testing. The decision is made to offer an empiric trial of amoxicillin/clavulanate, and the patient's diarrhea subsequently resolved.

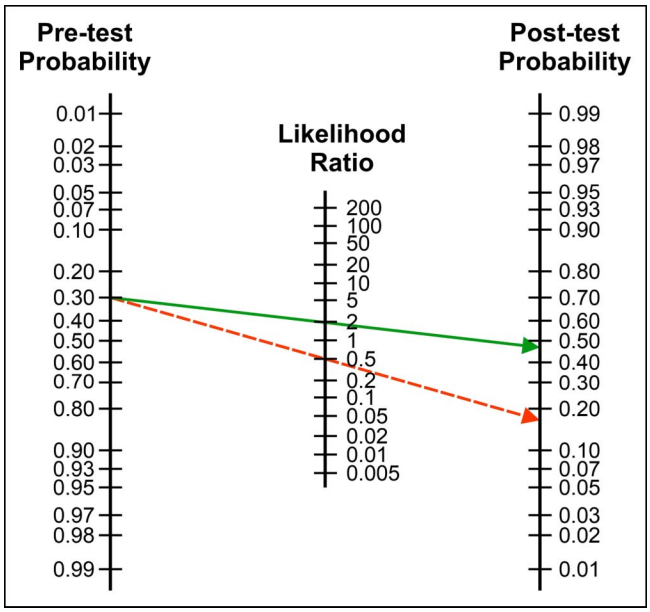

Figure 4. Fagan nomogram example. Starting point at pretest probability—in this example, 28% for symptomatic persons with celiac disease despite gluten-free diet (Table 4). Line drawn based on the likelihood ratio of the positive or negative breath test. Green arrow if the glucose breath test is positive (positive likelihood ratios 2.45) resulting in posttest probability 48.8%; red dashed arrow if the glucose breath test is negative (negative likelihood ratios 0.60) resulting in posttest probability 18.9%.

Real-world application of methane breath testing. As detailed previously, clinical guidelines support the distinction of patients with increased methane gas levels as having IMO (1). Limited data are available on the prevalence of IMO in different patient populations, as most initial clinical studies that report breathing test results did not separately report test positivity due to hydrogen or methane levels. Table 7 summarizes the prevalence of IMO in normal individuals and patients with IBS or inflammatory bowel

Table 7. Methane-positive breath test (intestinal methanogen overgrowth) prevalence in IBS and IBD populations

	IMO prevalence
IBS total	20.0% (95% CI 17.3%–22.7%) Similar to controls 22.0% (95% CI 19.0%–25.3%)
IBS-C	37.7% (95% CI 33.5%–42.1%)
IBS-mixed	24.3% (95% CI 17.4%–32.3%)
IBS-D	12.4% (95% CI 10.2%–14.9%)
IBD total	7.4% (95% CI 5.4%–9.8%) Less than controls 23.5% (95% CI 19.8%–27.5%)
Crohn's disease	5.3% (95% CI 3.0%–8.5%)
Ulcerative colitis	20.2% (95% CI 12.8%–29.4%)

Based on a systematic review using a random-effects model of case-control studies (50), 1,653 patients with IBS with 713 controls (70 symptomatic and 643 healthy asymptomatic; 7 studies) and 558 adult patients with IBD with 497 healthy asymptomatic controls (6 studies). There is an inverse relationship between diarrhea and methane-predominant abnormal breath test with increased prevalence in IBS-C and lower prevalence in IBD-D. Due to significant heterogeneity when prevalence studies (studies without a control population) were combined, only pooled data of case-control studies are presented. Additional sensitivity analysis evaluating IBS studies with only healthy controls revealed similar findings (IBS small intestinal bacterial overgrowth [SIBO] prevalence 18.9%, healthy controls SIBO prevalence 20.5%, $P = 0.404$).
CI, confidence interval; IBD, irritable bowel disease; IBS, irritable bowel syndrome; IBS-C, irritable bowel syndrome with constipation; IBS-D, irritable bowel syndrome with diarrhea; IMO, intestinal methanogen overgrowth.

Table 8. Consensus practice guidelines on small intestinal bacterial overgrowth (SIBO) and breath testing

Group	Topic	Year published	Citation
European H_2-CH_4-breath test group (EAGEN, ESNM, and ESPGHAN)	Hydrogen-methane breath testing	2022	Hammer et al (15)
ESPGHAN	Pediatric breath testing	2022	Broekaert et al (37)
Asian-Pacific consensus (INMA)	SIBO	2022	Ghoshal et al (7)
ACG	SIBO	2020	Pimentel et al (1)
AGA	SIBO	2020	Quigley et al (11)
North American Consensus	Hydrogen-methane breath testing	2017	Rezaie et al (3)
Rome Consensus	Hydrogen breath testing	2009	Gasbarrini et al (18)

ACG, American College of Gastroenterology; AGA, American Gastroenterological Association; EAGEN, European Association for Gastroenterology, Endoscopy and Nutrition; ESNM, European Society of Neurogastroenterology and Motility; ESPGHAN, European Society for Paediatric Gastroenterology Hepatology and Nutrition; INMA, Indian Neurogastroenterology and Motility Association.

disease (50). As can be seen, there is a signal that IMO is more prevalent in constipated patients, which is similar to other reported data (4,47–49,114). Unfortunately, there are insufficient available data to determine the test characteristics of breath testing in diagnosing IMO (115). Thus, the decision to use breath testing specifically to detect IMO should be influenced by patient symptoms and reserved for patients with constipation as the dominant phenotype (1,11,15).

Relevant billing and cost information. The procedure codes (Current Procedural Terminology) for hydrogen-methane breath testing are 82542 and 91065. Practitioners can also charge for the professional component of breath testing using the procedure code 91065 with a modifier of −26. The most commonly used *International Classification of Diseases, Tenth Revision* codes associated with the testing include R10.84 generalized abdominal pain; R14.0 abdominal distension (gaseous); R19.7 diarrhea, unspecified; K59.0 constipation, unspecified; K58.0 irritable bowel syndrome with diarrhea, and A04.9 bacterial intestinal infection, unspecified. The typical out-of-pocket cost to patients without insurance is generally $250 or less in the United States. Several companies offer at-home testing, which is a practice alternative for patients who may have limited access to on-site breath testing. Regardless of testing at home or on site, it is imperative that patients are compliant with the preparations before testing. Reinforcing these instructions with patients before testing should help improve test accuracy.

CONCLUSION

Breath testing is a useful, safe, and noninvasive tool to diagnose SIBO. Culture of intestinal fluid aspirates remains the gold standard test but is limited by its invasiveness and cost, limited access to required equipment, and questions about diagnostic cutoffs (3,15). Because of these barriers, breath testing remains widely used in general practice (11). Significant heterogeneity in breath test protocols among various centers has resulted in multiple recent consensus guidelines (Table 8) to provide more uniform test protocols, which will further improve the reliability and accuracy of breath testing. The clinical decision to use a breath test and the test's interpretation is influenced by multiple factors, including the patient population, presenting symptoms, and the choice of test substrate. Pretest probability serves as an important guide in identifying patients most likely to benefit from breath testing where patients with moderate risk for SIBO will most benefit from breath testing to provide supportive rule-in or rule-out information that guide management decisions. Further research is needed to improve our understanding of breath test performance in detecting SIBO in different patient care scenarios, but the breath testing paradigm is ideally positioned for routine clinical care and will continue to influence clinical practice for years to come.

CONFLICTS OF INTEREST

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