Tricks for interpreting and making a good report on hydrogen and ^{13}C breath tests

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Abstract. Breath tests (BT) represent a valid and non-invasive diagnostic tool in many gastroenterological disorders. Their wide diffusion is due to the low cost, simplicity and reproducibility and their common indications include diagnosis of carbohydrate malabsorption, Helicobacter pylori infection, small bowel bacterial overgrowth, gastric emptying time and orocaecal transit time.

The review deals with key points on methodology, which would influence the correct interpretation of the test and on a correct report.

While a clear guideline is available for lactose and glucose breath tests, no gold standard is available for Sorbitol, Fructose or other H_2 BTs.

Orocaecal transit time (OCTT) defined as time between assumption of 10 g lactulose and a peak > 10 ppm over the baseline value, is a well-defined breath test. The possible value of lactulose as a diagnostic test for the diagnosis of small bowel bacterial overgrowth is still under debate.

Among ^{13}C breath test, the best and well characterized is represented by the urea breath test. Well-defined protocols are available also for other ^{13}C tests, although a reimbursement for these tests is still not available.

Critical points in breath testing include the patient preparation for test, type of substrate utilized, reading machines, time between when the test is performed and when the test is processed. Another crucial point involves clinical conclusions coming from each test. For example, even if lactulose could be utilized for diagnosing small bowel bacterial overgrowth, this indication should be only secondary to orocaecal transit time, and added into notes, as clinical guidelines are still uncertain.

Key words:

Breath test report, H2 breath test, C13 breath test.

Introduction

Breath tests (BT) represent a valid and non-invasive diagnostic tool in many gastroenterological disorders. Hydrogen-breath tests are based on the fact that gases produced by colonic bacterial fermentation diffuses into the blood and are excreted in breath samples, where it can be easily quantified. There is a growing interest in breath tests but many differences persist in the methodology used.

Hydrogen Breath test (HBT) has become an important diagnostic tool in many Gastroenterological conditions, characterized by abdominal pain, discomfort, bloating etc. Its wide diffusion is due to the low cost, simplicity and reproducibility: it represents a valid and non-invasive test to diagnose carbohydrate malabsorption, small bowel bacterial overgrowth, and for the determination of orocaecal transit time.

^{13}C-breath tests use ^{13}C-labelled substrates in order to perform a safe non-invasive evaluation of several metabolic pathways. The turnover of the ^{13}C-substrate throughout the specific explored pathway is assessed by measuring $^{13}CO_2$ in exhaled air. The availability of plenty of ^{13}C-substrates gives these tests a wide range of potential applications, although the routine clinical use of certain substrates may be sometimes affected by their high costs. The most used ^{13}C breath test is the one for diagnosing *Helicobacter pylori* infection.

In this paper we summarize key element on assessing and interpreting H_2 and major ^{13}C breath tests, and we will provide a guide for an accurate breath test report.

Tricks on hydrogen and methane excretion

Hydrogen isn't produced in healthy humans fasting and at rest, but it is only generated during

anaerobic metabolism, which occurs, for example, during bacterial fermentation. Anaerobic bacteria metabolize sugar molecules, producing CO_2, short-chain fatty acid (SCFA) and Hydrogen[1]. Unabsorbed carbohydrates are so fermented by colonic bacteria and the hydrogen, like other gases produced within the intestine, is absorbed through the intestinal wall, passes in the blood circulation, reaches the lungs, and finally it is released and exhaled in the breath samples. The amount of hydrogen produced by carbohydrate fermentation varies according to the individual's microbiota, as hydrogen is also used by bacteria for other metabolic processes, including conversion to methane.

In fasting state, normal hydrogen baseline values are reported to be 7 ± 5 ppm[2], if it results too high (>10-16 ppm), breath test shouldn't be performed[3]. The ingestion of rice flour and meat is not associated with a detectable increase in breath hydrogen excretion, so they are eaten the evening before the test[4,5]. In response to the test, some people produce huge amount of hydrogen in response to sugars, some produce lower amounts, and about 15% produce apparently none (designated 'non-hydrogen producers'), as the expired hydrogen throughout the test dose not reach the limit detection rate. In some case of apparent hydrogen non production, methane determination could represent a valid alternative, although insufficient data are presented in this topic. In patients that do not produce methane neither hydrogen (a condition present in about 5% of the population), there is no indication of performing breath tests[6] and no indication exists on whether increase test time would exert any role. This condition is mainly due to a different intestinal flora composition, which in turn is influenced by many factors including age, susceptibility to infections, dietary habits, immunological factors, intraluminal pH, interaction among components of intestinal flora and fermentable substrates, recent antibiotic therapy, use of laxatives (preparation for colonoscopy), or for the predominance of methane producing bacteria in the colon[7-9]. Some groups try to overcome this problem by testing initially lactulose: this synthetic disaccharide cannot be absorbed in humans and is, therefore, always fermented producing hydrogen, except for the "non-H_2-producer". It is, therefore, conceivable that a slow gastrointestinal transit of the substrate may be responsible for false negative results. It was shown that the prolongation of measurement from 2 to 4 hours induce a significant reduction of the prevalence of "H_2 non producer"[8].

