Diagnostic Approaches for SIBO A Scoping Review on Breath Testing

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Abstract

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Small Intestinal Bacterial Overgrowth (SIBO) has gained increasing attention in recent years due to its association with various gastrointestinal diseases. Accurate, accessible, and standardized diagnostic tools are therefore essential. While breath testing has become a widely used non-invasive alternative to jejunal aspirate culture, its diagnostic performance and consistency remain debated.

The purpose of this thesis was to examine the diagnostic role of breath testing for SIBO in comparison to jejunal aspirate culture, the traditional gold standard. The aim was to evaluate the effectiveness, methodological variation, and clinical applicability of breath testing through a scoping review.

This study followed the PRISMA-ScR framework and involved structured searches in four databases covering literature from 2004 to 2024. Ten studies were included after applying defined inclusion and exclusion criteria and performing critical appraisal using the CASP and SANRA checklists.

Hydrogen and methane breath tests were the most commonly used methods. However, significant variability was observed across studies in terms of substrate types, dosages, test durations, sampling intervals, and diagnostic thresholds. Glucose-based tests tended to show higher specificity, while lactulose-based tests offered greater sensitivity. Hydrogen sulfide breath testing was identified as a promising, though currently insufficiently validated, emerging method.

Although breath testing is less invasive and more practical than jejunal aspiration, its diagnostic accuracy is limited by inconsistent protocols. These methodological inconsistencies affect clinical reliability and hinder comparability across studies. The findings highlight the need for standardization to enhance the diagnostic utility of breath testing for SIBO in both research and clinical practice.

Keywords: Small intestinal bacterial overgrowth, breath test, scoping review

Contents

1 Introduction

Small Intestinal Bacterial Overgrowth (SIBO) presents a mounting concern in Western societies and urbanising countries, with its prevalence showing an increasing from 2.5 to 22% depending on the source (Radomańska and Cukrowska 2022; Tansel and Levinthal, 2023). Despite its growing significance in public health discourse, the precise factors driving this upward trend remain elusive. While this data does not directly pertain to SIBO, it highlights the broader economic burden of digestive diseases in general. According to a report on the economic impact of digestive diseases across 31 UEG member countries, the cost of inpatient health service delivery for digestive diseases, excluding treatment and diagnostics, was, on average, 0.12% of GDP. This figure reflects the significant strain placed on healthcare systems by digestive diseases as a whole (Rose et al. 2022). Furthermore, the estimated costs of lost output due to morbidity and premature mortality related to digestive diseases were, on average, 0.22% and 0.25% of GDP, respectively. These figures underscore the profound impact digestive diseases have not only on individuals' health but also on national economies.

The potential for reducing these costs is considerable. If premature mortality related to digestive diseases were reduced by 25%, 50%, or 75% in 2019, the estimated savings in productivity losses across the 31 countries would have amounted to €11.4 billion, €22.8 billion, and €34.2 billion, respectively. For example, reducing premature mortality by 25% in 2019 could have resulted in savings of €2.97 billion in Germany, €1.77 billion in the UK, and €1.4 billion in France (Rose et al. 2022). These findings highlight the far-reaching economic and societal benefits of addressing digestive diseases more effectively, even though they do not specifically focus on conditions like SIBO.

The multifaceted nature of SIBO's aetiology has led to divergent perspectives within the scientific community, hindering the establishment of a unified understanding. Nonetheless, the impact of SIBO extends far beyond its direct manifestations, with mounting evidence suggesting its involvement in a spectrum of gastrointestinal disorders. From irritable bowel syndrome to inflammatory bowel disease, SIBO's intricate interplay with various gastrointestinal conditions underscores its central role in digestive health.

Yet, despite its clinical relevance, the current diagnostic landscape for SIBO within the Danish healthcare framework is fragmented and lacks standardization. Conventional diagnostic methods for SIBO are marked by invasiveness, high costs, and logistical complexities, posing significant barriers to widespread adoption and accessibility (Rezaie et al. 2017). However, recent advancements in medical technology have opened new avenues for non-invasive testing through breath analysis. By leveraging the detection of specific gases associated with SIBO, breath testing offers a promising alternative that aligns with the principles of patient-centred care and resource optimization.

Nevertheless, the journey towards establishing breath testing as a reliable diagnostic tool for SIBO is fraught with challenges. Variability in test protocols, inconsistencies in result interpretation, and limited accessibility present challenging hurdles that must be addressed (Rana 2017). Moreover, disparities in testing methodologies across healthcare facilities further compound the issue, highlighting the pressing need for standardized approaches.

2 Purpose and aim

The purpose of this thesis is to explore non-invasive breath testing as a diagnostic tool for SIBO, in comparison to the traditional small bowel aspirate and culture test. This thesis aimed to critically review existing literature to assess the effectiveness and potential of breath testing as a gold standard procedure for accurately diagnosing SIBO.

The research questions:
1: What breath testing methods are currently available for diagnosing SIBO?

2: Can breath testing effectively diagnose SIBO compared to small bowel aspirate and culture test?

3 Background

3.1 Pathophysiology of SIBO

In SIBO, an abnormally high number of bacteria colonize the small intestine, although this overabundance of bacteria does not always directly correlate with disease activity (Sachdev and Pimentel 2013). Clinical manifestations typically arise when inflammation is induced by invasive strains of bacteria (Pimentel 2017). These strains can produce enzymes or endotoxins that damage the epithelial cell layer, leading to the onset of symptoms (Ghoshal et al. 2020). Among the most commonly identified bacterial species in SIBO are Escherichia coli, Aeromonas, and Klebsiella species (Quigley and Pimentel, 2019). Anaerobic bacteria are particularly implicated in directly causing epithelial injury and producing enterotoxins, while aerobic bacteria tend to produce enterotoxins that contribute to intestinal inflammation (Ghoshal et al. 2020). The overgrowth of bacteria can also impact bowel motility, further exacerbating symptoms (Sachdev and Pimentel 2013).

Endoscopically and histopathologically, the mucosa in SIBO typically appears healthy, although there may be nonspecific changes such as mucosal edema, loss of vascularity, patchy erythema, or occasionally ulceration ((Sachdev and Pimentel 2013). Histopathological findings may include villous blunting, cryptitis, intraepithelial lymphocytosis, and eosinophilia, but these are also nonspecific in nature (Pimentel 2017).

The development of bacterial overgrowth in SIBO is often facilitated by intestinal stasis, allowing coliform bacteria to proliferate locally (Khoshini et al. 2008). Mechanical stasis, frequently observed in patients with a history of gastrointestinal surgery, can occur due to surgical blind loops or other anatomical abnormalities (Bures et al. 2010). Additionally, conditions such as diabetes, scleroderma, intestinal diverticulosis, and intestinal obstruction can contribute to stasis and subsequent bacterial overgrowth (Quigley and Pimentel 2019). Medications like narcotics and proton pump inhibitors (PPIs) may also predispose individuals to intestinal slowing or reduced acid levels, creating an environment conducive to bacterial proliferation (Sachdev and Pimentel (2013)

The mechanism of diarrhea in SIBO is multifactorial and hypothetical. It may involve bacterial digestion of carbohydrates, resulting in the production of gas and osmotically active byproducts that promote osmotic diarrhea (Pimentel 2017). Bacterial byproducts and fatty acids can injure the mucosa, contributing to diarrhea, while mucosal injuries may also lead to lactase deficiencies (Ghoshal et al. 2020). Additionally, bacterial deconjugation of bile salts may interfere with fat absorption and the absorption of fat-soluble vitamins (Sachdev and Pimentel 2013)

3.2 Mechanism of SIBO

Several mechanisms contribute to the development of SIBO. Impaired gastrointestinal motility, such as intestinal dysmotility or conditions like intestinal pseudo-obstruction, can slow down transit time in the small intestine, allowing bacteria to proliferate. Structural abnormalities, such as strictures or adhesions, can also disrupt normal intestinal flow and promote bacterial overgrowth (Sachdev and Pimentel 2013). Dysfunction of the ileocecal valve, which normally prevents backflow of colonic contents into the small intestine, can further exacerbate SIBO by facilitating bacterial migration. The importance of the ileocecal valve in preventing bacterial overgrowth is well documented, particularly in patients with conditions such as Crohn's disease or after surgical resections.

Impaired immune function plays a crucial role in regulating the microbial populations in the gut and preventing bacterial overgrowth. Conditions that compromise immune function, such as HIV/AIDS, immunodeficiency disorders, or chronic inflammatory conditions like celiac disease or inflammatory bowel disease, can impair the host's ability to control bacterial colonization in the small intestine (Sachdev and Pimentel 2013). Additionally, impaired gastric acid secretion, such as achlorhydria or the use of proton pump inhibitors, can create an environment conducive to bacterial colonization in the small intestine. Reduced gastric acid, through mechanisms like impaired gastric sterilization, allows more bacteria to survive and proliferate in the small intestine.

