BMJ Open Gastroenterology

Characteristics of oxygen concentration and the role of correction factor in real-time GI breath test

Siu Man Lee, Imogen H E Falconer, Trudi Madden, Peter O Laidler

To cite: Lee SM, Falconer IHE, Madden T, *et al*. Characteristics of oxygen concentration and the role of correction factor in real-time GI breath test. *BMJ Open Gastro* 2021;**8**:e000640. doi:10.1136/bmjgast-2021-000640

Received 2 March 2021
Accepted 5 May 2021

Check for updates

© Author(s) (or their employer(s)) 2021. Re-use permitted under CC BY-NC. No commercial re-use. See rights and permissions. Published by BMJ.

Medical Physics & Bioengineering, Royal United Hospitals Bath NHS Foundation Trust, Bath, UK

Correspondence to
Dr Siu Man Lee;
siuman.lee@nhs.net

ABSTRACT

Objective A high quality end-expiratory breath sample is required for a reliable gastrointestinal breath test result. Oxygen (O_2) concentration in the breath sample can be used as a quality marker. This study investigated the characteristics of O_2 concentration in the breath sample and the impact of using a correction factor in real-time breath measurement.

Design This study includes two separate groups of patient data. Part 1 of the study analysed the patient's ability to deliver end-expiratory breath samples over a 2-year period (n=564). Part 2 of the study analysed a separate group of patients (n=47) with additional data to investigate the O_2 characteristics and the role of correction factor in breath test.

Results The results indicated 95.4% of 564 patients were able to achieve an O_2 concentration below 14% in their end-expiratory breath. Part 2 of the study revealed that the distribution of O_2 concentration was between 9.5% and 16.2%. Applying a correction factor to predict the end-expiratory H_2 and CH_4 values led to an average measurement error of −36.4% and −12.8%, respectively.

Conclusion The majority of patients are able to deliver a high quality end-expiratory breath sample, regardless of age or gender. The correction factor algorithm is unreliable when predicting the end-expiratory result at 15% O_2 and it would have resulted in false negative result for 50% of the positive cases in this study. It has also indicated that the continuous O_2 measurement is essential to ensure breath sample quality by preventing secondary breathing during real-time breath collection.

Summary box

What is already known about this subject?

- H_2/CH_4 breath test is useful for diagnosing small intestinal bacterial overgrowth (SIBO) or carbohydrate malabsorption but its accuracy can suffer from a range of uncertainties when the breath measurement is collected. Oxygen or carbon dioxide is now recommended as a breath sample quality factor.

What are the new findings?

- This study showed that the majority of patients are able to provide good quality end-expiratory breath samples (below 14% oxygen), regardless of age or gender. Less than 5% of combined H_2/CH_4 breath test cases over a period of two years collected sub-optimal breath samples which potentially required correction factor (CF) to predict the end-expiratory H_2 and CH_4. A CF is often applied to overcome a poor quality breath sample or to reduce breath sampling time. However, the role of CF is relatively insignificant in real-time measurement due to the high compliance rate. Furthermore, there is an indication if the end-expiratory breath measurements are not obtained, it can lead to false negative result. Oxygen concentration measurement is an essential quality indicator during breath sample acquisition.

How might it impact on clinical practice in the foreseeable future?

- H_2/CH_4 breath test is useful for diagnosing SIBO or carbohydrate malabsorption but its accuracy can suffer from a range of uncertainties when the breath measurement is collected. Oxygen or carbon dioxide is now recommended as a breath sample quality factor.

INTRODUCTION

Breath test has been widely used as a diagnostic tool to identify conditions related to the gastrointestinal (GI) tract. It is a non-invasive, low cost and functional diagnostic test. Depending on the type of carbohydrate administered during the test, it can provide useful information to assist diagnosis of conditions like lactose maldigestion, using lactose, and small intestinal bacterial overgrowth, using glucose or lactulose. The bacterial colonies in the digestive tract metabolise the carbohydrate and produce hydrogen (H_2) or methane (CH_4). These trace gases are absorbed in the intestine, returned to the lungs and equilibrated with air in the alveoli. The concentration of these trace gases can then be detected in the breath.[1–3]