Tricks on sampling H_2 from alveolar expirates for H_2 breath testing

We can measure hydrogen concentrations in end-expiratory breath samples using gas chromatography or electrochemical cells. Stationary dedicated gas chromatographs represent the gold standard for hydrogen determinations in breath, as they were previously validated in comparison with non-dedicated instruments in terms of linearity and reproducibility of results[10-12]. Stationary dedicated gas-chromatographs could be made with solid state sensors, and they represent the gold standard for hydrogen determination, or with electrochemical sensor, which also show a good accuracy, although they will undergo to a natural "drift" over time.

Gas measurement must be performed in alveolar air, avoiding the interference represented by respiratory dead space air. The three systems are available to collect alveolar air: the modified Haldan-Priestley tube, the Y-Piece device and the two bag-system[13]. The most efficient method to remove dead space air and guarantee complete respiratory exchanges is to inhale maximally, hold the inhalation for 15 s and expire into the bag[14]. It could be also considered as a marker of correct sampling the concentration of CO_2, which levels in alveolar air are stable around 5-5.5%:, it is possible to reduce variability due to the death space, measuring CO_2 in breath sample and correcting for the ideal value of CO_2[15].

Breath samples are currently stored in plastic syringes, an inexpensive method allowing the analysis of gases with no further handling. Because of the high diffusion capacity of hydrogen, samples should be analyzed as soon as possible, avoiding storage longer than 12 h; however, simple refrigeration of plastic syringes is sufficient to ensure the stability of hydrogen concentrations for a long time. At room temperature, after 5 days, the hydrogen concentration is reduced up to 30%, while at –20 °C the reduction is equal to 5% and only 7% after 15 days. Moreover, at –20 °C no hydrogen loss is detectable for 2 days[13]. Hydrogen concentration in breath samples must, therefore, be determined within 6 h of sampling.

Tricks on Lactose breath test interpretation

Lactose breath test shows good sensitivity (mean value of 77.5%) and excellent specificity (mean value of 97.6%) for lactose intolerance in patients which display or not symptoms[16, 17]. False negative tests, however, can be due to "hydrogen non-producers"; false positive breath tests are less frequent and are mainly secondary to of small

bowel bacterial overgrowth. LBT was considered positive when the peak of hydrogen was 20 ppm over the baseline. In children some laboratories have used > 10 ppm as cutoff value, but a rise of > 20 ppm over baseline correlates better with symptom development[18].

If the value rises more than 10 ppm but less than 20 ppm above the base value, the test could be considered as "borderline"[19]. In this condition, it must be verified when this peak occurs: if the hydrogen value seems to increase at the end of the test, should be considered the possibility of a prolonged test, to augment the sensibility, reducing "false negative" due to a possible slow intestinal transit time.

Tricks on other carbohydrates breath test interpretation

Sorbitol is a sugar alcohol widespread in plants, particularly in fruits and juices, which is only partially absorbed. Sorbitol BT is effective in detecting small bowel damage, especially in celiac disease[20]. Tursi et al published many papers on Sorbitol Breath test (5 g) for the diagnosis of subclinical or silent celiac disease, and for the asses histological recovery after gluten-free diet[21,22].

Fructose, widely used as a sweetener in different foods, is a monosaccharide, which is absorbed by a specific receptor for fructose (GlUT5 fructose transporter mediated facilitative diffusion), which occurs along the entire small bowel and it is slow; and by a second receptor which is rapid and efficient, but occurs only together with glucose[6]. The mean daily fructose intake is about 16 g per day, but eating enriched food and drinks can achieve up to 100 g per day[23,24].

The clinical importance of sorbitol and fructose malabsorption remains to be evaluated, so BT should not be recommended in clinical practice in both adults and children, according to current guidelines[14].

Tricks on small intestinal bacterial overgrowth interpretation

Small intestinal bacterial overgrowth (SIBO) is usually defined as a growth of $>10^5$ colony forming units (CFU)/ml in fluid aspirated from the jejunum[14], which could be associated to abdominal pain, bloating, diarrhea and/or signs of malabsorption, similar to those observed in irritable bowel syndrome (IBS)[14,25,26]. Glucose Breath test (GBT) is currently used for the diagnosis of SIBO. Glucose is rapidly absorbed in the proximal small bowel and usually does not reach the

colon, so it is a suitable substrate to detect proximal small bowel overgrowth. A rise in H_2, after the assumption of the substrate, means that glucose meets bacteria in the small bowel, before its absorption. Because of its early absorption, GBT may not able to diagnose SIBO of the distal small intestine (ileum). Sensitivity and specificity are 62.5% and 77.8 %, respectively compared to the gold standard (jejunal culture)[27].

Lactulose has also been used for diagnosis SIBO. Many criteria have been established to try to detect SIBO using Lactulose: some authors suggested that a rise in breath H_2 of 20 ppm above base levels within 90 minutes after ingestion of lactulose should be considered as diagnostic of SIBO[26], but this criterion has not been validated. Actually the most diffuse criterion is the presence of a hydrogen "double peak", the earlier representing presence of bacteria fermenting in the small bowel, the second and bigger one linked with the flora usually colonizing the colon. However, it is difficult to distinguish between an early H_2 peak caused by SIBO and a fast transit; thus, the non-standardized criteria, and lower diagnostic accuracy (55 versus 71 of Glucose[14]) makes lactulose breath test not indicated to diagnostic for SIBO.