Conditions that disrupt intestinal motility, such as diabetic neuropathy, scleroderma, or intestinal pseudo-obstruction, can lead to stasis of intestinal contents, providing an opportunity for bacterial overgrowth (Sachdev and Pimentel 2013). Structural abnormalities in the gastrointestinal tract, such as strictures, diverticula, or surgical adhesions, can alter the normal flow of intestinal contents and create pockets where bacteria can proliferate, which can worsen the condition. Dysfunction of the ileocecal valve, which can occur due to Crohn's disease or surgical resection, can facilitate the migration of colonic bacteria into the small intestine, leading to SIBO.

Finally, dysbiosis an imbalance in the gut microbiota can predispose individuals to SIBO. Factors such as antibiotic use, PPIs, and dietary changes can disrupt the delicate balance of microbial communities in the gut, creating an environment conducive to bacterial overgrowth (Sachdev and Pimentel 2013). Additionally, conditions that compromise immune function, such as immunodeficiency disorders or intestinal inflammation, further impair the host’s ability to control bacterial colonization, leading to SIBO.

3.3 Navigating the challenges of SIBO diagnosis

Diagnosing SIBO has historically been challenging due to the lack of a universally accepted gold standard test (Bushyhead and Quigley 2022). Traditional methods, such as small bowel aspirate culture, involve direct sampling of the small intestine but are invasive and impractical for routine clinical use. As a result, these methods are often reserved for research settings rather than day-to-day patient care.

In contrast, breath testing has emerged as a promising non-invasive alternative, offering a simpler approach to diagnosis. This method measures the production of hydrogen (H_2) and methane (CH_4) gases by bacteria in the small intestine after a patient ingests a substrate like lactulose or glucose (Rezaie et al. 2017). However, despite its non-invasive nature, breath testing comes with its own set of challenges. There is a lack of consensus on how to best conduct these tests, including variations in apparatus, the types of gases measured, fasting protocols, testing intervals, and the use of specific substrates (Bushyhead and Quigley 2022).

These inconsistencies in methodology can impact the accuracy, reproducibility, and standardization of breath testing, leading to variability in results and a degree of diagnostic uncertainty (Rezaie et al. 2017). For example, differences in fasting protocols or testing intervals can yield varying levels of gas production, complicating the interpretation of results.

Improved knowledge of the diagnostic accuracy and reliability of breath testing for SIBO could significantly influence clinical practice. If validated through standardized protocols, breath testing could provide a reliable non-invasive option, allowing for earlier detection and more effective patient care (Bushyhead and Quigley 2022). This, in turn, could lead to more targeted treatment strategies for individuals with symptoms suggestive of SIBO. Standardizing protocols across healthcare settings would enhance consistency, potentially improving diagnostic accuracy and patient outcomes on a broader scale.

3.4 Variability in testing methodologies

The lack of consensus regarding SIBO testing methodologies is a significant barrier to accurate diagnosis and effective management of the condition. Disagreements persist concerning several aspects of breath testing, including the choice of apparatus, types of gases measured, fasting protocols, testing intervals, and the addition of substrates. While the QuinTron Breath Tracker SC Analyzer is commonly recommended for breath testing in diagnosing SIBO, there remains a lack of consensus regarding the preferred apparatus across clinical settings. This absence of standardized recommendations for device selection can lead to variations in test results and impede the establishment of uniform diagnostic criteria for SIBO, as observed by Shrestha et al. (2019).

In practice, any device capable of accurately measuring gases such as hydrogen and methane could theoretically be utilized for breath testing. However, the absence of specific recommendations or guidelines regarding apparatus selection underscores the need for greater standardization in this aspect of SIBO diagnosis. Without standardized protocols for device selection and calibration, clinicians may encounter challenges in comparing results across different testing facilities and interpreting the significance of test outcomes. Addressing the variability in apparatus selection may require collaborative efforts among researchers, clinicians, and medical device manufacturers to establish consensus guidelines or recommendations (Ponziani et al. 2022). Such guidelines could help ensure consistency in testing methodologies, improve the reproducibility of results, and facilitate the development of evidence-based diagnostic criteria for SIBO.

In traditional breath testing for SIBO, the focus has primarily been on measuring methane and hydrogen gases, as their presence serves as indicators of bacterial overgrowth in the small intestine (Ponziani et al. 2022). These gases are produced by the fermentation of carbohydrates by bacteria in the gut and are subsequently absorbed into the bloodstream and exhaled through the lungs. Methane and hydrogen are typically measured using gas analysers that are calibrated to detect specific concentrations of these gases in exhaled air. During breath testing, patients are instructed to consume a substrate, such as lactulose or glucose, which serves as a fermentable source for gut bacteria. As bacteria ferment the substrate, they produce methane and hydrogen gases, which are then measured at various time intervals following substrate ingestion (Bushyhead and Quigley 2022).

While methane and hydrogen have been the primary focus of breath testing for SIBO, emerging research has highlighted the potential role of hydrogen sulfide in the pathogenesis of the condition. Hydrogen sulfide is another byproduct of bacterial fermentation in the gut and has been implicated in gastrointestinal disorders such as inflammatory bowel disease and irritable bowel syndrome. However, unlike methane and hydrogen, the detection capability for hydrogen sulfide is not currently incorporated into most gas analysers used in routine clinical practice (Shrestha et al. 2019). The lack of standardized guidelines for measuring and the range of the gases mentioned above presents a challenge for clinicians and researchers interested in exploring its role in SIBO.

Interpreting breath test results requires careful consideration of various factors, including baseline gas levels, peak gas concentrations, and the presence of multiple gas peaks. Recommendations for defining positive test results vary among studies, with proposed criteria based on changes in gas concentrations relative to baseline levels and specific time intervals following substrate ingestion (Ponziani et al. 2022). Advancements in SIBO testing methodologies, including the development of novel detection techniques and standardized testing protocols, are needed to improve diagnostic accuracy and reliability. Collaborative efforts among researchers, clinicians, and industry stakeholders are essential for advancing our understanding of SIBO pathophysiology and refining diagnostic strategies to better serve patient needs (Ponziani et al. 2022; Bushyhead and Quigley 2022).

Advancements in SIBO testing methodologies, including the development of novel detection techniques and standardized testing protocols, are needed to improve diagnostic accuracy and reliability. Collaborative efforts among researchers, clinicians, and industry stakeholders are essential for advancing our understanding of SIBO pathophysiology and refining diagnostic strategies to better serve patient needs.

3.5 Breath testing

Small bowel aspirate culture remains the gold standard for diagnosing SIBO, as it provides a direct and precise method of bacterial identification. However, this traditional method requires invasive endoscopy and time-consuming microbial culture, making it less accessible in clinical practice. For this reason, non-invasive hydrogen and methane breath tests are now the most frequently used diagnostic tools for SIBO. These tests are based on the fact that many species of eubacteria in the lower gut produce hydrogen gas (H2) as a by-product of anaerobic metabolism. In some individuals, methane gas (CH4) is also produced, but exclusively by archaebacteria (Ghoshal et al. 2020).

The microbial hydrogen and methane produced in the gut are readily transferred to the bloodstream through the vessels of the gut wall. Once they reach the lungs, these gases can be detected in the exhaled breath, making them measurable for diagnostic purposes (Sanjeevi et al. 2021).

Hydrogen is produced by specific bacteria during carbohydrate fermentation. When substrates like lactulose or glucose are ingested, the bacteria in the small intestine that can ferment these sugars will produce hydrogen. This gas is then absorbed into the bloodstream and eventually exhaled. A significant rise in hydrogen levels, typically within 90 minutes of ingesting the substrate, suggests that fermentation is occurring in the small intestine, which is a key indicator of SIBO. Elevated hydrogen levels can also indicate malabsorption, as unabsorbed carbohydrates lead to increased fermentation.

Methane is produced by a distinct group of bacteria known as methanogens. Unlike hydrogen, which is produced by eubacteria, methane is produced by archaebacteria. The presence of methane can be especially valuable in diagnosing specific types of bacterial overgrowth, such as that associated with constipation-predominant irritable bowel syndrome (IBS). Breath tests that measure methane, in addition to hydrogen, offer more comprehensive insights into the microbial composition of the small intestine, helping clinicians distinguish between different types of SIBO and tailoring treatment strategies accordingly (Ghoshal et al. 2020).

Breath testing involves measuring the baseline levels of hydrogen and methane, then tracking the changes in these levels following the ingestion of a carbohydrate substrate. An increase in either gas following ingestion points to fermentation in the small intestine and suggests the presence of SIBO. The non-invasive nature and ease of breath testing make it an appealing alternative to the invasive small bowel aspirate culture, particularly in outpatient settings.

