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Authors' Affiliation:
[1]Voivodeship Hospital in Tarnów, Lwowska Street 178A, 33-100 Tarnów, Poland
[2]Voivodeship Hospital in Płock, Medyczna Street 19, 09-400 Płock, Poland
[3]Ministry of the Interior and Administration Hospital, Północna Street 42, 91-425 Łódź, Poland
[4]7th Military Naval Hospital, Polanki Street 117, 80-305 Gdańsk, Poland
[5]Copernicus PL Sp. z o.o., St. Adalbert Hospital, Aleja Jana Pawła II 50, 80-462 Gdańsk, Poland
[6]Medical University of Łódź, Aleja Tadeusza Kościuszki 4, 90-419 Łódź, Poland
[7]Cardinal Stefan Wyszyński University in Warsaw, Wóycickiego Street 1/3, 01-938 Warsaw, Poland
[8]Medical University of Warsaw, Żwirki i Wigury Street 61, 02-091 Warsaw, Poland
[9]Jagiellonian University Collegium Medicum, Świętej Anny Street 12, 31-008 Kraków, Poland

[*]**Corresponding author:**
Jakub Majcherek,
Voivodeship Hospital in Tarnów, Lwowska Street 178A, 33-100 Tarnów, Poland,
E-mail: lek.jakub.majcherek@gmail.com

ORCID List:

Jakub Majcherek:	0009-0006-6713-0901
Tomasz Karwowski:	0009-0009-8610-2916
Maciej Świerczyna:	0009-0008-8253-7165
Agata Olecka:	0000-0002-2101-9603
Filip Gałązka:	0009-0008-1301-7326
Fryderyka Orawczak:	0009-0005-6988-6940
Julia Gałązka:	0009-0000-5062-1778
Mateusz Mazurek:	0009-0004-5391-1008
Mikołaj Kotusiewicz:	0009-0001-2610-7493
Zuzanna Czuba:	0009-0002-4393-050X

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Breath testing in SIBO and IMO: interpretation and evidence-informed clinical approach

Jakub Majcherek[1]*, Tomasz Karwowski[2], Maciej Świerczyna[3], Agata Olecka[4], Filip Gałązka[5], Fryderyka Orawczak[6], Julia Gałązka[7], Mateusz Mazurek[8], Mikołaj Kotusiewicz[9], Zuzanna Czuba[8]

ABSTRACT

Interpretation of breath tests is, however, difficult, because positive results need to be interpreted, including the whole clinical presentation. Tests do not directly show the exact localization of fermentation. These results are estimated based on intestinal transit time. This narrative review gathers currently available guidelines, meta-analyses, and systematic reviews to present a wide spectrum of information about the biological basis, indications, process of testing, and clinical meaning in the context of SIBO and IMO. Difficult interpretation of positive results restrains the indications of glucose and lactulose breath tests to symptomatic patients. For many patients, there are additional factors like motility issues, altered anatomy, history of surgeries or presentation with predominant constipation or diarrhea phenotype. Both glucose and lactulose testing protocols have their own specification that affects interpretation. For example, lactulose testing is more prone to being affected by intestinal transit because it is not absorbed from the intestinal lumen. Intestinal methane overgrowth (IMO) should be considered as another entity. Studies show that, in patients with constipation, the presence of methanogenic archaea is associated. All of this shows that the results of breath testing need to be interpreted with the patient's whole clinical presentation, but technical factors like baseline gas values, transit-related factors, or used substrate need to be included. Future studies should focus on standardization, the three-gas testing method, and the development of validation methods.