Tricks on orocaecal transit time interpretation

Lactulose is a manufactured disaccharide, consisting of galactose and fructose, which is not metabolized by the human organism, producing always fermentation in the colon. Thus, the time from ingestion of lactulose to the hydrogen increase in breath samples reflects the orocaecal transit time. An increase >20 ppm compared to baseline is considered significant in diagnostic routine[28]. The orocaecal transit time in healthy subjects ranges between 40 and 170 min for a lactulose meal (192-232 min for a solid meal). A delayed orocaecal transit has been reported in constipation, alcoholism, depression, diabetes mellitus, IBS, pregnancy, obesity, cholecystectomy, cirrhosis, scleroderma, acromegaly and dyspepsia. Meanwhile, a fast transit in partial gastrectomy, post vagotomy diarrhea, hyperthyroidism, IBS, alcoholics. LHBT is also useful to demonstrate drug effects on orocaecal transit.

Tricks for sampling and measuring $^{13}C/^{12}C$ ratio

^{13}C stable isotope is used in breath testing to label specific substrates. The ^{13}C-labelled tracer probe, once ingested, is cleaved in the gastroin-

testinal lumen, transported and degraded following the same metabolic pathways of its native isoform. The final result is the excretion of $^{13}CO_2$ through the alveolocapillary membrane into the exhaled air, in which $^{13}CO_2$ amount is measured.

Since ^{13}C stable isotope is commonly found in nature – accounting for 1.11% of all carbon atoms – and its amount may vary among individuals, it is necessary to refer results to a baseline value in order to get accurate results. Thus, total CO_2 production at baseline must be measured or estimated in each patient. Usually it is calculated with 300 mmol CO_2/m^2 body surface area/h[29]. Moreover, factors influencing the endogenous CO_2 production (e.g. food ingestion, physical activity, respiratory diseases, thyroid dysfunctions, fever) should be considered.

Breath samples are collected at baseline and for a variable number of times and at different time intervals depending on the type of ^{13}C breath test performed.

The tracer could be detected and expressed as a static variable, e.g. the delta over baseline [DOB: $\delta_{sample} - \delta_{basal\ value\ before\ tracer\ application}$] in urea breath test, or as a dynamic one, in tests where $^{13}C/^{12}C$ is expressed as recovery rate per hour or percent dose recovery and more than 2 dosages are necessary and special algorithms are used. Moreover, in order to get accurate results, measurement or estimation of basal ^{13}C value is recommended. ^{13}C recovery is never complete as a substantial amount of tracer is retained in the carbon pool of the body. Thus, ^{13}C breath test analysis is a semi-quantitative diagnostic tool.

Breath samples are analyzed by high-resolution mass spectrometers, which are able to measure the slight mass difference of one neutron between ^{13}C-labelled carbon dioxide and the naturally most common carbon dioxide with the carbon isotope ^{12}C, on the base of different light absorbance between the two isotopes. The precision of high-resolution mass spectrometers is very high, so very low isotopic quantity can be detected, permitting the use of small samples of exhaled air. On the other hand, mass spectrometers are highly expensive, they have relatively long analysis time and they require well-trained personnel for their use. The introduction of infrared technologies (non-dispersive isotope selected infrared spectrometers, NDIRS), based on the use of photoacustic detectors, made this goal cheaper with adequate accuracy. Operating and handling of NDIRS is easy, even for non-experienced users. This enables not only the

performance, but also the diffusion of ^{13}C-breath tests in primary care settings. Generally, there is a good agreement between mass spectroscopy and infrared based technology, for $^{13}C/^{12}C$ ratio estimate, particularly for Urea breath test[30,31]. The agreement of infrared technologies on dynamic ^{13}C breath tests is not well addressed and some concerns remains on reproducibility and accuracy.

Tricks on Helicobacter pylori breath test

Helicobacter pylori (H. pylori) is a common Gram-negative pathogen bacterium colonizing the gastric mucosal environment in about 50% individuals. ^{13}C-urea breath test [UBT] is considered the gold standard for non-invasive diagnosis of *H. pylori* infection, because of its accuracy and its easy execution[32]. Urea hydrolysis can be generally entirely accounted to the presence of *H. pylori,* since it is the most common gastric pathogen expressing urease[33]. Several studies reported the high diagnostic reliability of UBT, that reaches values >95% both for sensitivity and specificity prior and after eradication therapy[34-36].

^{13}C-UBT protocol underwent multiple modifications through years. Nowadays, 75 mg ^{13}C-urea in 200 ml water with the adjunction of 1-4 g citric acid[37] represents the most commonly accepted protocol. Adding citric acid *enhances H. pylori* urea hydrolisation by both increasing intra-citoplasmic urea availability and enhancing urease activity[38]. Hence it allows to spare urea and to contain costs. Breath samples are collected through a straw in a glass phial prior to the substrate administration and after 20 or 30 minutes.