4 Methodological approach

4.1 Defining research scope and methodology

This thesis aimed to explore current research on breath testing as it relates to SIBO within the field of health sciences. Rather than conducting a systematic review, which requires precise and structured methods, a scoping review was chosen. This approach allows for a broader examination of the existing literature as it stood at the time of writing. Such a review is particularly well-suited for describing and discussing the scientific understanding of a specific topic from both theoretical and contextual perspectives, making it a fitting choice for a master’s level dissertation.

The goal of a literature review is to generate new insights through the analysis, critical assessment, and synthesis of existing research on a given topic or area. As noted by it is crucial to prioritize the most robust evidence when assembling a literature review to ensure the validity of the findings.

When defining the scope of a scoping review, it’s essential to understand that there isn’t a one-size-fits-all approach. Scoping reviews have become an increasingly popular research methodology, particularly when the objective is to explore the breadth and depth of existing literature on a specific topic. This makes them ideal for examining emerging or complex topics where a comprehensive overview of the existing evidence is necessary (Peters et al. 2015). Scoping reviews are especially effective in mapping key concepts, identifying gaps in research, and synthesizing evidence across a wide range of study designs. They are particularly useful in fields where the literature is vast or fragmented, providing a preliminary assessment that can inform the direction of future research, including whether a full systematic review is warranted (Munn et al. 2018).

Unlike exploratory studies, which focus on investigating new or less understood topics, scoping reviews aim to provide a comprehensive overview of existing research. They offer a detailed account of the state of knowledge, much like descriptive research, but with a broader focus. While correlational studies look at relationships between variables and explanatory studies seek to understand causality, scoping reviews are designed to explore a wide range of evidence and highlight areas for further investigation. The scope of a scoping review should align with the research problem and objectives, ensuring a thorough and methodologically sound exploration of the topic (Hernández-Sampieri 2021).

The flexibility of scoping reviews is one of their key strengths. They can incorporate various study designs and methodologies, making them adaptable to a wide range of research questions. This flexibility is particularly valuable in multidisciplinary fields, such as healthcare, where understanding the full scope of research is crucial for informing policy, clinical practice, and future research directions. Scoping reviews are effective in identifying research gaps, synthesizing diverse evidence, and providing a foundation for subsequent research, including systematic reviews or primary research studies (Tricco et al. 2016).

Conducting a scoping review in a master's program requires careful attention to several key steps, even when working independently. The process began by formulating a clear and focused research question, broad enough to allow for a comprehensive exploration but specific enough to guide the review effectively. A preliminary literature search is then conducted to refine the research question and evaluate the availability of relevant studies. This involves selecting appropriate keywords, Medical Subject Headings (MeSH), and databases, with adjustments made as needed based on initial search results (Tricco et al. 2016). Although working alone, it's beneficial to seek advice from subject matter experts or consult a librarian to ensure the search strategy is robust and well-developed.

To ensure transparency and rigor in the review process, it is recommended to follow established frameworks, such as the PRISMA-ScR (Preferred Reporting Items for Systematic Reviews and Meta-Analyses extension for Scoping Reviews), which provides guidance on conducting and reporting scoping reviews systematically (Tricco et al. 2018).

Breath testing is a non-invasive diagnostic method that measures specific gases in the exhaled breath to evaluate gastrointestinal conditions, particularly SIBO. The procedure begins with the patient fasting for a specified period to ensure baseline levels of breath gases are established. After fasting, the patient ingests a substrate, usually lactulose or glucose, which serves as a fermentable carbohydrate (Ghoshal et al. 2020). Following ingestion, breath samples are collected at regular intervals, usually every 15 to 20 minutes, for a duration of up to three hours. These samples are analysed for the presence of hydrogen and methane, gases produced by bacterial fermentation in the small intestine.

4.2 Search strategy

As the use of breath testing for SIBO remains relatively new and alternative within scientific circles, a preliminary literature search was crucial for refining the research question and assessing the availability of relevant studies. The process followed a framework, beginning with the formulation of a PICO model, continuing with development and testing of search terms, and concluding with the application of inclusion and exclusion criteria. In this scoping review, the population of interest consisted of individuals with small intestinal bacterial overgrowth (see table 1). The intervention was breath testing, including variations such as breath test, hydrogen testing, and related terms. The comparison element included alternative diagnostic methods, such as jejunal aspirate culture, lactulose hydrogen breath test, and glucose hydrogen breath test. This framework was essential for guiding the identification and combination of appropriate search terms.

Table 1. PICO search

PICO	
Population	Small intestinal bacterial overgrowth
Intervention	Breath testing OR Breath test OR Hydrogen testing
Comparison	Alternative diagnostic methods (e.g., jejunal aspirate culture, lactulose hydrogen breath test, glucose hydrogen breath test)

The initial search produced a substantial amount of literature, making it necessary to reassess the keywords and search strategy to eliminate irrelevant studies. When a high number of unrelated studies appeared in the results, the search parameters were adjusted to remove terms that added unnecessary noise (Siddaway et al. 2021). Through iterative refinement of the search terms and criteria, a more focused collection of studies was identified, ensuring they directly addressed the role of breath testing in diagnosing SIBO.

Given the evolving nature of this field, standardization concerning pathologies, gas detection, protocols, and other specific aspects is still lacking (Sanjeevi et al. 2021). Therefore, the literature search was not only aimed at refining the research question but also at providing an overview of the current research landscape and identifying prevalent methodologies. Using resources like PubMed, the goal was to establish a detailed search protocol for identifying high-quality studies, which would help map out both existing knowledge and gaps in this.

Search terms were generated based on the PICO components and refined through preliminary background reading and several practice searches. To ensure comprehensive coverage, a wide range of terms was considered, reflecting different ways in which SIBO and breath testing are described in the literature. Boolean operators were used to combine these terms effectively. The operator AND was used to ensure both core concepts were included in the search results, such as SIBO AND breath testing, while OR was applied to capture synonyms and variations, such as breath test OR hydrogen test OR methane test. Truncation symbols were applied where necessary to increase the breadth of the search while maintaining relevance.

The database used was PubMed, Wiley, Cochrane and ProQuest selected for its comprehensive indexing of biomedical and clinical literature. Initial practice searches produced an excessive number of irrelevant results, leading to iterative adjustments of the search terms and combinations. The search was limited to articles published between 2004 and 2024 and restricted to English-language studies.

To ensure that only relevant and high-quality evidence was included, specific inclusion and exclusion criteria were applied. Studies were included if they focused on adult individuals with a confirmed or suspected diagnosis of SIBO and used breath testing as part of the diagnostic process. Additionally, studies comparing breath testing with established diagnostic methods such as jejunal aspirate culture or alternative breath test substrates were prioritized. Excluded studies were those involving children under 14, studies not focused on SIBO or breath testing, publications prior to 2004, or those lacking a diagnostic comparison (see table 2).

Table 2. Inclusion and excluding criteria

Including	Excluding	Rationale
Individual suspected or diagnosed with small intestinal bacterial overgrowth (SIBO).	Individuals without the diagnosis of small intestinal bacterial overgrowth.	Focus on studies relevant to the target condition, ensuring that the population of interest is properly represented.
Use of breath testing as a diagnostic tool for SIBO.	Absence of breath testing as a diagnostic tool for SIBO.	Breath testing is a key diagnostic method for SIBO, providing relevant diagnostic accuracy and practical application information.
Studies published between 2004 and 2024.	Studies published before 2004.	Ensuring the inclusion of recent studies to reflect the latest research, methodologies, and clinical guidelines.
Studies published in English.	Studies not published in English.	Limitation due to language proficiency and to ensure consistent interpretation of data.
Comparison with established diagnostic methods (e.g., jejunal aspirate and culture).	Studies without comparison with established diagnostic methods.	To assess the validity and reliability of breath tests in comparison to standard diagnostic methods, ensuring

		robust and comparative analysis.
Studies conducted in a clinical environment for adults in any geographical location.	Studies focusing on children under age 14.	To ensure that the findings are applicable to the adult population, as diagnostic accuracy and presentation may differ in pediatric populations.

4.3 Data selection

The initial database search identified 118 records across four databases, with no duplicates found or removed. Titles and abstracts were screened for relevance based on the inclusion and exclusion criteria, resulting in the exclusion of 98 records. The remaining 20 articles were retrieved for full-text review, though two were not accessible. A total of 18 articles were assessed for full-text eligibility, and 8 were excluded because they did not compare breath testing to aspirate culture. Ultimately, 10 studies were included for critical appraisal.

The study selection process followed the PRISMA 2020 guidelines. The flow of information through the various stages of the review is depicted in the PRISMA flow diagram (see figure 1).