However, although GI breath test is simple and well tolerated by patients, there are a number of uncertainties within the test result, mainly related to the quality of the breath samples collected, as well as the patient preparation procedures. Such uncertainties can adversely affect the accuracy of the result. There are criticisms among clinicians who do not consider hydrogen breath test (HBT) as

a reliable diagnostic test.[4 5] Significant effort has been spent on standardising and refining the protocol in order to make the test more reliable, such as the Rome Consensus and North American Consensus.[6 7] A common understanding in the test protocol is that alveolar air or end-expiratory breath is critical to the accuracy of the test result.[2 8–12] In addition, it is well-known that CH_4 can be produced instead of H_2 when the patient possesses methanogenic bacteria that converts H_2 to CH_4. It has been estimated that between 5% and 15% of the population is affected.[3]

Traditionally, only H_2 concentration is measured. This may be due to the availability of a cost-effective detection system with precision down to part-per-million (ppm). More modern breath analysers are now commercially available and they are able to concurrently measure both H_2 and CH_4. This equipment often includes measurement of oxygen (O_2) or carbon dioxide (CO_2) as a quality indicator of the breath sample. The rationale that defined the CO_2 concentration of an end-tidal breath as 5% was published in the 70s.[13] This value was widely adopted in subsequent research in breath tests. O_2 concentration of alveolar air was approximated as 14% but it was estimated mathematically using the alveolar gas equation.[14] This equation has been widely used in studies on sustainable breathing, such as safety limit for hypoxemia.[15] However, the application of the alveolar gas equation can be limited by the conditions with which the equation was derived. It may not be applicable to the single and maximum exhalation in GI breath test conditions.

The main technologies employed in the breath analysers are gas chromatography, electrochemical sensing and optoelectronic sensing. There are a range of different types of analyser designs commercially available. However, they can be categorised into either point-of-care (POC) systems or laboratory systems. POC analysers can take either real-time measurement or can use a collection bag to collect breath sample from patients. Real-time measurement collects a breath sample and concurrently analyses the trace H_2/CH_4 concentration, so no sample storage is required. It avoids volume normalisation, sample contamination and storage issues which can affect its accuracy. Laboratory-based analysers require breath samples to be collected in a vessel and the batch of breath samples will be analysed at the same time.

This study aims to investigate the characteristics of O_2 concentration in breath samples taken in combined H_2/CH_4 breath test (CBT), in relation to the end-expiratory H_2/CH_4 concentration. We will analyse the relationship between patient's demographics and O_2 concentrations in their breath samples. The impact of applying correction factor (CF) to predict the actual H_2/CH_4 values and the role of CF in real-time CBT measurement will also be evaluated.

METHODS

The clinical data in this study were obtained from the service audit data of CBT results at the Royal United Hospitals (RUH) Bath NHS Foundation Trust, UK. Anonymised breath test data are taken regularly for audit purposes. This service data aims to evaluate the value of CBT against H_2 only BT. The data include the test date, patient demographics, actual H_2 measurement, actual CH_4 measurement and the suggested CF.

The patients had followed the standard preparation protocol included a fasting period of 12 hours prior to the test as described in the North American Consensus.[6]

Breath samples were collected in real-time measurement. In order to obtain an end-expiratory breath sample, every patient undergoing a CBT test followed a strict instruction during each breath sample collection. Patients were asked to breathe in normally; hold their breath for 5 s and breathe out completely. When the patient was breathing into the analyser, the flow rate was strictly monitored and maintained at optimum level as specified by the manufacturer. There was no noticeable leakage around the mouth piece and nose while the patients were encouraged to breathe out completely. A breath sample was taken every 20 min, and the H_2, CH_4 and O_2 level, as well as the CF from the breath analyser were recorded. Typically, there were five samples collected for glucose breath test and six samples collected for a lactose mal-digestion investigation.