The coexistence of some factors might alter UBT results affecting its accuracy. In particular, the assumption of proton pump inhibitors reduces UBT sensitivity in 31%[39] since it reduces the bacterial load especially in the antrum[32,40,41].

False-positive tests may be provided by urease expressed by oropharyngeal flora. Mouth washing or administering the substrate with a straw or in pills may be helpful strategies to avoid this disadvantage.

Although performing the test in a non-fasting condition has been associated with a higher rate of both false-negative and false-positive results[42], several other studies found only small differences between DOB values in fasting and non-fasting status[43,44] or even no differences[45]. However, it seems prudent to perform the test in a fasting status until this issue is better clarified.

The choice of a reliable cut-off to define the positivity of the test is another incompletely addressed question. A unique DOB cut-off is not definable because it has to be adapted to different factors, such as the test meal, doses and types of urea, or the pre-/post-treatment setting in which the test is employed[40]. Although, because positive and negative UBT results tend to cluster outside of the DOB range between 1.8 and 5‰, a change in cut-off value within this range would be expected to have little effect on clinical accuracy of the test[41].

Tricks on ^{13}C octanoic acid breath for studying gastric emptying time

Gastric emptying is a complex process requiring variable durations, depending on quantity and quality of food ingested, and on the presence of certain pathologies. In a clinical setting, a gastric emptying study might be particularly useful to assess how gastric physiology varies depending on age and diet, to study functional dyspepsia, diabetic gastroparesis and to evaluate the effect of prokynetic therapy. Radioscintigraphy represents the gold standard for gastric emptying studies.

Breath testing for gastric emptying assessing is justified by the assumption that the time limiting step for $^{13}CO_2$ to appear in the exhalation is represented by the passage of the test meal into duodenum, whilst the subsequent processes occur negligibly fast or at a constant rate[29].

^{13}C-octanoate and ^{13}C-acetate are the most used substrate in breath testing, respectively used to label solid and liquid components of test meals.

In the original description by Ghoos et al, ^{13}C-octanoic acid is mixed together with egg yolk and baked. The frying process anchors the molecule in the solid phase of the egg yolk[46]. After its administration, when the meal has passed through the pylorus, the ^{13}C-octanoic acid is rapidly absorbed in the duodenum and jejunum and is oxidised to ^{13}C-labelled carbon dioxide, then eliminated by lungs. Collecting breath samples for more than 4 h gives reproducible $^{13}CO_2$-kinetics in breath, which reflects gastric emptying; thus the appearance of $^{13}CO_2$ in breathed air reflects the transit of the test meal from the stomach through the duodenum, where this is digested and absorbed with the final production of ^{13}C-labelled carbon dioxide.

Several parameters are calculated to evaluate gastric emptying and this issue, requiring mathematical formulae, makes breath testing an indirect technique for the study of gastric emptying. Half-emptying gastric time [$t_{1/2}$] indicates the time required to dismiss half of the gastric content; gastric emptying coefficient [GEC] is an index of the gastric emptying rate; lag phase [tlag] is the time occurring for the solid meal to be triturated into <2 mm^3 fragments[46].

Test protocols vary depending on the test meal used. The main variations regard the formulation of test meals, caloric burden, time intervals between sample collections and the total duration of sampling. There is a reasonable agreement on a 4-6 hours-long sampling at time intervals of 15 or 30 minutes[47-50]. Regarding the test meal, the standardisation of the test meals is required but to date it is still lacking. To this purpose, Perri et al. proposed a new standardised test meal in form of a gluten-, lactose-, glucose-free muffin[51].

^{13}C-acetate is used as marker in liquid phase studies because of the hydrophilic character of the molecule[52] and has showed an excellent correlation toward scintigraphy[53]. Liquid phase studies are particularly useful for research purposes, whilst in a clinical setting only certain neurological pathologies affect gastric emptying for the liquids.

Breath tests can be easily performed by children, pregnant women and critically ill patients. Their analysis does not require expert personnel operating in the same place where they are performed, thanks to the easy transportability of the samples. Although, breath-test and scintigraphy results are not identical[46,54]. Different results originate from different approaches in describing the gastric emptying. Radioactive tests study the progressive tracer disappearing from the stomach and are affected by tissue attenuation, angle of detection, radiation decay. ^{13}C-Breath-tests study absorption of ^{13}C-labelled molecules in the small bowel and are delayed pulmonary excretion and partial retention of the tracer amount in the body and is potentially influenced by a delayed pulmonary excretion and partial retention of the tracer amount in the body[29].

On the other hand, simultaneous labelling of solid and liquid phases, although could be done in nuclear medicine, is possible in breath-testing only with the addition of ^{14}C[55], whose clinical use is obsolete because of its radioactivity.

Tricks on ^{13}C mixed triglyceride acid breath exploring exocrine pancreatic function

Except for secretin-pancreozymin test and secretin-enhanced magnetic resonance pancre-

atography [s-MRP] that are both able to reach good levels of sensitivity and specificity, functional studying for the diagnosis of chronic pancreatitis plays a minor role today[56]. Nevertheless, their high costs and invasiveness, makes useful to develop cheaper tests with similar accuracy.