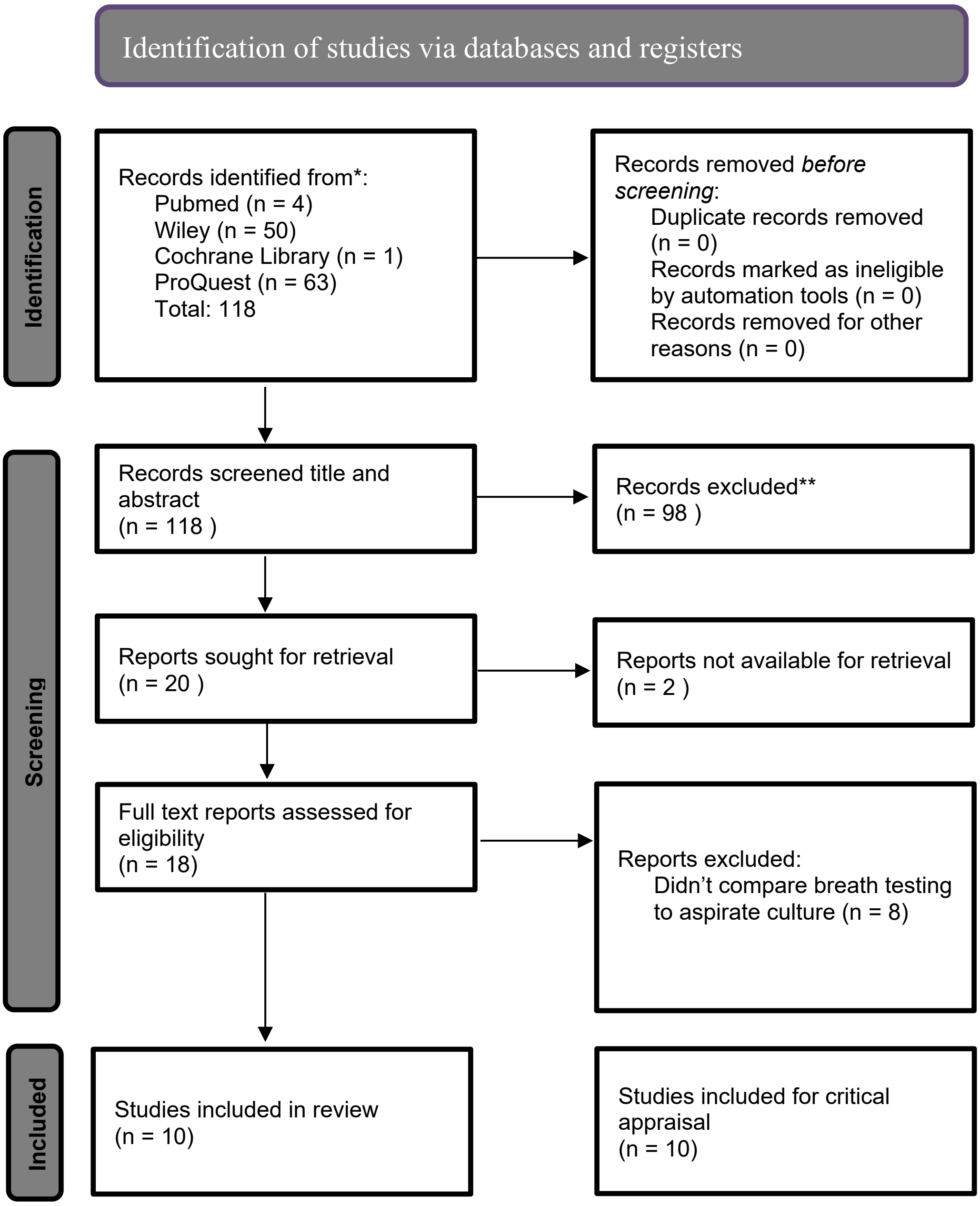
Identification of studies via databases and registers
Identification
Records identified from*:
Pubmed (n = 4)
Wiley (n = 50)
Cochrane Library (n = 1)
ProQuest (n = 63)
Total: 118
Records removed before screening:
Duplicate records removed (n = 0)
Records marked as ineligible by automation tools (n = 0)
Records removed for other reasons (n = 0)
Screening
Records screened title and abstract (n = 118)
Records excluded** (n = 98)
Reports sought for retrieval (n = 20)
Reports not available for retrieval (n = 2)
Full text reports assessed for eligibility (n = 18)
Reports excluded:
Didn't compare breath testing to aspirate culture (n = 8)
Included
Studies included in review (n = 10)
Studies included for critical appraisal (n = 10)

Figure 1. Selection process for included studies.

4.4 Critical Appraisal

To ensure methodological rigor, this review employed several critical appraisal tools, including the CASP Diagnostic Study Checklist, CASP Systematic Review Checklist, SANRA Checklist, and the CASP Checklist for Systematic Reviews with Meta-Analysis of Observational Studies (see table 3). Although formal quality appraisal is not a mandatory component of scoping reviews (Wickstrom et al. 2023), it was deliberately included in this study to strengthen the credibility and reliability of the findings. A total of 10 articles met the inclusion criteria and were selected for critical appraisal. Each article underwent a structured quality assessment prior to inclusion in the final synthesis.

The CASP checklists were used to assess qualitative and empirical studies, focusing on key domains such as research design, methodological transparency, data collection, ethical considerations, and coherence of findings. These checklists function as qualitative assessment tools, with each item answered using "Yes," "No," or "Can't tell." While the CASP tools do not provide a formal scoring system, a semi-quantitative approach is occasionally adopted in systematic reviews, whereby the number of affirmative responses can be used to categorize the methodological quality of studies as high, moderate, or low (Munn et al. 2018).

In this assignment, the CASP checklist has been used to assess the methodological quality of the included studies. Studies with more "Yes" responses are considered methodologically stronger, while studies with many "No" or "Uncertain" responses may have a higher risk of bias and lower reliability.

The CASP assessment of diagnostic studies included four articles, all rated as moderate methodological quality. These studies provided valuable diagnostic insights; however, some limitations were identified, including potential bias due to unclear blinding, restrictions in patient selection criteria, and incomplete reporting of confidence intervals. No studies were classified as high or low quality.

For narrative review articles, the SANRA checklist was used to assess the justification of the topic, comprehensiveness of literature coverage, quality of sources, and clarity of conclusions. The SANRA assessment of narrative reviews included six studies, all rated as high quality. Scores ranged from 8/12 to 11/12. These reviews demonstrated well-reasoned arguments, strong referencing, and appropriate data presentation. However, a common limitation was the lack of explicit transparency in the literature search, which affected reproducibility.

Table 3. Critical appraisal

Study	Checklist	Score/classification
Ghoshal et al. (2017)	SANRA	9/12
Rao & Bhagatwala (2019)	SANRA	9/12
Eamonn M. M. Quigley (2019)	SANRA	8/12
Sharab & Rezaie (2024)	SANRA	11/12
Achufusi et al. (2020)	SANRA	9/12
Skrzydło-Radomańska and Cukrowska (2022)	SANRA	10/12
Erdogan et al. (2015)	CASP Diagnostic Study	Moderate
Sundi et al. 2018	CASP Diagnostic Study	Moderate
Sanjeevi et al. (2021)	CASP Diagnostic Study	Moderate
Ghoshal et al. (2019)	CASP Systematic Review / Meta-Analysis	High

The necessity of quality assessment in scoping studies remains a subject of debate. The absence of methodological quality assessment may challenge the interpretation of findings, while failure to conduct quality appraisal may reduce the applicability of scoping study results in policy and practice (Levac et al. 2010; Grant and Booth 2009). Although this study does not directly engage with this debate, it acknowledges the complexity of evaluating methodological rigor across diverse sources, including published and grey literature. Furthermore, challenges persist regarding the assessment of stakeholder consultation evidence within the framework of scoping reviews (Levac et al. 2010).

4.5 Data Analysis

The data extracted from the included studies were analysed using both numerical and thematic approaches, following the principles outlined by Mak and Thomas (2022). A numerical summary of the studies was first compiled, presenting key characteristics such as study design, population, and main findings in tabular format. This quantitative overview provided a structured foundation for further thematic analysis.

For the thematic analysis, an inductive coding process was applied to identify patterns, similarities, and differences across the studies. Relevant text segments were extracted and assigned initial codes that captured their meaning in relation to the research question. These codes were iteratively refined and grouped into broader categories, allowing overarching themes to emerge (Mak and Thomas 2022). To ensure a systematic and transparent approach, a coding framework was developed and adjusted throughout the process.

Unlike research teams, where coding reliability can be strengthened through collaborative discussion and consensus-building, this master's thesis was conducted independently. Therefore, particular attention was given to self-reflection and consistency in coding decisions to maintain analytical rigor. The final themes were derived through a continuous process of comparison and synthesis, ensuring that they accurately represented the key insights from the included studies (see figure 2).