Keywords: small intestinal bacterial overgrowth; intestinal methanogen overgrowth; breath testing; hydrogen; methane

1. INTRODUCTION

Small intestinal bacterial overgrowth (SIBO) is a set of clinical symptoms that are often non-specific, such as bloating, abdominal pain, flatulence, and altered bowel habits. Historically, this disease has been mostly diagnosed among patients with anatomical abnormalities and impaired intestinal motility resulting in maldigestion, malabsorption, diarrhea, and nutritional deficiencies (Pimentel et al., 2026). Today, it's diagnosed by breath testing, a method that indirectly detects products of gut

microbiome fermentation. This shift in diagnosis was made possible by the expansion of breath testing. The definition of SIBO is still debated due to the quantitative limitations of the small-intestine microbiota (Bushyhead and Quigley, 2022). No specific symptoms are recognized; they may overlap with, e.g., irritable bowel syndrome, functional diarrhea. Such limitations create a need for a non-invasive, relatively inexpensive, straightforward, and widely available tool: breath testing (Pimentel et al., 2026).

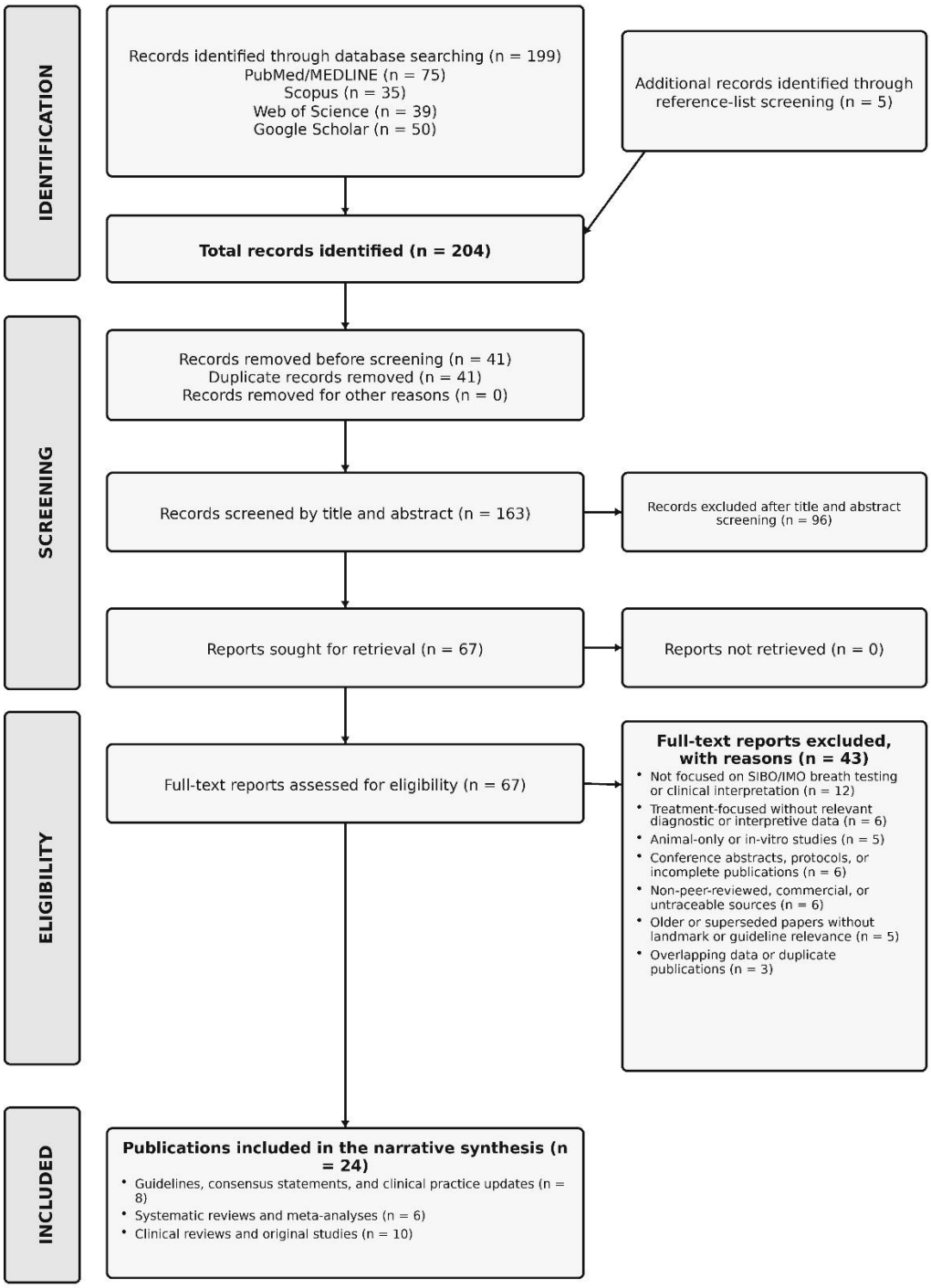

Figure 1. PRISMA-based flow diagram of literature identification, screening, eligibility assessment, and inclusion in the narrative review.

Breath testing is a non-invasive, relatively cheap, and widely available method of diagnosis. However, it is also not a fully objective way to diagnose patients; it might be affected by the procedure of collecting samples, the substrate used, and the diagnostic criteria (Tansel and Levinthal, 2023). Breath testing for SIBO should be considered in symptomatic patients rather than used for indiscriminate screening.

Glucose or lactulose breath testing may be advised in patients with gastrointestinal symptoms, especially when intestinal dysmotility or previous luminal abdominal surgery is present in the patient's medical history. In patients presenting predominantly with constipation, methane measurement should also be considered to identify intestinal methanogen overgrowth, which should be distinguished from bacterial overgrowth when interpreting the test result (Pimentel et al., 2026).

The aim of the review is to provide evidence-based knowledge on SIBO breath tests. Other issues elaborated in this article are indications, risk assessment, and interpretation. This paper is predominantly written for medical professionals; the authors intended to create a review-like summary to help physicians.

2. REVIEW METHODS