Several substrates have been studied for assessing exocrine pancreatic disorders, e.g. ^{13}C-starch[57], ^{13}C-egg white[58-60] and ^{13}C-peptides[61] demonstrated low sensitivity because of the non-exclusivity of exocrine pancreas to digestion processes of carbohydrates and proteins. Thus, the most suitable breath tests for the study of exocrine pancreatic disorders seem to be those exploring fat maldigestion. This kind of studies reach sufficiently good accuracy even in the study of early or mild disease mainly because lipase is the first enzyme reduced during pancreatic insufficiency; gastric lipase participates only for 15% to intestinal lipolysis; moreover, gastric lipase is not able to adequately compensate the reduced pancreatic lipase activity[62].

The best investigated triglyceride breath test uses 1,3-distearyl, 2-[^{13}C-carboxyl] octanoyl glycerol and is known as ^{13}C-mixed triglyceride breath test[63,64] administered at the doses of 200-300 mg. Breath samples are collected before the test meal and for the subsequent 5 hours at time intervals of 30 minutes[65]. Labeled triglycerides are metabolized in the duodenum under physiological conditions; they are easily absorbed by mucosal wall and metabolized in the liver cells with production of ^{13}C labeled carbon dioxide, excreted by lungs. Low ^{13}C/^{12}C ratio in expired air indicate a not completed digestion of marked substrates due to impaired pancreatic secretion.

Sensitivity and specificity of the mixed triglyceride breath test compared to the stimulated lipase output in the pancreocymin test are 89% and 81%, respectively[64-66].

It is important that the patient follows certain indications prior to the test: food naturally rich in ^{13}C (i.e. corn, pineapple, cane sugar) should be avoided at least from 2 days before the test; a 12 hours fasting is recommended, with the exception of natural water. Moreover, smoking, sleeping or doing physical exercise should be avoided from 30 minutes before the test through the entire duration of the test.

Even if breath tests are valid methods for evaluate pancreatic function, the costs of the substrates, the high time expenditure and the lack of standardization limit the utilization and the diffusion in common clinical settings.

Drawing a good and complete report

The medical report is necessary to reduce variability between tests performed in different centers and to give the opportunity to experienced gastroenterologist to fully interpret results, particularly in the case of tests not fully standardized.

It must include all the methodologies and clinical characteristics to let the test interpretable also by non-specialists.

In addition to patient data, it should be specified the substrate administrated, in term of dosage and concentration, the methods of sample collecting (intervals and duration of the test) and machine used for the measurement.

It is good to indicate some clinical notes, like for instance the clinical indications for requiring the test (ie suspect lactose intolerance, diarrhea, constipation, bloating, etc.).

If necessary, on the basis of the results, gastroenterologist or family doctors could require further exams, to complete the diagnostic process.

In Figure 1 we summarized in a virtual format all informations, which should be clearly written in a good report.

We think that, dealing with H_2 breath testing, the key points for the validity of the test would be the distance between the day of the breath sampling and the day of breath assessment. Type of machines utilized, status of the machines, being the "age" of the machine, a limiting step particularly for gas-chromatography with electrochemical sensor.

Duration of the test, contemporary measurement of CO_2 and/or methane is also another important parameter. Even for ^{13}C breath test, a clear indication of protocol used for the test is crucially important, particularly for non conventional breath tests. Despite H_2 breath testing, samples for ^{13}C breath testing, when kept in proper tubes, are more stable, being ^{13}C a stable isotope. The indication of the machine utilized could be of interest, as mass spectroscopy has the highest accuracy and, usually, highest precision.

Institution, address, clinical indication

Date
(when the test was performed)

Date
(when the test was analyzed)

NAME, SURNAME
IDENTIFICATION NUMBER,
OF BIRTH DATE

TYPE OF TEST,
protocol used, type of substrate and dose, machine utilized

Patients baseline assessment:
(if the patients did a correct preparation for the test, etc)

Clinical notes during excution test

Graph and results

CLINICAL MEANING AND POSSIBLE INTERPRETATION

Clinical suggestion

Figure 1. Schematic representation of a complete breath test report

References

1) LEVITT MD. Volume and composition of human intestinal gas determined by means of an intestinal washout technic. N Engl J Med 1971; 25: 1394-1398.

2) PERMAN JA, MODLER S, BARR RG, ROSENTHAL P. Fasting breath hydrogen concentration: normal values and clinical application. Gastroenterology 1984; 6: 1358-1363.

3) GHOSHAL UC. How to interpret hydrogen breath tests. J Neurogastroenterol Motil 2011; 3: 312-317.

4) ANDERSON IH, LEVINE AS, LEVITT MD. Incomplete absorption of the carbohydrate in all-purpose wheat flour. N Engl J Med 1981; 15: 891-892.

5) LEVITT MD, HIRSH P, FETZER CA, SHEAHAN M, LEVINE AS. H2 excretion after ingestion of complex carbohydrates. Gastroenterology 1987; 2: 383-389.

6) BARRETT JS, GIBSON PR. Fructose and lactose testing. Aust Fam Physician 2012; 5: 293-296.

7) Hao WL, Lee YK. Microflora of the gastrointestinal tract: a review. Methods Mol Biol 2004; 268: 491-502.