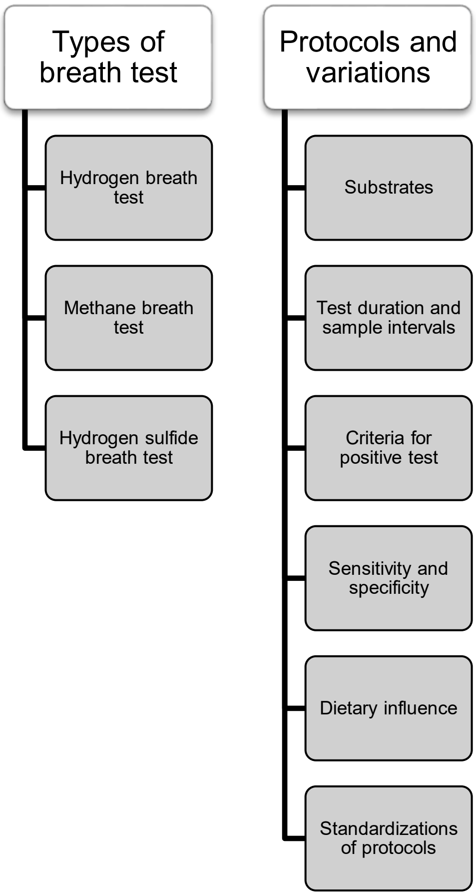

Figure 2. Selection process for included studies.

5 Results

This section presents the findings of the scoping review, which examines the available breath testing methods for diagnosing SIBO and their diagnostic accuracy compared to small bowel aspirate culture. The themes were derived through thematic analysis and structured to directly reflect the scope of the research questions. The first research question, which explores the different types of breath tests available for diagnosing SIBO, is addressed by Theme 1: Types of Breath Tests**,** encompassing the subcategories of hydrogen, methane, and hydrogen sulfide testing. The second research question, Protocols and variations, which examines the diagnostic effectiveness of breath testing compared to small bowel aspirate culture, is addressed through Themes 2**.** These include subcategories as Substrates**,** Test Duration and Sampling Intervals**,** Criteria for Positive Test**,** Sensitivity and Specificity**,** Dietary Influence, and Standardizations of Protocols, all of which relate to test performance, variability, and clinical applicability. This thematic structure ensures alignment between the research aims and the extracted data, allowing a nuanced synthesis of evidence across methodological and diagnostic dimensions.

To systematically address the first research question “What breath testing methods are currently available for diagnosing SIBO?”, this section presents a thematic analysis of the existing literature. The findings are organized into key themes, including different breath test modalities, variations in testing protocols, and emerging diagnostic approaches, to provide a comprehensive overview of current methodologies.

A comprehensive summary of the included studies, including author and year, study design, intervention and participants, and key findings (see appendix 1). This table provides the extracted data that formed the basis for the thematic analysis described in the results section.

5.1 Types of breath tests.

5.1.1 Hydrogen Breath test.

Hydrogen breath testing (HBT) is widely reported across the included studies as a primary method for the non-invasive diagnosis of SIBO. It is considered both practical and commonly used in clinical settings, particularly following the administration of glucose or lactulose substrates (Sharab and Rezaie 2024). Several studies emphasize the ease and non-invasive nature of the procedure. For example, one study describes breath tests as “popular, non-invasive and patient-friendly methods used increasingly for diagnosis of SIBO” (Achufusi 2020; Ghoshal et al. 2020; Ghoshal et al. 2021; Quigley 2020; Sanjeevi et al. 2021; Sharab and Rezaie 2024).

The effectiveness of hydrogen breath testing is closely linked to the substrate used. Achufusi (2020) explain that "the diagnostic role of hydrogen breath tests largely depends on the type of substrate used," noting that glucose and lactulose are commonly employed for diagnosing bacterial overgrowth, whereas lactose HBTs are more relevant in carbohydrate malabsorption.

The test procedure typically involves the oral administration of a substrate followed by serial measurements of hydrogen concentration in exhaled breath. The use of a hydrogen breath analyser for conducting the breath analysis is specified (Sanjeevi et al. 2021). Hydrogen is often measured alongside methane in contemporary clinical practice, which enhances diagnostic sensitivity (Quigley 2020).

Furthermore, elevated hydrogen levels may also be observed in patients with functional gastrointestinal disorders. “Patients with IBS have also been shown to produce more hydrogen in fasting state as well as after administration of fermentable substrates as compared with healthy controls,” indicating potential overlap in hydrogen production between SIBO and IBS populations, which may affect diagnostic specificity (Ghoshal et al. 2020).

5.1.2 Methane Breath test.

Methane breath testing is frequently mentioned in the literature as a useful addition to hydrogen measurement in breath analyses. Its relevance lies in the fact that methane is produced only by archaebacteria, which are present in a subset of the population (Sundin et al. 2018). This specificity is important, as relying solely on hydrogen levels may lead to false-negative results in individuals who primarily produce methane.

Estimates suggest that approximately 5–15% of the general population are methane producers (Sanjeevi et al. 2021). In studies where methane measurement was excluded, this was considered a limitation, since the presence of methanogens in some patients could have led to an underestimation of SIBO prevalence (Sanjeevi et al. 2021).

Some researchers argue that incorporating methane measurement into breath testing protocols may enhance diagnostic accuracy. For example, an increase in methane levels above 10 parts per million (ppm) is proposed as a threshold for a positive test outcome (Achufusi 2020).

Methane is typically analysed in conjunction with hydrogen using gas chromatography techniques, which enable the detection of both gases in exhaled breath samples (Erdogan et al. 2015).

Despite this, the overall utility of methane testing is still debated. Some studies indicate that including methane does not necessarily increase the diagnostic yield for SIBO, suggesting that its contribution should be interpreted in the specific clinical and research context (Ghoshal et al. 2017).

5.1.3 Hydrogen sulfide Breath test.

Hydrogen sulfide (H_2S) breath testing represents a relatively recent and experimental approach within the broader category of non-invasive diagnostics for SIBO. Unlike hydrogen and methane, which are routinely assessed, the use of H_2S in breath analysis remains under investigation and is not yet part of validated clinical practice (Skrzydło-Radomańska and Cukrowska 2022).

Preliminary findings indicate that H_2S could have diagnostic relevance in selected patient groups. Specifically, an overgrowth of sulphate-reducing bacteria has been hypothesised to contribute to symptoms in individuals with irritable bowel syndrome (IBS), particularly those with diarrhea-predominant subtypes (IBS-D). This bacterial activity may influence the development of SIBO, potentially through mechanisms involving visceral hypersensitivity. Based on these associations, H_2S has been proposed as a non-invasive biomarker for SIBO in such populations (Ghoshal et al. 2017).

Despite these initial insights, the integration of H_2S breath testing into routine diagnostic protocols is currently limited by a lack of robust validation, underscoring the need for further research to determine its clinical utility.

5.2 Protocols and variations.

To explore the second research question “Can breath testing effectively diagnose SIBO compared to small bowel aspirate culture tests? this section examines the diagnostic performance of breath tests relative to the gold standard methods. The analysis encompasses factors such as sensitivity, specificity, and the prevalence of false positives and negatives. Additionally, practical considerations, including clinical applicability, patient compliance, and external influences on test accuracy, are discussed to provide a nuanced evaluation of breath testing as a diagnostic tool for SIBO.

5.2.1 Substrates

The type and dosage of substrates used in breath testing are crucial factors influencing diagnostic outcomes. The most commonly used substrates in the reviewed studies were glucose and lactulose, with occasional mention of other sugars such as lactose and fructose.

Several studies reported the use of glucose in specific, standardized doses as part of the breath testing procedure. A common protocol involved administering 75 grams of glucose dissolved in 250 mL of water following an overnight fast (Sanjeevi et al. 2021; Erdogan et al. 2015). Another study used a slightly higher concentration, with 90 grams of glucose dissolved in 100 mL of water (Sundin et al. 2018).

These dosages are consistent with published consensus guidelines, which outline standard amounts for various substrates used in breath testing. For example, glucose is recommended at 75 g, lactulose at 10 g, lactose at 25 g, and fructose at 25 g (Achufusi 2020).

The preference for either glucose or lactulose as test substrates is supported by evidence from a meta-analysis of 14 clinical studies, where the dosages most commonly used were 75 g of glucose or 10 g of lactulose, depending on the testing protocol (Skrzydło-Radomańska and Cukrowska 2022).

These findings demonstrate consistency across studies regarding substrate selection and dosage, highlighting glucose and lactulose as the dominant substrates for breath testing in the diagnosis of SIBO.