This paper was created as a narrative review with a structured search. Searched databases included: PubMed/MEDLINE, Scopus, Web of Science, Google Scholar. Search period: January 2000 – April 2026, with priority to January 2020 – April 2026. Core terms: "small intestinal bacterial overgrowth", "SIBO", "intestinal methanogen overgrowth", "IMO", "hydrogen breath test", "methane breath test", "glucose breath test", "lactulose breath test", "diagnostic accuracy", "interpretation", "rifaximin", "neomycin", "hydrogen sulfide". Core terms were used in different combinations as a search tool across different medical databases. Articles had to fulfill strict inclusion criteria, and all papers had to be consistent with current medical knowledge. The reference lists of cited articles were analyzed to double-proof the scientific cohesion. We put most of our attention on official guidelines with emphasis on European and American recommendations. American College of Gastroenterology (ACG), European Association for Gastroenterology, Endoscopy and Nutrition (EAGEN); European Society of Neurogastroenterology and Motility (ESNM); European Society for Pediatric Gastroenterology, Hepatology and Nutrition (ESPGHAN) are organizations whose recommendations were mostly taken into consideration while preparing citation mapping of this article.

Systematic reviews, meta-analyses, clinical reviews, and diagnostic accuracy studies were also included in the reference list to ensure a broad perspective on the subject. We excluded papers not compliant with the evidence-based approach. The analyzed source did not include non-scientific webpages, laboratory brochures, commercial test advertisements, case reports unless uniquely relevant, animal-only studies, or studies not directly related to breathe testing/interpretation. The literature identification and selection process is presented in Figure 1.

3. RESULTS AND DISCUSSION

Breath testing is the most common method for diagnosing SIBO. Results of breath tests are affected by multiple factors, including substrate choice, the patient's preparation process, colonic transit rate, baseline gas levels, and the patient's individual predispositions, such as altered gastrointestinal anatomy. IMO is caused by a different group of microorganisms and has a specific presentation of dominant constipation. For those reasons, it should be treated as another entity. Positive or negative results of breath testing need to be considered with the whole clinical presentation. They are not definitive, and physicians need experience to properly interpret the results. In future studies, researchers will need to focus on standardization and the accuracy of localization of the fermentation process. Improved intestinal aspirate sampling will help to better estimate gas production.