CBT was performed by the GastroCH$_4$ECK Gastrolyzer (V.1) (software version: V.11.0), manufactured by Bedfont Scientific, UK. This instrument provides continuous real-time measurement of H_2, CH_4 and O_2 concentration, in ppm, during an episode of breath sample collection. It also provides a ratiometric CF according to the end-expiratory O_2 (EEO_2). Typically in a breath collection episode, the H_2 and CH_4 concentration will rise from zero; while the O_2 concentration will drop from the atmospheric concentration (20.9%) to EEO_2 which is typically below 14%. The manufacturer regards the ideal EEO_2 as 13.9% (<14%).[16] The EEO_2 value is used to produce the ratiometric CF. The CF is applied to the H_2 and CH_4 measurement and attempts to compensate for the non-end-expiratory breath sample (ie, EEO_2 above 14%). The predicted H_2 (CFH_2) and predicted CH_4 ($CFCH_4$) values are expressed as the product of the actual H_2/CH_4 measurement and the CF. When the EEO_2 has not yet reached the manufacturer specified compliant level during a breath collection episode, a predicted value will be calculated. If EEO_2 is below 14%, CF will be equal to 1 so $CFH_2/CFCH_4$ remains the same as the actual H_2/CH_4 measured. The maximum value of the CF is 2.33 which corresponds to 18% O_2 in the breath sample. If the O_2 concentration in a breath sample is above 18%, it is usually regarded as inadequate and should not be used in the analysis.

Part 1: ability to deliver end-expiratory breath sample

There were 1344 CBT cases carried out since the introduction of the CBT service in 2015. The desirable sample size from the total number of cases, estimated using Cochran's Formula with a 95% confidence level, is 384

cases. A sample of 564 patients which is the total cases carried out at the RUH for 2 years, without any exclusion, was used in this study. The analysis has taken into account the patient demographics, EEO_2 and the end-expiratory H_2 (EEH_2) and end-expiratory CH_4 ($EECH_4$) concentrations.

Part 2: O_2 concentration in breath samples and evaluation of CF predicted values

Followed by a recent addition to the service data which records extra H_2 and CH_4 readings when O_2 was at 15%, it enabled an evaluation on the impact of applying CF predicted values in CBT. It has been suggested that an appropriate sample size for a proof-of-concept study is between 24 and 36 cases.[17 18] A sample of further 47 patients who generated 293 samples were studied in greater detail. This dataset was taken continuously over a period of 12 weeks, without exclusion.

For both parts of the study, the proportion of patients who were able to comply EEO_2 below 14% was studied with their demographics. When the datasets were expressed as a categorical variable, the compliance by gender was analysed using a χ^2 test with MS Excel 2016.

As part 2 of this study consists of additional data, the EEO_2 distribution was tested for its normality using Shapiro-Wilks test which is suitable for smaller sample studies, using the statistical computing software R, V.4.0.5. A subsequent t-test was carried out to assess the mean EEO_2 by gender, also using R. Statistical significance was assessed at the 5% level in this study. The H_2/CH_4 errors between end-expiratory measurement (EEH_2 and $EECH_4$) and the predicted values (CFH_2 and $CFCH_4$) using CF were assessed. The effect of CF to the diagnostic results was also evaluated by comparing the CF predicted diagnostic indication with the actual results using end-expiratory measurements. The CBT results were analysed according to the North American Consensus.[6]

RESULTS

In part 1 of this study, the results indicated that an average of 95.4% of the 564 patients who had undergone the CBT achieved EEO_2 below 14% (table 1). The compliance between men and women is 96.3% and 94.9%, respectively. Applying a χ^2 test on the level of compliance by gender, there is no significant difference in the level of compliance between men and women (p=0.44).

The result showed that none of the age groups between 11 and 80 years old had any difficulty delivering EEO_2 below 14% in their end-expiratory breath samples (table 1). The 81–90 years old group has a reduction in delivering EEO_2 below 14% but still achieves a 73.3% success rate. However, this may be due to the smaller sample in this group.

In part 2 of this study, an enhanced dataset with a further 47 patients and a total of 293 end-expiratory breath samples were analysed, as shown in table 2. The overall compliance rate is 88.1% and it is slightly lower than the 95.4% average in part 1 of this study. The breakdown between men and women is 96.6% and 82.4%, respectively. However, the difference in the rate of compliance may be due to the smaller sample size. Applying a χ^2 test on the compliance rate by gender, there is no significant difference in the level of compliance between men and women (p=0.43). This reflected the same indication as part 1 of this study.