5.2.2 Test Duration and Sample Intervals

The duration of breath tests and timing of sample collection vary across studies, with protocols generally ranging from 90 to 180 minutes and sampling intervals typically between 15 and 20 minutes (see table 4).

The duration and sampling intervals for breath testing vary across studies but generally follow standardized protocols. One common approach involves measuring hydrogen levels every 20 minutes over a total period of 120 minutes (Sanjeevi et al. 2021). In other studies, the test duration is extended to 180 minutes, starting with two baseline samples to establish initial gas concentrations before continuing with 20-minute intervals (Sundin et al. 2018).

Slightly shorter testing intervals are also reported. For instance, some studies describe collecting breath samples every 15 minutes for a total test duration of 90 to 120 minutes, particularly when using the glucose breath test (Rao and Bhagatwala 2019; Erdogan et al. 2015).

Consensus guidelines recommend using a hydrogen increase of 20 parts per million (ppm) within 90 minutes as a diagnostic threshold (Achufusi 2020). However, this timing-based criterion has been challenged in the literature. Critics argue that relying on a 90-minute cut-off assumes uniform mouth-to-cecum transit times across individuals, which may not be accurate and could lead to diagnostic misclassification (Ghoshal et al. 2017).

These findings reveal a general pattern of 2- to 3-hour testing durations and 15- to 20-minute sampling intervals, though there remains debate around the interpretation of timing relative to intestinal transit variability.

Table 4. Duration and Sampling Intervals

Paper	Duration (min)	Interval (min)	Notes
Sanjeevi et al. (2021)	120	20	Standard GHBT; hydrogen measured every 20 minutes
Sundin et al. (2018)	180	20	Extended test with two baseline

			samples; glucose administered
Erdogan et al. (2015)	120	15	Glucose used; alveolar gas analyzed for hydrogen and methane
Rao and Bhagatwala (2019)	90-120	15	None
Achufusi et al. (2020)			Cites consensus guidelines; no specific protocol described
Ghoshal et al. (2017)			Criticizes 90-minute cutoff; no protocol details provided
Remaining studies (n=4)			Did not explicitly report breath test duration or sampling protocols

5.2.3 Criteria for positive test

Criteria for interpreting hydrogen and methane breath tests vary slightly across studies, though a general threshold of a ≥20 ppm rise in hydrogen from baseline within a defined time frame is widely accepted. For methane, a rise of ≥10 ppm is typically used as the diagnostic cutoff.

Diagnostic thresholds for glucose hydrogen breath testing (GHBT) vary across the literature, though several key criteria appear recurrent. Some studies define a positive test result as a hydrogen peak of at least 12 parts per million (ppm) above the baseline level following glucose administration (Sanjeevi et al. 2021; Ghoshal et al. 2017).

However, a threshold of 20 ppm is more commonly used. A rise in hydrogen exceeding 20 ppm or a methane increase greater than 10 ppm at any time after glucose ingestion is frequently considered indicative of SIBO (Sundin et al. 2018; Rao and Bhagatwala 2019). These criteria are consistent with published consensus guidelines, which recommend interpreting a hydrogen increase of more than 20 ppm within 90 minutes or a methane rise above 10 ppm within two hours as positive (Achufusi 2020). Some sources further specify that elevated baseline levels do not in themselves confirm a positive result unless the defined increase thresholds are met (Erdogan et al. 2015).

Additional criteria include a minimum increase of 20 ppm for hydrogen or 15 ppm for methane above baseline, or a combined increase of at least 20 ppm for both gases (Erdogan et al. 2015). These thresholds are echoed across multiple sources, including the North American consensus and recent reviews, which reinforce the ≥20 ppm hydrogen increase within 90 minutes as the standard for both glucose and lactulose breath testing (Skrzydło-Radomańska and Cukrowska 2022; Quigley 2020; Sharabi and Rezaie 2024).

Nonetheless, there remains some debate in the literature regarding these diagnostic cut-offs. For instance, some authors question the clarity of current criteria, noting that elevated baseline hydrogen levels above 20 ppm, especially when combined with a flatline pattern and absence of methane production, may also suggest the presence of SIBO despite not meeting standard increase thresholds (Ghoshal et al. 2020).

Together, the articles illustrate general agreement on a 20 ppm rise in hydrogen and a 10 ppm rise in methane as diagnostic criteria, typically within 90 minutes of substrate ingestion. Nevertheless, some studies employ alternative cut-offs, and there remains ongoing discussion around baseline values and flatline patterns.

5.2.4 Sensitivity and specificity

The diagnostic accuracy of breath testing for SIBO shows considerable variability across studies, particularly regarding the sensitivity and specificity of glucose hydrogen breath testing (GHBT) and lactulose hydrogen breath testing (LHBT).

Several studies report GHBT as a highly specific but relatively insensitive diagnostic tool. A sensitivity of 44.4% and a specificity of 80% was reported (Sanjeevi et al. 2021), while slightly lower sensitivity (42%) but higher specificity (84%) was found in another study (Erdogan et al. 2015). Broader ranges based on existing literature were provided, with GHBT sensitivity varying between 15.7% and 62%, and specificity between 78% and 97% (Achufusi 2020). This pattern was reinforced by the description of GHBT as "highly specific (78% to 97%)" but "quite insensitive (15.7% to 62%)" (Ghoshal et al. 2017).

In contrast, LHBT was generally described as more sensitive but less specific. LHBT sensitivity ranged from 31% to 68%, and specificity from 44% to 100% (Rao & Bhagatwala 2019). This was echoed in another source, noting sensitivity between 31% and 68%, and specificity ranging from 65% to 97.9% (Achufusi 2020). Similarly, the conventional double-peak criterion for LHBT was described as lacking sensitivity (31%–68%), and the newer early-peak criterion was noted too often yield false positives, with specificity between 65% and 97.9% (Ghoshal et al. 2017).

The widespread use of breath testing due to its non-invasive and cost-effective nature was acknowledged, although it was emphasized that "its sensitivity and specificity are limited" (Skrzydło-Radomańska and Cukrowska 2022). The lack of a clear consensus on cut-off values was also highlighted, further complicating interpretation and potentially influencing test accuracy (Ghoshal et al. 2017).

Collectively, these findings underline a trade-off between sensitivity and specificity across substrate types. GHBT tends to minimize false positives at the cost of false negatives, whereas LHBT may identify more true positives but risks overdiagnosis due to lower specificity. The diagnostic performance is further influenced by the variability in test protocols, cut-off thresholds, and interpretation criteria.

5.2.5 Dietary influence

Pre-test dietary preparation plays a significant role in ensuring the accuracy of breath test results, as diet influences the baseline levels of fermentable substrates and thereby affects hydrogen and methane production.

Most studies emphasized adherence to a specific diet in the 24 hours preceding testing. A low-fermentation or low-carbohydrate diet the day before the test was consistently recommended (Rao and Bhagatwala 2019; Achufusi 2020; Erdogan et al.

2015; Skrzydło-Radomańska and Cukrowska 2022). Specifically, patients were advised to avoid complex carbohydrates, fibre, dairy products, and other fermentable foods such as beans and tofu (Sundin et al. 2018; Rao and Bhagatwala 2019; Achufusi 2020; Erdogan et al. 2015; Skrzydło-Radomańska and Cukrowska 2022). It was underlined that fermentable foods, including complex carbohydrates, should be excluded, and that fasting for 8–12 hours prior to the test was recommended (Rao and Bhagatwala 2019). Similarly, it was advised that no dietary fibre or alcohol should be consumed within 24 hours of testing, and a 12-hour fasting period was emphasized (Skrzydło-Radomańska and Cukrowska 2022).

Several articles also highlighted the importance of limiting external factors that could influence gas production or test accuracy. Subjects were instructed not to smoke or sleep during testing, and oral bacteria were minimized by brushing teeth and using mouthwash before the test (Sundin et al. 2018). The recommendation to avoid smoking and physical exertion on the day of the test was echoed (Rao and Bhagatwala 2019; Skrzydło-Radomańska and Cukrowska 2022).

Beyond test preparation, dietary management may also have implications for post-treatment maintenance. The theoretical benefits of a low-fermentation diet in maintaining remission were discussed, drawing from data in IBS studies (Sharabi and Rezaie 2024). It was suggested that reducing fermentable carbohydrates, such as FODMAPs, may lower the risk of symptom recurrence by minimizing substrate availability for bacterial fermentation. Additionally, structured eating patterns such as spacing meals at least five hours apart and avoiding overnight eating may promote the activity of migrating motor complexes, which facilitate intestinal clearance (Sharabi and Rezaie 2024).

Overall, consistent dietary protocols before testing are essential to reduce variability in test outcomes. Meanwhile, dietary interventions may also play a role in both symptom management and the prevention of relapse in patients diagnosed with SIBO.