Biological basis of breath testing

Gases detected in SIBO breath testing originate from bacterial overgrowth in the intestines; human cells cannot produce hydrogen or methane. Diffused in mucosal tissue gases come from bacterial fermentation, a process during which carbohydrate substrates are fermented and converted into hydrogen or methane. Without a substrate, gas production is low, dependent on diet, and difficult to standardize. Key hydrogen-producing taxa include Bacteroides, Clostridium, Escherichia coli, and Klebsiella (Pimentel et al., 2026).

After the administration of substrate (lactulose or glucose), a series of gas concentration measurements creates a breath-gas curve. Analysis of this curve allows us to estimate the source of fermentation. The source of fermentation is estimated localization, which part of the intestines is affected with bacterial overgrowth. Measuring the time required to reach the peak hydrogen concentration in

exhaled air can indirectly indicate whether fermentation occurred in the small intestine. Tests are based on estimates, which may vary depending on intestinal motility (Tansel and Levinthal, 2023).

Methane is another measured metabolic product; however, it is produced by Methanobrevibacter smithii, an archaeon, not a bacterial genus. These archaea might consume hydrogen, which creates interesting implications for test analysis. Methane-positive patients may have low hydrogen levels, which impairs proper analysis of the breath-gas curve (Pitcher et al., 2022).

Excess methane production is more appropriately called intestinal methanogen overgrowth (IMO). Unlike SIBO, IMO is not limited to the small intestine because methanogens may be present in both the small and large bowel (Lim and Rezaie, 2023). Moreover, sulfur-reducing bacteria also consume hydrogen, which may flatten the curve of gas extraction (Pimentel et al., 2020) All these factors need to be considered before diagnosing patients with SIBO or IMO. Struggles with the interpretation of these tests have brought more attention to finding new gases to analyze.

Substrates and protocols

As previously mentioned in this paper, there are two main substrates in SIBO breath-gas tests. Both substrates are saccharides, but are processed differently in organisms. they differ in absorption, intestinal exposure and susceptibility to colonic fermentation. Protocols are not fully uniform across consensus documents, specifically about the glucose dose (Hammer et al., 2022).

Glucose breath test

Glucose is rapidly absorbed from the intestine, so it does not normally reach the colon. Because of that, the results analysis can be more precise (Tansel and Levinthal, 2023). Contrary to glucose, lactulose is not absorbed at all, which is why the glucose breath test offers higher specificity (Lim and Rezaie, 2023). False-negative results might occur when overgrowth is predominantly in the distal segments (Tansel and Levinthal, 2023).

A retrospective study showed a big variability of positive glucose breath tests depending on the dosage of the substrate. Increasing it from 50 g to 75 g resulted in the following increase from 22 to 36%. Interpretation might be inconsistent because improvement in sensitivity might stem from more false-positive results (Pitcher et al., 2022).

In case of SIBO breath test, an often-used reference standard is small-intestine aspirate culture - direct evidence of bacterial overgrowth. A meta-analysis using this method has shown sensitivity and specificity of the glucose breath test were 54.5% and 83.2%, respectively (Losurdo et al., 2020). High specificity, with moderate sensitivity, means that positive results are rather certain, while a negative one does not exclude it reliably. This is important for a proper interpretation of clinical symptoms and potential treatment, following an uncertain diagnosis.

Lactulose breath test

Lactulose is an indigestible disaccharide that is also not absorbed in the small intestine. As a result, the substrate is fermented by microorganisms in the intestines (Lim and Rezaie, 2023).