8) CORAZZA G, STROCCHI A, SORGE M, BENTAI G, GASBARRINI G. Prevalence and consistency of low breath H2 excretion following lactulose ingestion. Possible implications for the clinical use of the H2 breath test. Dig Dis Sci 1993; 11: 2010-2016.

9) VERNIA P, CAMILLO MD, MARINARO V, CAPRILLI R. Effect of predominant methanogenic flora on the outcome of lactose breath test in irritable bowel syndrome patients. Eur J Clin Nutr 2003; 9: 1116-1119.

10) SOLOMONS NW, VITERI FE, HAMILTON LH. Application of a simple gas chromatographic technique for measuring breath hydrogen. J Lab Clin Med 1977; 5: 856-862.

11) Christman NT, Hamilton LH. A new chromatographic instrument for measuring trace concentrations of breath-hydrogen. J Chromatogr 1982; 2: 259-265.

12) Solomons NW, Hamilton LH, Christman NT, Rothman D. Evaluation of a rapid breath hydrogen analyzer for clinical studies of carbohydrate absorption. Dig Dis Sci 1983; 5: 397-404.

13) Corazza GR, Sorge M, Maurino E, Strocchi A, Lattanzi MC, Gasbarrini G. Methodology of the H2 breath test. I. Collection and storage for gas measurement. Ital J Gastroenterol 1990; 4: 200-204.

14) Gasbarrini A, Corazza GR, Gasbarrini G, Montalto M, Di Stefano M, Basilisco G, Parodi A, Usai-Satta P, Vernia P, Anania C, Astegiano M, Barbara G, Benini L, Bonazzi P, Capurso G, Certo M, Colecchia A, Cuoco L, Di Sario A, Festi D, Lauritano C, Miceli E, Nardone G, Perri F, Portincasa P, Risicato R, Sorge M, Tursi A, st Rome HBTCCWG. Methodology and indications of H2-breath testing in gastrointestinal diseases: the Rome Consensus Conference. Aliment Pharmacol Ther 2009; 29: 1-49.

15) Niu HC, Schoeller DA, Klein PD. Improved gas chromatographic quantitation of breath hydrogen by normalization to respiratory carbon dioxide. J Lab Clin Med 1979; 5: 755-763.

16) Ojetti V, Gabrielli M, Migneco A, Lauritano C, Zocco MA, Scarpellini E, Nista EC, Gasbarrini G, Gasbarrini A. Regression of lactose malabsorption in coeliac patients after receiving a gluten-free diet. Scand J Gastroenterol 2008; 2: 174-177.

17) Metz G, Jenkins DJ, Peters TJ, Newman A, Blendis LM. Breath hydrogen as a diagnostic method for hypolactasia. Lancet 1975; 7917: 1155-1157.

18) Veligati LN, Treem WR, Sullivan B, Burke G, Hyams JS. Delta 10 ppm versus delta 20 ppm: a reappraisal of diagnostic criteria for breath hydrogen testing in children. Am J Gastroenterol 1994; 5: 758-761.

19) Eisenmann A, Amann A, Said M, Datta B, Ledochowski M. Implementation and interpretation of hydrogen breath tests. J Breath Res 2008; 4: 046002.

20) Tursi A, Brandimarte G, Giorgetti GM, Inchingolo CD. Effectiveness of the sorbitol H2 breath test in detecting histological damage among relatives of coeliacs. Scand J Gastroenterol 2003; 7: 727-731.

21) Tursi A, Brandimarte G, Giorgetti GM. Sorbitol H2-breath test versus anti-endomysium antibodies for the diagnosis of subclinical/silent coeliac disease. Scand J Gastroenterol 2001; 11: 1170-1172.

22) Tursi A, Brandimarte G, Giorgetti GM. Sorbitol H2-breath test versus anti-endomysium antibodies to assess histological recovery after gluten-free diet in coeliac disease. Dig Liver Dis 2002; 12: 846-850.

23) Glinsmann WH, Bowman BA. The public health significance of dietary fructose. Am J Clin Nutr 1993; 5 Suppl: 820S-823S.

24) Gibson PR, Newnham E, Barrett JS, Shepherd SJ, Muir JG. Review article: fructose malabsorption and the bigger picture. Aliment Pharmacol Ther 2007; 4: 349-363.

25) Singh VV, Toskes PP. Small bowel bacterial overgrowth: presentation, diagnosis, and treatment. Curr Treat Options Gastroenterol 2004; 1: 19-28.

26) Pimentel M, Chow EJ, Lin HC. Eradication of small intestinal bacterial overgrowth reduces symptoms of irritable bowel syndrome. Am J Gastroenterol 2000; 12: 3503-3506.

27) Corazza GR, Menozzi MG, Strocchi A, Rasciti L, Vaira D, Lecchini R, Avanzini P, Chezzi C, Gasbarrini G. The diagnosis of small bowel bacterial overgrowth. Reliability of jejunal culture and inadequacy of breath hydrogen testing. Gastroenterology 1990; 2: 302-309.

28) Wilberg S, Pieramico O, Malfertheiner P. [The H2-lactulose breath test in the diagnosis of intestinal transit time]. Leber Magen Darm 1990; 3: 129-137.