The results showed a wide range of EEO_2, from the best sample of 9.5% to the worst at 16.2%. The mean EEO_2 for the total of 293 samples is 12.9%, with an SD of 1.1%. The EEO_2 dataset resembles a normal distribution, as shown in figure 1. By descriptive statistics, the Kurtosis value is −1.8 and it is within the −2.0 threshold for normal distribution; and skewness is 0.35, indicating relatively good symmetry as expected in normal distribution. Applying the Shapiro-Wilks test for normality, the result has met the threshold of normal distribution (p=0.06).

The means of EEO_2 between men and women are 12.6% O_2 (SD: 0.88%) and 13.1% O_2 (SD: 1.18%), respectively.

Table 1 The demographic of the patients who were able to deliver EEO_2 below 14% in part 1 study

				Male		Female	
Age group	No. of patients	No. of patients below 14% O_2	% of patients below 14% O_2	No. of patients below 14% O_2	% of patients below 14% O_2	No. of patients below 14% O_2	% of patients below 14% O_2
11–20	19	17	89.5	7	100.0	10	83.3
21–30	76	73	96.1	24	100.0	49	94.2
31–40	74	74	100.0	26	100.0	48	100.0
41–50	87	83	95.4	31	93.5	52	96.3
51–60	118	112	94.9	28	96.6	84	94.4
61–70	99	97	98.0	36	94.7	61	100.0
71–80	76	71	88.2	27	96.4	44	91.7
81–90	15	11	73.3	5	83.3	6	66.7
Total	564	538	95.4	189	96.3	373	94.9

EEO_2, end-expiratory O_2; O_2, oxygen.

Table 2 The demographic of the patients who were able to deliver EEO_2 below 14% in part 2 study

		Total		Male		Female	
	No. of samples	No. of samples below 14% O_2	% of samples below 14% O_2	No. of samples below 14% O_2	% of samples below 14% O_2	No. of samples below 14% O_2	% of samples below 14% O_2
21–30	46	40	87.0	N/A	N/A	40	87.0
31–40	41	24	58.5	5	71.4	19	55.9
41–50	75	73	97.3	38	100.0	35	94.6
51–60	61	57	93.4	50	96.2	7	77.8
61–70	56	52	92.8	12	100.0	40	87.0
71–80	6	4	66.7	N/A	N/A	4	66.7
81–90	8	8	100.0	8	100.0	N/A	N/A
Total	293	258	88.1	113	96.6	145	82.3

EEO_2, end-expiratory O_2; O_2, oxygen.

A t-test was carried out to compare the means of EEO_2 by gender, the result indicates male patients are able to deliver a breath sample with lower O_2 concentration (p=0.0005).

In order to evaluate the efficacy of the CF, the EEH_2 and $EECH_4$ values were compared with the CFH_2 and $CFCH_4$ values. The CFH_2 and $CFCH_4$ were calculated from the real-time H_2 and CH_4 measurement when the O_2 concentration of the breath sample dropped to 15%. CF at 15% O_2 is 1.19. The range of difference recorded was between −30 ppm and 114 ppm.

The percentage of error on the predicted values for H_2 and CH_4 is shown in figure 2A,B, respectively. The results show the predicted values (CFH_2 and $CFCH_4$) often underestimated the actual measurements (EEH_2 and $EECH_4$).

The average error for CFH_2 was −42.7%, while the average error for $CFCH_4$ was 7.8%. However, it was noted that the percentage of error might be skewed when the measurement was at low level, for example, CFH_2=0 and EEH_2=1 will lead to 100% error. As the minimum threshold for positive result in GI breath test is 10 ppm, the average error was adjusted by excluding any EEH_2 below 10 ppm. The adjusted average errors for CFH_2 and $CFCH_4$ were −36.4% and −12.8%, respectively.

When evaluating the impact of applying the CF predicted values in the CBT analysis, there was no false positive CBT result found in the 47 cases in this study. This may be due to the fact that CF predicted values have a trend of underestimating the actual end-expiratory measurements.