Similarly to the glucose test, in the retrospective comparison, the exact dose of lactulose resulted in more positive results when 10 g was used, as opposed to 16 g. In this study, the authors tried to identify the reason for this, explaining that a higher dose of lactulose increases intestinal transit (Pitcher et al., 2022).

Moreover, guidelines do not agree on exact dosage, while American guidelines are more consistent and recommend 10 g of lactulose, European ones are vaguer and describe used doses between 10 to 20g (Rezaie et al., 2017). The authors suggested that normal fermentation processes might be detected and wrongfully misclassified as positive results, due to lactulose-driven increased transit. The higher the dose, the faster the transit (Pitcher et al., 2022). However, this issue is still speculative and requires more studies.

Dependence on orocecal transit time, which might be individually differentiated. Especially prone to false positive results are patients with diarrhea-predominant functional bowel symptoms. In a study, a 90-minute diagnostic window was enough for lactulose dosage to reach the cecum and, for that reason give false-positive results (Kashyap et al., 2024). For this reason, when transit is not measured simultaneously, glucose as a test substrate is preferred by the European guideline (Hammer et al., 2022).

In the meta-analysis by Losurdo et al., (2020) the lactulose breath test showed a sensitivity of 42.0% and a specificity of 70.6%. However, these estimates were limited by accuracy issues and substantial heterogeneity and imperfections of aspirate culture as the reference standard. Lactulose testing should therefore be interpreted cautiously and always in relation to transit, symptoms, anatomy, and pre-test probability.

Hydrogen sulfide measurement

Hydrogen sulfide is an example of a more experimental approach; it is available as a system quantifying H_2S and methane in breath samples. The three-gas breath testing system is based on the same rules, with samples being collected after glucose or lactulose dosage (Pimentel et al., 2026).

Patients with higher H_2S concentration in a breath test reported greater severity of diarrhea, defecatory urgency, and abdominal pain. No final threshold or universally accepted concentration was defined (Pimentel et al., 2026). Therefore, currently there is not enough evidence to support a diagnosis based on Hydrogen sulfide. Moreover, breath-testing estimates anatomical localization of gas production based on time after digestion, making this method prone to accuracy mistakes.

Preparation before testing

Breath tests are non-invasive methods; however, they require proper preparation to ensure validated results. Preparation has its purpose in minimizing background fermentation. Hard-to-digest carbohydrates get fermented, producing gases that will affect the baseline and peak of the breath-gas curve. Rapid colonic transit will also influence the results, which is why high-fiber products should also be avoided at least one day before testing. Moreover, an 8-hour fasting period should be included (Hammer et al., 2022). Studies have shown that fiber and fermentable food was associated with lower hydrogen baseline and methane concentrations, which are elements of proper interpretation. This resulted in less positive glucose breath tests (Mattio et al., 2024). Testing in patients who underwent antibiotic therapy in the last 4 weeks should be delayed.

Medication that causes altered intestinal motility should be avoided for at least 24 hours before testing. This includes especially medications like prokinetics, laxatives, and probiotics. Another aspect of testing is proper ventilation. Physical activity affects ventilation, increasing the volume of exhaled gases, which causes a lower gas concentration in exhaled air. The European guideline also underlines that patients should avoid smoking for at least 2 hours before testing (Hammer et al., 2022).

Table 1. Commonly used breath-test criteria and major limitations.