5.2.6 Standardizations of protocols

One of the major challenges in the clinical application of breath testing for SIBO is the lack of standardized protocols across studies and practices. This variability limits the comparability of findings and complicates the interpretation of results.

Several articles highlighted significant methodological heterogeneity. Although hydrogen breath tests are easy to administer, their interpretation is often ambiguous due to differences in substrates, cutoff thresholds, and sampling intervals used across studies (Sanjeevi et al. 2021). This concern was reinforced: “There is currently no standard methodology for breath testing,” and glucose or lactulose breath testing “requires further standardization and validation” (Rao and Bhagatwala 2019). Methodological inconsistencies were also outlined, including the use of different glucose doses (ranging from 50 g to 100 g) and variations in interpretation criteria, with positive results defined based on a rise of 10, 12, 15, or 20 parts per million (ppm) in hydrogen over baseline (Erdogan et al. 2015).

The lack of consensus regarding whether to measure hydrogen alone or in combination with methane adds further variability. Some studies have focused exclusively on hydrogen despite evidence suggesting that methane measurements may improve diagnostic accuracy (Erdogan et al. 2015).

Nonetheless, efforts to unify practices are emerging. A recently published North American consensus proposed standardized cutoff values for breath hydrogen testing, offering clinicians a more consistent framework for interpreting results (Achufusi, 2020). However, this consensus also implies the need for broader adoption and validation of the proposed guidelines.

Additionally, sampling protocols for both breath tests and small bowel aspirates remain unstandardized. In the case of aspirates, up to 20% of samples may be contaminated by oropharyngeal microbes, calling into question the reliability of this so-called gold standard (Sharabi and Rezaie 2024).

6 Discussion

The studies included in this scoping review consistently reported that breath testing is the most widely adopted non-invasive method for diagnosing SIBO, owing to its ease of administration, patient acceptability, and cost-effectiveness (Chang et al. 2023). However, the diagnostic performance of breath testing is highly dependent on the substrate used typically glucose or lactulose. Glucose-based tests tend to offer high specificity but are prone to false negatives due to limited reach into the distal small intestine. In contrast, lactulose tests provide greater sensitivity but carry an increased risk of false positives, particularly in patients with rapid intestinal transit times (Chang et al. 2023).

In the studies included in this review, the incorporation of complementary methane testing was found to enhance sensitivity in individuals with methanogenic microbiota, though the overall diagnostic yield remained inconsistent. The emerging role of hydrogen sulfide as a biomarker is promising, particularly for patients with diarrheal-predominant irritable bowel syndrome (IBS-D), although this approach currently lacks sufficient clinical validation for routine use (Das and Pal 2020).

All the studies included in this review reported that false positives and negatives in breath testing and aspirate culture are influenced by factors such as gut motility, substrate type, and individual physiological differences. Furthermore, a broad range of external and comorbid factors can affect test results. For instance, dental problems, asthma, mental and other conditions can alter hydrogen and methane concentrations, potentially leading to diagnostic inaccuracies (Das and Pal, 2020). These findings underscore the complexity of interpreting breath test and aspirate culture results in a clinically meaningful way.

Recent comparative studies have demonstrated that hydrogen and methane breath tests are approaching the diagnostic accuracy of jejunal aspirate cultures, traditionally considered the gold standard for diagnosing SIBO. Chang et al. (2023) conducted a study involving 40 adult outpatients, where both breath tests and jejunal aspirate cultures were performed. The results showed that the glucose breath test (GBT) had a good agreement with jejunal aspirate culture, and the lactulose breath test (LBT) had a moderate agreement. These findings suggest that, when standardized, breath tests can serve as reliable, non-invasive alternatives to jejunal aspirate cultures, offering advantages in terms of patient comfort, cost, and accessibility. However, the study also emphasizes the need for consistent guidelines on substrate choice and test duration to enhance diagnostic accuracy and comparability across clinical settings.

Across the ten studies analysed in this scoping review, a major challenge identified was the substantial variability in breath testing protocols. Test durations range from 90 to 180 minutes, sampling intervals typically fall between 15 and 20 minutes, and diagnostic thresholds vary, with a ≥20 ppm rise in hydrogen or ≥10 ppm rise in methane most commonly used. These discrepancies contribute to wide-ranging sensitivity and specificity values, ultimately undermining the reliability and clinical utility of breath testing as a diagnostic tool.

This lack of standardization has been highlighted in expert consensus as a critical barrier to clinical utility. For instance, Ghoshal et al. (2022) emphasize that the absence of harmonized procedures in test preparation, substrate dosing, and interpretation contributes significantly to inconsistent diagnostic outcomes. Similarly, Rezaie et al. (2024) underline the variability across laboratories and studies as a key reason for the divergent prevalence rates of SIBO and inconsistent patient management strategies.

There is a clear and pressing need for the development of consistent, evidence-based guidelines to standardize critical methodological components, including substrate selection, dosage, test duration, sampling intervals, and interpretation criteria. Uniform protocols would not only improve diagnostic accuracy and reproducibility but also enhance the comparability and synthesis of future research findings (Das and Pal, 2020; Rezaie et al. 2024). Such standardization would facilitate the establishment of robust reference values and diagnostic cutoffs, tailored to specific patient populations and clinical contexts (Ghoshal et al. 2022).

Several studies included in this review noted that the interpretation of breath test results is further complicated by the overlap in gas production profiles between SIBO and other gastrointestinal disorders, particularly irritable bowel syndrome (IBS). This overlap increases the risk of misclassification and may lead to inappropriate or delayed treatment. In many cases, patients with IBS-like symptoms may receive conflicting or inconclusive breath test results, highlighting the limitations of relying solely on gas concentration changes for diagnosis.

Furthermore, individual factors such as intestinal transit time, diet, recent antibiotic or probiotic use, and underlying motility disorders can influence test outcomes, adding another layer of complexity to clinical interpretation. These confounding variables emphasize the need for a more nuanced, integrative approach that considers both clinical presentation and complementary diagnostic tools.

In light of these methodological challenges, future research should prioritize the validation of standardized protocols across diverse populations and clinical settings. Integrating breath testing with symptom assessment, microbiome profiling, and emerging biomarkers may provide a more comprehensive and accurate diagnostic framework for conditions such as SIBO and IBS.

Although breath testing remains the most practical diagnostic tool for SIBO in many clinical settings, its limitations must be acknowledged. The absence of standardized protocols contributes to inconsistent outcomes and poses a barrier to widespread clinical adoption.

However, its affordability and non-invasive nature makes it a preferable option compared to endoscopic aspirate culture, which are more expensive, invasive, and technically demanding. The latter also suffers from sampling variability, which can affect diagnostic accuracy (Mooney et al. 2023).

From a patient-centred perspective, the relative comfort of breath testing enhances patient compliance and facilitates repeated testing, when necessary, an advantage not shared by invasive procedures. Hou et al. (2022) emphasize that the human microbiome is subject to substantial day-to-day variability, influenced by a wide array of internal and external factors. These include diet, stress, medications, infections, sleep patterns, and even genetic predispositions all of which can significantly alter microbial composition and diversity within short time frames. For instance, dietary changes can rapidly shift microbial communities, while stress and antibiotic use may disrupt microbial balance. Likewise, sleep quality and genetic factors further contribute to these fluctuations, underscoring the highly dynamic nature of the gut microbiota.

This variability presents a major challenge for diagnostic tools that rely on a single measurement, as they risk capturing only a limited and potentially unrepresentative snapshot of gut health. A key limitation of breath testing is precisely this: its reliance on single time-point measurements of intestinal gas concentrations. These gas levels, including hydrogen, methane, and potentially hydrogen sulfide, fluctuate in response to numerous physiological and environmental factors such as recent food intake, physical activity, circadian rhythms, and psychological stress. As such, the results of a single breath test may not accurately reflect the overall metabolic activity of the gut.
Given the dynamic interplay between host metabolism and microbial function, as highlighted by Hou et al. (2022), there is a clear rationale for diagnostic approaches that allow for repeated, longitudinal assessments. Breath testing, by virtue of being non-invasive, low cost, and well tolerated, is particularly well-suited for such repeated

use unlike invasive methods such as jejunal aspirate culture, which are less feasible for routine monitoring.

Furthermore, psychosocial factors including stress, anxiety, and overall mental health can modulate gastrointestinal physiology and influence breath test outcomes. These variables are rarely accounted for in clinical studies, yet they may significantly confound diagnostic interpretations if overlooked. Future research should therefore aim to integrate these contextual influences into study designs and analytical frameworks to enhance the reliability and clinical utility of breath testing in gut health assessment.