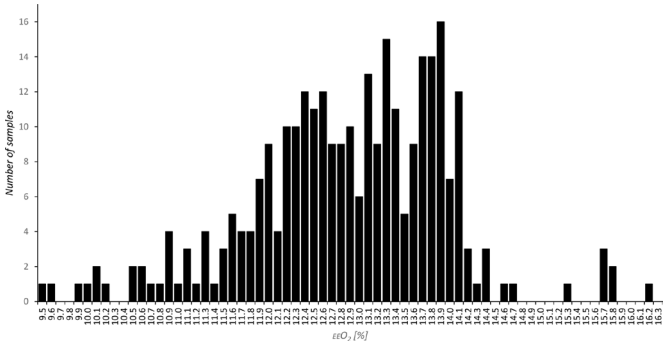


Figure 1 The distribution of 293 end-expiratory breath samples (EEO_2).

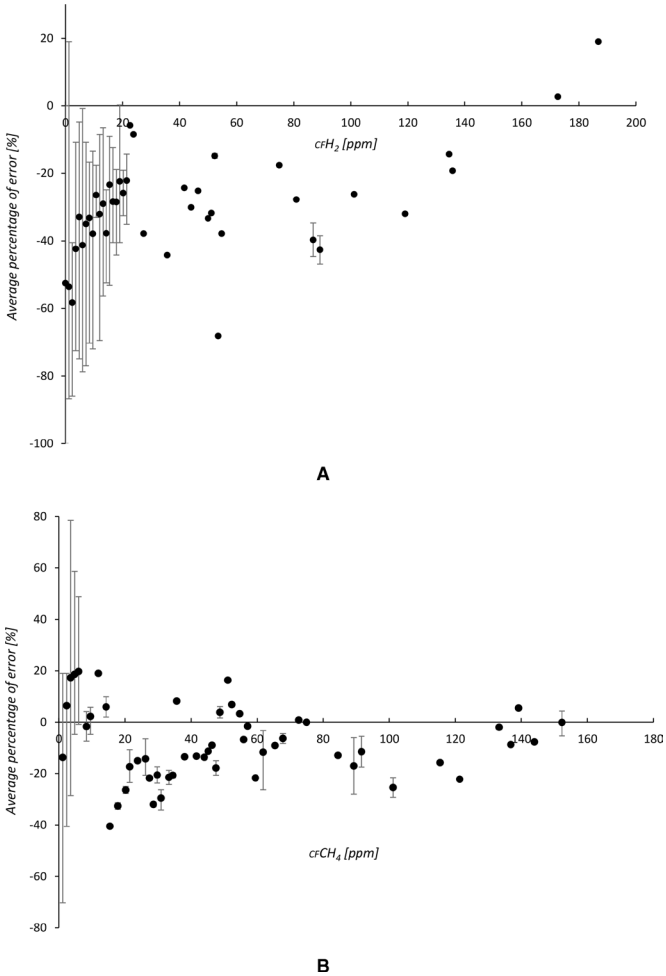
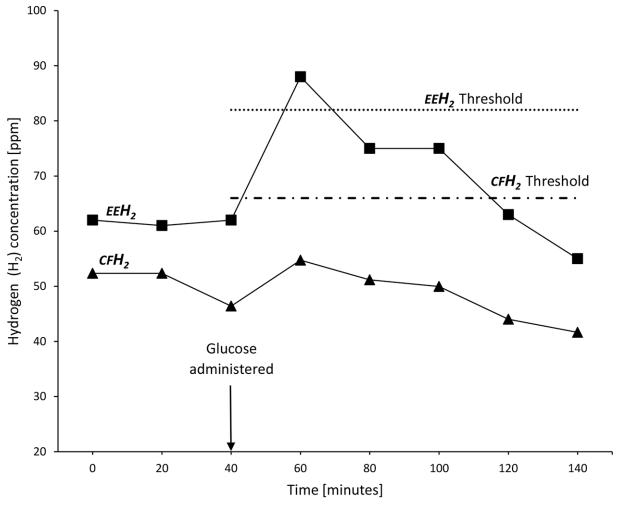


Figure 2 (A) Percentage of error for CFH_2 compared with EEH_2. (B) Percentage of error for $CFCH_4$ compared with $EECH_4$. $CFCH_4$, predicted methane; CFH_2, predicted hydrogen; $EECH_4$, end-expiratory methane; EEH_2, end-expiratory hydrogen.