29) Braden B. Methods and functions: Breath tests. Best Pract Res Clin Gastroenterol 2009; 3: 337-352.

30) Braden B, Haisch M, Duan LP, Lembcke B, Caspary WF, Hering P. Clinically feasible stable isotope technique at a reasonable price: analysis of $^{13}CO2/^{12}CO2$-abundance in breath samples with a new isotope selective-nondispersive infrared spectrometer. Z Gastroenterol 1994; 12: 675-678.

31) Savarino V, Mela GS, Zentilin P, Bisso G, Pivari M, Mansi C, Mele MR, Bilardi C, Vigneri S, Celle G. Comparison of isotope ratio mass spectrometry and nondispersive isotope-selective infrared spectroscopy for ^{13}C-urea breath test. Am J Gastroenterol 1999; 5: 1203-1208.

32) Malfertheiner P, Megraud F, O'Morain CA, Atherton J, Axon AT, Bazzoli F, Gensini GF, Gisbert JP, Graham DY, Rokkas T, El-Omar EM, Kuipers EJ, European Helicobacter Study G. Management of Helicobacter pylori infection—the Maastricht IV/ Florence Consensus Report. Gut 2012; 5: 646-664.

33) Perri F, Festa V, Clemente R, Quitadamo M, Andriulli A. Methodological problems and pitfalls of urea breath test. Ital J Gastroenterol Hepatol 1998; S315-319.

34) Roberts AP, Childs SM, Rubin G, de Wit NJ. Tests for Helicobacter pylori infection: a critical appraisal from primary care. Fam Pract 2000; S12-20.

35) Ohara S, Kato M, Saito M, Fukuda S, Kato C, Hamada S, Nagashima R, Obara K, Suzuki M, Honda H, Asaka M, Toyota T. Comparison between a new ^{13}C-urea breath test, using a film-coated tablet, and the conventional ^{13}C-urea breath test for the detection of Helicobacter pylori infection. J Gastroenterol 2004; 7: 621-628.

36) Gatta L, Ricci C, Tampieri A, Osborn J, Perna F, Bernabucci V, Vaira D. Accuracy of breath tests using low doses of ^{13}C-urea to diagnose Helicobacter pylori infection: a randomised controlled trial. Gut 2006; 4: 457-462.

37) Graham DY, Runke D, Anderson SY, Malaty HM, Klein PD. Citric acid as the test meal for the ^{13}C-urea breath test. Am J Gastroenterol 1999; 5: 1214-1217.

38) Agha A, Opekun AR, Abudayyeh S, Graham DY. Effect of different organic acids (citric, malic and ascorbic) on intragastric urease activity. Aliment Pharmacol Ther 2005; 9: 1145-1148.

39) Klein PD, Graham DY. Minimum analysis requirements for the detection of Helicobacter pylori infection by the ^{13}C-urea breath test. Am J Gastroenterol 1993; 11: 1865-1869.

40) Gisbert JP, Pajares JM. Review article: ^{13}C-urea breath test in the diagnosis of Helicobacter pylori infection — a critical review. Aliment Pharmacol Ther 2004; 10: 1001-1017.

41) Graham DY, Opekun AR, Jogi M, Yamaoka Y, Lu H, Reddy R, El-Zimaity HM. False negative urea breath tests with H2-receptor antagonists: interactions between Helicobacter pylori density and pH. Helicobacter 2004; 1: 17-27.

42) Epple HJ, Kirstein FW, Bojarski C, Frege J, Fromm M, Riecken EO, Schulzke JD. ^{13}C-urea breath test in Helicobacter pylori diagnosis and eradication. Correlation to histology, origin of 'false' results, and influence of food intake. Scand J Gastroenterol 1997; 4: 308-314.

43) Braden B, Duan LP, Caspary WF, Lembcke B. More convenient ^{13}C-urea breath test modifications still meet the criteria for valid diagnosis of Helicobacter pylori infection. Z Gastroenterol 1994; 4: 198-202.

44) Mana F, Franken PR, Ham HR, Reynaert H, Urbain D. ^{13}C urea breath test with nondispersive isotope-selective infrared spectrometry: reproducibility and importance of the fasting status. Helicobacter 2000; 2: 104-108.

45) Ng FH, Lai KC, Wong BC, Wong WM, Wong SY, Chow KC, Yuen ST, Leung SY, Lam SK. [^{13}C]-urea breath test without prior fasting and without test meal is accurate for the detection of Helicobacter pylori infection in Chinese. J Gastroenterol Hepatol 2002; 8: 834-838.

46) Ghoos YF, Maes BD, Geypens BJ, Mys G, Hiele MI, Rutgeerts PJ, Vantrappen G. Measurement of gastric emptying rate of solids by means of a carbon-labeled octanoic acid breath test. Gastroenterology 1993; 6: 1640-1647.

47) Zahn A, Langhans CD, Hoffner S, Haberkorn U, Rating D, Haass M, Enck P, Stremmel W, Ruhl A. Measurement of gastric emptying by ^{13}C-octanoic acid breath test versus scintigraphy in diabetics. Z Gastroenterol 2003; 5: 383-390.