Gas/result	Commonly used criterion	Interpretation	Main limitation
Hydrogen	Rise ≥20 ppm above baseline within 90 minutes after glucose or lactulose ingestion	Supports a hydrogen-positive breath-test pattern compatible with SIBO (Rezaie et al., 2017)	Consensus-derived and not universal; affected by substrate, dose, orocecal transit and protocol
Methane	CH_4 ≥10 ppm at any time, including baseline	Supports intestinal methanogen overgrowth, particularly in a constipation phenotype (Tansel and Levinthal, 2023)	Does not localize methanogens to the small intestine; methanogens are archaea and may occur in the small or large bowel
Flat-line pattern	No methane, fixed H_2 ≤3 ppm and no rise >1 ppm above baseline	May reflect hydrogen consumption through H_2S-producing pathways, delayed substrate delivery, recent antibiotic exposure or technical factors (Lim and Rezaie, 2023)	Not a validated diagnostic entity and does not independently diagnose H_2S overproduction
Hydrogen sulfide	No consensus diagnostic threshold; 1.5- and 2-ppm values have been used for study-specific analyses	Higher H_2S may indicate increased activity of H_2S-producing organisms and is associated with diarrhea-related phenotypes (Villanueva-Millan et al., 2025)	Limited availability, uncertain anatomical contribution and lack of validated thresholds or treatment algorithms

Interpretation criteria

Breath-test results are indirect markers of microbial fermentation and do not measure bacterial burden or its anatomical location directly (Tansel and Levinthal, 2023). Organizations worldwide recommend different approaches to diagnostic criteria. For example, a rise of at least 20 ppm above baseline, 90 minutes after substrate digestion. On the other hand, the North American threshold is considered a positive result, which is a rise of at least 20 ppm within 90 minutes after substrate dose (Rezaie et al., 2017).

European guidelines, on the other hand, are vaguer, underlying that the rise in concentration of exhaled gases needs to occur before the substrate reaches the cecum. More direct criteria that are commonly used are increments of approximately 10–12 ppm (Hammer et al., 2022). The recent Asian monograph proposed a rise of at least 12 ppm, especially highlighting rapid cecal transit as a factor to be considered, while analyzing the results (Xiao et al., 2026). Usually, the limit value is 10 ppm at any moment throughout the test, which supports IMO diagnosis.

Absence of excessive methane and baseline concentration above 20 ppm has been commonly described in studies. However, the diagnostic value is not yet fully validated. (Lim and Rezaie, 2023). Another article has proposed a reason for the uncertain diagnostic value of breath testing. They included incomplete preparation, oral fermentation, ongoing intestinal fermentation, and foregut dysmotility (Tansel and Levinthal, 2023). Alegre et al., (2022) have repeatedly shown that elevated gas concentration was reduced after light walking. This method is, however, not recommended as an established SIBO protocol.

Absent methane with low hydrogen remaining below 3 ppm is described as a flat-line pattern. (Lim and Rezaie, 2023). Those flat-line patterns are associated with fermentation; however, caution is advised because they may also be caused by hydrogen consumption, delayed substrate transit, or technical inaccuracies. Hydrogen-sulfide measurement may clarify low-hydrogen concentration, but currently, there is no direct established threshold (Villanueva-Millan et al., 2025).

It is important to underline the factors influencing the results, as these may reflect the final diagnosis. Different established thresholds for diagnosis across studies and guidelines affect patients' therapeutic management. The commonly used breath-test criteria and their principal interpretative limitations are summarized in Table 1.

Diagnostic performance and limitations

Breath testing fulfills many requirements of perfect diagnostic methods like non-invasiveness and relatively low cost to perform, and is much easier compared with small-bowel aspirate, which requires invasive sampling (Tansel and Levinthal, 2023). Breath testing is a commonly accepted method of SIBO diagnosis; nevertheless, a diagnostic accuracy study comparing glucose and lactulose breath testing methods with small-intestinal aspirate culture as the reference. In the analyzed meta-analysis, the glucose breath test had a sensitivity of 54.5% and specificity of 83.2%, while the corresponding values for the lactulose breath test were 42.0% and 70.6% (Losurdo et al., 2020).

The cited article, however, uses small-bowel aspirator culture, which is also not a perfect reference standard. Samples are getting easily contaminated, resulting in false-positive tests. Moreover, bacteria may be distributed unevenly. However, a reference standard in the form of a biopsied aspirate is also not a perfect solution, because samples might become contaminated during the process of obtaining them. Another aspect is that symptoms might be caused by unevenly distributed bacteria, causing false-negative results (Bushyhead and Quigley, 2022).