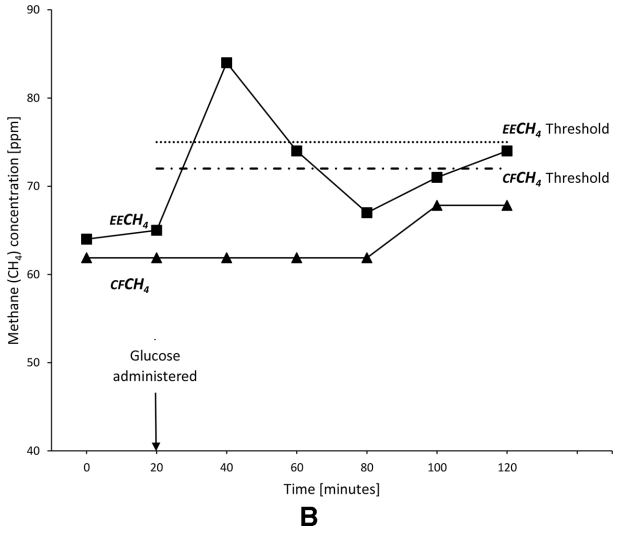


Figure 3 (A) Difference between EEH_2 and CFH_2 values in a GI breath test (glucose). (B) Difference between $EECH_4$ and $CFCH_4$ values in a GI breath test (glucose). $CFCH_4$, predicted methane; CFH_2, predicted hydrogen; $EECH_4$, end-expiratory methane; EEH_2, end-expiratory hydrogen; GI, gastrointestinal.

The proportion of positive cases in the part 2 study is 17.4%. It is similar to the number of average positive cases reported over 3 years at the same centre, using the same breath analyser.[19] Due to the inherently high proportion of negative results, it is useful to evaluate the results separately from the CBT negative group. Otherwise, it may give a false impression of the accuracy from the overall result; for example, equipment with poor sensitivity that is unable to detect any positive result would indicate a pseudo-accuracy of 82.6% in this study.

When CF predicted values were applied in the positive cases, it has revealed that the predicted values (CFH_2 or $CFCH_4$) led to a false negative result in four out of eight positive cases. This indicates an error of 50%. Two such cases are shown in figure 3A (H_2 false negative) and figure 3B (CH_4 false negative) for a CBT (glucose) test. They began with a high but stable baseline reading.

In figure 3A, the EEH_2 peaked within 20 min (after glucose administered at 40 min) and raised above the positive threshold of 20 ppm from baseline. The H_2 peak measurement, EEH_2, was 88 ppm (26 ppm increased from baseline). The predicted value (CFH_2), however, indicated only 55 ppm (9 ppm increased from baseline), resulting a false negative result.

In figure 3B, the $EECH_4$ peaked within 20 min (after glucose administered at 20 min) and raised above the positive threshold of 10 ppm from baseline. The CH_4 peak measurement, $EECH_4$, was 84 ppm (19ppm increased from baseline). The predicted value ($CFCH_4$), however, indicated 66 ppm (0 ppm increased from baseline), resulting a false negative result.

DISCUSSION

The results indicated that the majority of patients who have undergone CBT with real-time measurement are able to deliver an end-expiratory breath sample with oxygen concentration (EEO_2) better than the recommended 14%. The compliance level did not appear to be affected by the patients' age or gender. Although there is an indication that the age group over 80 years old may have lower rate of compliance, this group consists of a smaller number of samples which may account for this reduction.

The analysis indicated that EEO_2 of the breath samples was not constant. In fact, it varied by a large degree, ranging from 9.5% to 16.2%. Nevertheless, the manufacturer has predefined the ideal or target EEO_2 at 14%. The CF algorithm built into the GastroCH$_4$ECK is similar to the method used in the CO_2 CF employed in gas chromatography breath analysers.[13] The published data indicated the end-tidal breath should ideally contain 5% CO_2 and suggested a specific CF algorithm which compensates for a range of CO_2 levels between 2% and 7%. The CF from the GastroCH$_4$ECK only compensates O_2 level when the breath sample has an EEO_2 level above 14%.