48) Pfaffenbach B, Wegener M, Adamek RJ, Wissuwa H, Schaffstein J, Aygen S, Hennemann O. [Non-invasive ^{13}C octanoic acid breath test for measuring stomach emptying of a solid test meal—correlation with scintigraphy in diabetic patients and reproducibility in healthy probands]. Z Gastroenterol 1995; 3: 141-145.

49) Duan LP, Braden B, Caspary WF, Lembcke B. Influence of cisapride on gastric emptying of solids and liquids monitored by ^{13}C breath tests. Dig Dis Sci 1995; 10: 2200-2206.

50) Schadewaldt P, Schommartz B, Wienrich G, Brosicke H, Piolot R, Ziegler D. Application of isotope-selective nondispersive infrared spectrometry (IRIS) for evaluation of [^{13}C]octanoic acid gastric-emptying breath tests: comparison with isotope ratio-mass spectrometry (IRMS). Clin Chem 1997; 3: 518-522.

51) Perri F, Bellini M, Portincasa P, Parodi A, Bonazzi P, Marzio L, Galeazzi F, Usai P, Citrino A, Usai-Satta P. (13)C-octanoic acid breath test (OBT) with a new test meal (EXPIROGer): Toward standardization for testing gastric emptying of solids. Dig Liver Dis 2010; 8: 549-553.

52) Uchida M, Shimizu K. ^{13}C-acetic acid is more sensitive than ^{13}C-octanoic acid for evaluating gastric emptying of liquid enteral nutrient formula by breath test in conscious rats. Biol Pharm Bull 2007; 3: 487-489.

53) Braden B, Adams S, Duan LP, Orth KH, Maul FD, Lembcke B, Hor G, Caspary WF. The [^{13}C]acetate breath test accurately reflects gastric emptying of liquids in both liquid and semisolid test meals. Gastroenterology 1995; 4: 1048-1055.

54) Braden B, Peterknecht A, Piepho T, Schneider A, Caspary WF, Hamscho N, Ahrens P. Measuring gastric emptying of semisolids in children using the ^{13}C-acetate breath test: a validation study. Dig Liver Dis 2004; 4: 260-264.

55) Maes BD, Ghoos YF, Geypens BJ, Mys G, Hiele MI, Rutgeerts PJ, Vantrappen G. Combined carbon-13-glycine/carbon-14-octanoic acid breath test to monitor gastric emptying rates of liquids and solids. J Nucl Med 1994; 5: 824-831.

56) Dominguez Munoz JE. Diagnosis of chronic pancreatitis: Functional testing. Best Pract Res Clin Gastroenterol 2010; 3: 233-241.

57) Loser C, Mollgaard A, Aygen S, Hennemann O, Folsch UR. ^{13}C-starch breath test—comparative clinical evaluation of an indirect pancreatic function test. Z Gastroenterol 1997; 3: 187-194.

58) Evenepoel P, Hiele M, Geypens B, Geboes KP, Rutgeerts P, Ghoos Y. ^{13}C-egg white breath test: a non-invasive test of pancreatic trypsin activity in the small intestine. Gut 2000; 1: 52-57.

59) Geboes KP, Bammens B, Luypaerts A, Malheiros R, Buyse J, Evenepoel P, Rutgeerts P, Verbeke K. Validation of a new test meal for a protein digestion breath test in humans. J Nutr 2004; 4: 806-810.

60) Hiele M, Ghoos Y, Rutgeerts P, Vantrappen G. Starch digestion in normal subjects and patients with pancreatic disease, using a ^{13}CO2 breath test. Gastroenterology 1989; 2 Pt 1: 503-509.

61) Ishii Y, Kohno T, Ito A, Suzuki S, Kohno T, Takayama T, Asai S. Evaluation of pancreatic exocrine secretion using ^{13}C-dipeptide (benzoyl-L-tyrosyl-[1-(13)C]alanine) breath test: focusing on pancreato-duodenectomy cases. Pancreas 2007; 4: 313-319.

62) Braden B. (13)C breath tests for the assessment of exocrine pancreatic function. Pancreas 2010; 7: 955-959.

63) Vantrappen GR, Rutgeerts PJ, Ghoos YF, Hiele MI. Mixed triglyceride breath test: a noninvasive test of pancreatic lipase activity in the duodenum. Gastroenterology 1989; 4: 1126-1134.

64) Kalivianakis M, Verkade HJ, Stellaard F, van der Were M, Elzinga H, Vonk RJ. The ^{13}C-mixed triglyceride breath test in healthy adults: determinants of the ^{13}CO2 response. Eur J Clin Invest 1997; 5: 434-442.

65) Loser C, Brauer C, Aygen S, Hennemann O, Folsch UR. Comparative clinical evaluation of the ^{13}C-mixed triglyceride breath test as an indirect pancreatic function test. Scand J Gastroenterol 1998; 3: 327-334.

66) Adamek RJ, Bodeker C, Szymanski C, Hagemann D, Pfaffenbach B. [^{13}C-mixed triglyceride CO2 exhalation test. Investigation with an isotope selective, non dispersive infrared spectrophotometer of indirect function of the exocrine pancreas]. Dtsch Med Wochenschr 1999; 5: 103-108.