Zhou et al. (2023) highlights the significant rise in inflammatory bowel diseases (IBD) globally, with projections showing a continued increase until at least 2050, particularly in regions undergoing industrialization and urbanization. This trend indicates a shift in global health challenges, where non-communicable gastrointestinal conditions are becoming dominant. As such, the need for scalable, non-invasive, and cost-effective diagnostic approaches, such as breath testing, becomes increasingly critical not only for SIBO but for a broader spectrum of gastrointestinal disorders.

The burden of gastrointestinal diseases extends well beyond the clinical setting. Zhou et al. highlight the significant economic impact, including rising healthcare expenditures, increased demand for specialist services, and indirect costs such as work absenteeism and reduced quality of life. These multifaceted challenges underscore the need for improved, accessible, and patient-friendly screening tools. In this context, breath testing holds promise as a valuable method for the early detection of functional symptoms that may precede or coexist with more serious conditions such as inflammatory bowel disease (IBD), thereby enabling earlier intervention and more efficient allocation of healthcare resources.

Based on the synthesis of included studies, breath testing appears to offer a non-invasive, cost-effective, and easily repeatable alternative to the more invasive and expensive jejunal aspirate culture. As Das and Pal (2020) emphasize, the standardization of breath analysis techniques can enhance the reliability and clinical utility of these tests, offering a more accurate reflection of an individual's ongoing health status rather than a single-point-in-time assessment. When used in conjunction with other diagnostic approaches, breath testing may contribute to a more holistic and accessible framework for monitoring gut health and microbiome-related disorders.

When contextualized within the broader literature, these findings align with previous reviews that have similarly emphasized the lack of consensus regarding diagnostic protocols. Variability in substrates, dosages, test durations, and threshold values, coupled with heterogeneity in study populations and methodological designs, significantly impairs the comparability of results. This methodological inconsistency has thus far prevented the development of universally accepted diagnostic guidelines and continues to limit the clinical utility of breath testing for SIBO.

Future research should aim to address existing methodological limitations through well-designed, large-scale trials that evaluate and standardize breath testing protocols. Comparative studies examining substrate types and dosages, test durations, sampling intervals, and diagnostic cut-offs are essential to establish protocols that deliver consistent and reproducible results across various populations. In parallel, longitudinal studies are crucial to assess how breath test outcomes correlate with clinical progression, symptom severity, and treatment response. These investigations should also include validation against gold-standard methods such as jejunal aspirate culture. In addition, the potential of emerging biomarkers particularly hydrogen sulfide warrants further exploration, especially in subgroups like patients with diarrheal-predominant IBS, where traditional hydrogen and methane testing may lack sensitivity. However, hydrogen sulfide measurement is not yet fully standardized, and more research is needed to understand its diagnostic and clinical significance.

Population-specific variables, such as age, dietary habits, comorbid conditions, medication use, and even regional microbiota differences, must also be considered in future trials to enhance the clinical relevance and global applicability of breath testing standards. Real-time, repeated breath testing could allow for more nuanced monitoring of disease activity and response to interventions, addressing the limitations of one-time snapshot diagnostics.

Furthermore, the integration of breath testing with digital health technologies such as mobile applications, wearable sensors, and machine learning algorithms may offer personalized tracking of gut health in real world settings. A multimodal diagnostic approach that combines breath testing with microbiome profiling, symptom scores, and other biomarkers has the potential to improve diagnostic precision, reduce misclassification, and support individualized patient management (Das and Pal, 2020).

7 Ethical considerations and validity

Ethical considerations are essential for maintaining the integrity, transparency, and academic credibility of a scoping review. Although no ethical approval was required for this thesis—due to the exclusive use of secondary data from publicly available literature the research was conducted in accordance with the ethical standards of Metropolia University of Applied Sciences.

To ensure transparency, the objectives, methodology, and scope of the review were clearly defined and documented following the PRISMA-ScR framework. A structured and comprehensive search strategy was developed using the PICO model and refined in consultation with a librarian from Metropolia University to ensure accuracy and relevance. Only peer-reviewed studies published between 2004 and 2024 in English were included. Inclusion and exclusion criteria were pre-established and strictly applied to maintain consistency in the selection process.

All sources were cited in accordance with Metropolia University's referencing guidelines. Full bibliographic information has been provided to ensure academic traceability, and proper citation of previous work was used to uphold intellectual property rights and avoid plagiarism. The originality of the thesis was verified using the Turnitin plagiarism detection service, with a similarity index of 11%, confirming the authenticity of the work.

To further support the writing process, OpenAI's ChatGPT was used as a writing aid to refine language, structure content, and clarify ideas. All interpretations, critical assessments, and conclusions were independently developed by the author.

Validity in this thesis was supported through multiple strategies. A systematic and multi-database search approach ensured a broad and representative evidence base. Studies were screened and selected based on pre-defined criteria, and a critical appraisal was conducted using validated checklists (CASP and SANRA) to assess methodological quality. Both numerical and thematic data analysis methods were used to enhance analytical rigor. The inclusion of a transparent PRISMA flow diagram and a structured data extraction table further support the reproducibility and trustworthiness of the findings. Although conducted independently, the author prioritized consistency, methodological transparency, and reflective evaluation throughout the process to support the overall validity of the review.

8 Conclusion

This scoping review mapped and thematically analysed the existing literature on breath testing for SIBO, with a particular focus on its diagnostic application compared to small bowel aspirate culture. Thematic synthesis revealed several key areas: variability and limitations in testing protocols, the evolving role of gas biomarkers (hydrogen, methane, and hydrogen sulfide), and ongoing challenges related to diagnostic accuracy and clinical interpretation.

Breath testing remains a non-invasive and widely accessible diagnostic tool; however, the evidence highlights significant inconsistencies in substrate types, sampling protocols, test durations, and diagnostic thresholds. These inconsistencies, combined with symptom overlap in functional gastrointestinal disorders, complicate interpretation and reduce comparability across studies.

The review also identified increasing interest in the diagnostic potential of methane and hydrogen sulfide, though further validation is needed. The lack of standardized guidelines emerged as a central barrier, limiting the broader clinical utility of breath tests for SIBO.

Overall, this review underscores the need for future research to establish standardized testing protocols, validate emerging biomarkers, and refine diagnostic criteria. A more consistent methodological framework may enhance diagnostic accuracy and facilitate the integration of breath testing into routine clinical practice.

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Appendix 1 Data extraction

Author and year	Design	Intervention and participants	Key findings
Achufusi et al. (2020)	Narrative literature review	Not applicable	Duodenal aspirate is the gold standard, but breath tests are more practical. Rifaximin is the most effective antibiotic. Future diagnostics may improve with metagenomics and molecular biology.
Eamonn M. M. Quigley (2019)	Literature review	Not applicable	The review examines the definition, diagnosis, and management of SIBO, evaluating the use of molecular techniques and real-time intestinal gas sampling to

			improve diagnostic accuracy.
Erdogan et al. (2015)	Prospective comparative study	All 135 participants underwent both a glucose breath test (GBT) and upper endoscopy with duodenal fluid aspiration for bacterial culture	The breath test missed more than half of culture-positive patients, leading authors to conclude it has low sensitivity and limited utility as a standalone diagnostic tool for SIBO
Ghoshal et al. (2019)	Meta-analysis	Studies applying tests for small intestinal bacterial overgrowth (SIBO) in subjects with irritable bowel syndrome (IBS)	Frequency of SIBO in patients with IBS using different tests (GHBT, LHBT, and upper gut aspirate culture
Ghoshal et al. (2017)	Review article	Not applicable	Jejunal aspirate is the gold standard, but hydrogen breath tests are widely used.
Rao & Bhagatwala (2019)	Review article	Not applicable	To review SIBO, including symptom patterns, predisposing risk factors, prevalence,

			specialized diagnostic testing, and potential therapeutic interventions
Sanjeevi et al. 2021	prospective single-center cross-sectional study	Jejunal fluid aspiration for bacterial cultures and glucose hydrogen breath test. 48 newly diagnosed chronic pancreatitis patients	Jejunal aspirate culture was positive in 18/48 (37.5%) patients, while GHBT indicated SIBO in 14/48 (29%) patients.
Sharab & Rezaie (2024)	Review article	Not applicable	To review the pathophysiology, risk factors, clinical behavior, diagnosis, and management of Small Intestinal Bacterial Overgrowth (SIBO)
Skrzydło-Radomańska & Cukrowska (2022)	review article	Not applicable	Breath tests are commonly used to diagnose SIBO but have limitations in accuracy and reliability.

Sundi et al. 2018	Prospective observational study	39 patients with suspected SIBO. Participants underwent a glucose-based hydrogen and methane breath test. Within 4 weeks before or after the breath test, they also underwent esophagogastroduodenoscopy (EGD) with duodenal aspirate collection for culture	study concludes that glucose-based hydrogen/methane breath tests show low sensitivity and moderate specificity for diagnosing SIBO using duodenal aspirate culture as reference