This study indicated that setting EEO_2 at 14% is likely to be too high, as the mean EEO_2 in this study was 12.9%. The rate of change in H_2/CH_4 values during an episode of breath sample collection will also significantly affect the validity of the CF and it expectedly varied to a large degree. Therefore, due to the highly inconsistent EEO_2 and the varying nature of the H_2/CH_4 in an episode of breath sample collection, the resulting predicted $CFH_2/CFCH_4$ value based on EEO_2 above 14% is often unreliable. It is indeed possible to produce a false negative result if $CFH_2/CFCH_4$ is used.

A major limiting factor to part 2 of this study is the small sample size. However, the result demonstrated the possibility of false negative result using the predicted $CFH_2/CFCH_4$ values in a short and random period of time. Further study is required to identify the prevalence of the false negative results.

For CBT with real-time measurement, quality of the end-expiratory breath is critical to the accuracy of the H_2 and CH_4 level. Although it is not the only factor, it must not be underestimated. Fortunately, clinicians are unlikely to use

CBT result in isolation. Patient's diet, medical history, comorbidity, medications (current or avoided), symptoms as well as the recorded symptoms during CBT will contribute to the final diagnosis. Nevertheless, a false negative indication from CBT test adds uncertainty and unnecessary complication to the diagnostic process. When there is an operator who can ensure the high quality end-expiratory breath sample is collected, CF may be redundant in majority of real-time GI breath measurement. Instead, CF should only be used as a last resort when dealing with patients who are genuinely unable to deliver high quality end-expiratory breath samples.

On the other hand, O_2 measurement is essential as a quality indicator for the breath sample. The continuous real-time trace of O_2 concentration on the GastroCH$_4$ECK Gastrolyzer (V.1) prevents the patient from accidentally breathing in during an episode of breath sample collection. Should secondary breathing be detected, the collection process can be aborted and restarted. This O_2 sensing feature ensures the quality of the breath sample by significantly reducing the uncertainties that arise from the sample collection stage and further exploiting the benefits of real-time breath measurement.

It may be important to note this study has focused on real-time breath measurement. Characteristics of O_2 with a bag collection system are likely to be different and may require further study. In addition, the breath holding time has a significant effect to the mixture of gases in the end-expiratory breath.[20] Hence, breath sampling procedure must be designed to avoid the confusion between genuine maximum exhalation and the sensation of dyspnoea due to excessive breath hold.

The majority of patients in this study were able to deliver breath samples below 14% O_2. O_2 concentration of an end-expiratory breath sample is largely unpredictable. The CF algorithm built-in to the GastroCH$_4$ECK proved to be unreliable and would have led to a false negative result of 50% of the positive cases in this study. Hence, with such uncertainty for the predicted H_2/CH_4 values, it is essential that the actual end-expiratory breath is collected and so CF may be redundant in real-time CBT. On the contrary, the on-screen continuous O_2 trace is highly valuable to ensure the quality of the end-expiratory breath sample collection. The findings in this study may be used to guide future development of GI breath analysers and may help to reduce uncertainties in the GI breath test result. It may also help to refine the breath collection protocol and further improve the accuracy of the test result.

Acknowledgements The authors would like to thank Rosie Nightingale, Lisa Hirst and Kate Gearon at the Academy Library, Royal United Hospitals Bath NHS Foundation Trust, for their assistance to carry out an initial literature search and obtaining the full text of a number of references in this study.

Contributors SML designed, planned, literature review, processing data, supervised and written up this study. IHEF carried out literature review, performed breath collections and processing data. POL and TM performed breath collections and carried out literature review.

Funding The authors have not declared a specific grant for this research from any funding agency in the public, commercial or not-for-profit sectors.

Competing interests None declared.

Patient consent for publication Not required.

Ethics approval This study is categorised as a Service Evaluation under the National Research Ethics Service (NRES) guideline, Heath Research Authority, UK. Therefore, approval from NRES is not required. Nevertheless, approval of this study is granted from the RUH Research Department.