European guidelines emphasize limitations of lactulose testing stemming from results influenced strongly by the pace of bowel transit. The faster lactulose reaches the colon, the higher the early rise in gas production - peak on the curve. This effect may be caused by fermentation and lead to a false diagnosis (Hammer et al., 2022). This underlines the importance of the association between clinical symptoms and test results; neither one of them is enough to establish a diagnosis. An extensive patients and family history, especially about motility disorders, previous surgeries, medication history, risk, and lifestyle factors, are essential, while taking care of patients presenting symptoms (Tansel and Levinthal, 2023).

Intestinal methanogen overgrowth

Intestinal methanogen overgrowth should be distinguished from SIBO. Methane is produced by methanogenic archaea rather than bacteria, and these microorganisms may be present in both the small and large intestine. The term IMO therefore more accurately describes the intestinal distribution of methanogens than methane-positive SIBO (Lim and Rezaie, 2023). To properly identify and diagnose patients, a methane concentration during a glucose or a lactulose breath test has to be considered with the clinical presentation. A recent meta-analysis found that patients with IMO had a higher prevalence and greater severity of constipation.

Diarrhea was less frequent than in patients without IMO (Mehravar et al., 2025). A methane concentration of at least 10 ppm at any point during a glucose or lactulose breath test has been considered a threshold (Lim and Rezaie, 2023). From the clinical point of view, IMO has been shown to often present a different phenotype of disease compared to SIBO. In a 2025 meta-analysis, those patients had a higher prevalence and greater severity of constipation, while diarrhea was less frequent (Mehravar et al., 2025). Researchers in a study measuring colonic transit showed that patients with IMO had experienced slower intestinal transit (Talamantes et al., 2024).

When to consider testing

Because of difficulties in the proper interpretation of results and Inter-patient variability, testing should be restricted. Glucose and lactulose tests are indicated for patients with predisposing morbidities or experiencing symptoms. Isolated bloating is not sufficient to justify a test recommendation. AGA guidelines recommend it only with patients with clear risk factors or severe symptoms (Moshiree et al., 2023). Symptoms described by patients suffering from SIBO are seen as subjective. They may often be mistaken or described in different ways depending on patient (Ahmed et al., 2023).

A 2022 meta-analysis has listed diseases to consider while diagnosing with IBS. Authors underlined that SIBO can be mistaken with IBS because symptoms are non-specific and overlapping. Chronic diarrhea, bloating, abdominal pain, and altered bowel habits may reflect several treatable mechanisms, not only SIBO (Poon et al., 2022). Constipation is not a deciding symptom IMO, but it was shown to be more prevalent in those patients. Although bloating, abdominal pain, diarrhea, and other symptoms may also occur (Mehravar et al., 2025). The symptom profile, therefore, increases clinical suspicion but cannot replace objective methane measurement.

PPI consumption has been associated with a higher prevalence of SIBO; however, a 2025 meta-analysis has shown inconsistent results across studies and does not support that there is enough evidence for testing solely based on taking this group of medications (Khurmatullina et al., 2025). Moreover, this is also underlined by ACG guidelines, which recommend testing when additional risk factors or symptoms occur. Asymptomatic patients should not undergo routine testing (Pimentel et al., 2020). This is why PPI use is a SIBO risk-modifier, not a testing indication.

Another issue with SIBO breath testing is the availability of at-home testing kits. Procedures based on which those test functions are similar to ambulatory ones. Patients drink substrate and measure methane and hydrogen concentration in exhaled gas. However, researchers underline that those tests are not standardized and may not reflect the thresholds recommended by the guidelines, leading to false assumptions among users (Lim and Rezaie, 2023). Moreover, such results may never be verified by a clinician.

How to act on results

As previously mentioned, positive results need to be interpreted with caution. Symptoms, risk factors, anatomy, medications, and the substrate used need to support the final diagnosis. Standard treatment is antimicrobial treatment, often consisting of rifaximin in adults and metronidazole in children. Antibiotics improve symptoms better than a placebo in Japanese studies. However, these conclusions are based on studies with small cohorts (Takakura et al., 2024). This is the reason why treatment should be advised based on the whole clinical presentation, not solely on breath-test results.

Methane positivity in a patient with constipation should direct a clinician to a possible IMO diagnosis. However, methane can be produced by methanogens in either the small or large intestine, and constipation is not present in every methane-positive patient. Clinical response must consequently be assessed using bowel frequency, stool form, straining, and related symptoms rather than gas concentration alone.

High baseline hydrogen should be a reason for additional review of the preparation process. The time of fasting, medication use, and motility disorders needs to be reevaluated to ensure proper diagnosis (Tansel and Levinthal, 2023). Hydrogen consumption by microorganisms previously mentioned can cause a flat pattern on a breath-test curve, leading to false-negative results (Lim and Rezaie, 2023).

Researchers have suggested taking a second breath sample before giving the substrate to patients who had their results with high values of the first baseline. Patients were told to take a one-hour break, take a walk, and then give another sample. If the second value was lower, the test can be validated, and this second sample could be used for calculating the results. If the values remained high, the test should be rescheduled, and the results are unreliable (Alegre et al., 2022).

Treatment implications

Treatment of patients with SIBO should be cause-oriented, in case of preexisting conditions like altered anatomy or motility issues, the original cause needs to be identified. For most patients experiencing symptoms and getting positive test results, antibiotics may be considered. A meta-analysis reported an eradication rate of rifaximin of 71%. Authors, however, underline the low quality of included studies (Gatta and Scarpignato, 2017).

Methane-positive patients may require a different treatment approach. The combination of rifaximin and neomycin has shown possible benefit, but the supporting evidence comes mainly from older uncontrolled or retrospective studies and should not be regarded as an established regimen for all patients with IMO (Quigley et al., 2020).

Treatment should also address the condition that predisposed the patient to overgrowth. Potentially correctable causes include obstruction, strictures, blind loops and surgically altered anatomy, while impaired motility related to diabetes or connective-tissue disease may require separate management (Quigley et al., 2020). Nutritional deficiencies should be corrected when maldigestion or malabsorption is present.

Recurrence of the symptoms or not a full recovery from symptoms should not lead to automatic repeated antibiotic treatment, but should be considered as an indication for review of the case. It may be common in patients with underlying anatomical or motility conditions (da Silva et al., 2024).

4. CONCLUSION

This review was focused on the clinical interpretation of SIBO and IMO breath tests. We found that practitioners may misinterpret the results, leading to an incorrect diagnosis. Superficial analysis of the baseline gas values can result in a wrong diagnosis; for example, low hydrogen concentration can stem from the methanogens' consumption. Factors influencing the shape of the breath-gas curve include substrate choice, inter-individual variability in intestinal transit time, and patient preparation. Moreover, new findings in this area, discrepancies between American, European, and Asian guidelines, and the need for holistic interpretation of the results, together with clinical presentation and symptoms experienced by patients, underline the importance of an evidence-informed approach to SIBO and IMO diagnosis.

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Authors' Contributions

- All authors reviewed and approved the final version of the manuscript and agreed to be accountable for all aspects of the work.
- Writing – review and editing: Mateusz Mazurek, Filip Gałązka
- Writing – original draft: Jakub Majcherek, Julia Gałązka
- Data analysis: Maciej Świerczyna, Fryderyka Orawczak
- Data extraction: Mikołaj Kotusiewicz, Agata Olecka
- Methodology: Tomasz Karwowski, Zuzanna Czuba
- Conceptualization: Tomasz Karwowski, Jakub Majcherek

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Conflict of interest

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Data and materials availability

All data associated with this study will be available based on the reasonable request to corresponding author.

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