Provenance and peer review Not commissioned; externally peer reviewed.

Data availability statement Data are available in a public, open access repository. Study data can be found in the Royal United Hospitals Bath website: https://www.ruh.nhs.uk/MPB/documents/Service_data_2020b.pdf.

Open access This is an open access article distributed in accordance with the Creative Commons Attribution Non Commercial (CC BY-NC 4.0) license, which permits others to distribute, remix, adapt, build upon this work non-commercially, and license their derivative works on different terms, provided the original work is properly cited, appropriate credit is given, any changes made indicated, and the use is non-commercial. See: http://creativecommons.org/licenses/by-nc/4.0/.

ORCID iD

Siu Man Lee http://orcid.org/0000-0002-2553-0986

REFERENCES

1 Braden B. Methods and functions: breath tests. *Best Pract Res Clin Gastroenterol* 2009;23:337–52.
2 Ghoshal UC. How to interpret hydrogen breath tests. *J Neurogastroenterol Motil* 2011;17:312–7.
3 de Lacy Costello BPJ, Ledochowski M, Ratcliffe NM. The importance of methane breath testing: a review. *J Breath Res* 2013;7:024001.
4 Saad RJ, Chey WD. Breath tests for gastrointestinal disease: the real deal or just a lot of hot air? *Gastroenterology* 2007;133:1763–6.
5 Simrén M, Stotzer P-O. Use and abuse of hydrogen breath tests. *Gut* 2006;55:297–303.
6 Rezaie A, Buresi M, Lembo A, *et al*. Hydrogen and Methane-Based breath testing in gastrointestinal disorders: the North American consensus. *Am J Gastroenterol* 2017;112:775–84.
7 Gasbarrini A, Corazza GR, Gasbarrini G, *et al*. Methodology and indications of H2-breath testing in gastrointestinal diseases: the Rome consensus conference. *Aliment Pharmacol Ther* 2009;29 Suppl 1:1–49.
8 Robb TA, Davidson GP. Advances in breath hydrogen quantitation in paediatrics: sample collection and normalization to constant oxygen and nitrogen levels. *Clinica Chimica Acta* 1981;111:281–5.
9 Hamilton LH. *Breath Tests & Gastroenterology*. Second Edition, 1998.
10 Lee WS, Davidson GP, Moore DJ, *et al*. Analysis of the breath hydrogen test for carbohydrate malabsorption: validation of a pocket-sized breath test analyser. *J Paediatr Child Health* 2000;36:340–2.
11 Anderson JC, Hlastala MP. Breath tests and airway gas exchange. *Pulm Pharmacol Ther* 2007;20:112–7.
12 Fleming SC. Evaluation of a hand-held hydrogen monitor in the diagnosis of intestinal lactase deficiency. *Ann Clin Biochem* 1990;27 (Pt 5:499–500.
13 Niu HC, Schoeller DA, Klein PD. Improved gas chromatographic quantitation of breath hydrogen by normalization to respiratory carbon dioxide. *J Lab Clin Med* 1979;94:755–63.
14 Wolfson MR, Shaffer TH. *Cardiopulmonary physical therapy*. Fourth Edition, 2004.
15 Wagner PD. The physiological basis of pulmonary gas exchange: implications for clinical interpretation of arterial blood gases. *Eur Respir J* 2015;45:227–43.
16 Bedfont Scientific Ltd. *GastroCH4ECK user manual*. UK, 2014.
17 Johanson GA, Brooks GP. Initial scale development: sample size for pilot studies. *Educ Psychol Meas* 2010;70:394–400.
18 Billingham SAM, Whitehead AL, Julious SA. An audit of sample sizes for pilot and feasibility trials being undertaken in the United Kingdom registered in the United Kingdom clinical research network database. *BMC Med Res Methodol* 2013;13:104.
19 Materacki L, Lee SM, Laidler P, *et al*. Is methane testing a useful adjunct to hydrogen breath testing? *BSG Annual Meeting 2018*; 4th – 7thJune, 2018. Liverpool, UK.
20 Levitt MD, Ellis C, Furne J. Influence of method of alveolar air collection on results of breath tests. *Dig Dis Sci* 1998;43:1938–45.