

European guideline on indications, performance and clinical impact of 13 C-breath tests in adult and pediatric patients: An EAGEN, ESNM, and ESPGHAN consensus, supported by EPC.

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Gastroenterology. <https://creativecommons.org/licenses/by-nc-nd/4.0/> This is an open access article under the terms of the <http://creativecommons.org/licenses/by-nc-nd/4.0/> License, which permits use and distribution in any medium, provided the original work is properly cited, the use is non-commercial and no modifications or adaptations are made. Abstract Introduction 13 C¹³breath tests are valuable, noninvasive diagnostic tests that can be widely applied for the assessment of gastroenterological symptoms and diseases. Currently, the potential of these tests is compromised by a lack of standardization regarding performance and interpretation among expert centers.

This consensus-based clinical practice guideline defines the clinical indications, performance, and interpretation of 13 C¹³breath tests in adult and pediatric patients. A balance between scientific evidence and clinical experience was achieved by a Delphi consensus that involved 43 experts from 18 European countries. Consensus on individual statements and recommendations was established if $\geq 80\%$ of reviewers agreed and $<10\%$ disagreed.

The guideline gives an overview over general methodology of 13 C¹³breath testing and provides recommendations for the use of 13 C¹³breath tests to diagnose *Helicobacter pylori* infection, measure gastric emptying time, and monitor pancreatic exocrine and liver function in adult and pediatric patients. Other potential applications of 13 C¹³breath testing are summarized briefly. The recommendations specifically detail when and how individual 13 C¹³breath tests should be performed including examples for well-established test protocols, patient preparation, and reporting of test results.

This clinical practice guideline should improve pan-European harmonization of diagnostic approaches to symptoms and disorders, which are very common in specialist and primary care gastroenterology practice, both in adult and pediatric patients. In addition, this guideline identifies areas of future clinical research involving the use of 13 C¹³breath tests.

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Breath tests are valuable, noninvasive diagnostic tests that are widely applied for the assessment of gastroenterological symptoms and diseases. 13 C¹³breath tests provide the opportunity to diagnose *Helicobacter pylori* (*H. pylori*) infection, document gastric emptying time, monitor pancreatic exocrine and liver function, and have several additional potential gastroenterological and nongastroenterological applications.

Currently, the potential of breath testing is compromised by a lack of standardization regarding performance and interpretation among expert centers. This is highly relevant because modifications of the volume and/or composition of the test meal, of test performance and of the evaluation of data may markedly influence test results, diagnosis and thus, clinical usefulness of the investigation.

This consensus-based clinical practice guideline is needed within the gastrointestinal (GI) community to enhance pan-European harmonization of diagnostic approaches to symptoms and disorders, which are very common in specialist and primary care gastroenterology practice, both in adult and in pediatric patients. The guideline can add significantly to quality of investigation and, thus, the welfare of gastroenterological patients because it will allow a more rational approach to diagnostic evaluation and treatment. The guideline also aims to minimize disparities between health care systems across Europe, to facilitate cooperation between expert groups and the performance of multicenter clinical trials.

The structured procedure, which was developed for the creation of this consensus-based clinical practice guideline, has previously been published. 1 Briefly, this procedure was initiated by three representatives of the contributing societies (heads of guideline, JK, HH, MF) and started with formation of a representative core group of experts nominated from all participating societies and associations. This core group developed statements and recommendations, which were then submitted to reviewers in a three-stage Delphi voting process. The heads of guideline and the core group members are listed as authors; the reviewers are listed as members of the European 13 C-breath test group.

The following key questions were addressed in the guideline: What is the role of 13 C breath tests in the detection of H. pylori infection, and in the measurement of gastric emptying, pancreatic exocrine and liver function?

What are the general technical requirements and operating procedures for performance of 13 C-breath tests, including preparation, dosage, breath sampling, technical analysis?

Are there areas of disagreement and research priorities?

A systematic literature search with the appropriate key words using Medline/Pubmed and the Cochrane database was performed. We limited our search to studies performed in humans, which were published between 01 January 2000 and 25 July 2019. The resulting 446 references were assessed and allocated to the following topics: general methodology, 13 C-urea breath tests (13 C-UBT), 13 C-gastric emptying breath tests (13 C-GEBT), 13 C-pancreatic function breath tests (13 C-PFBT), 13 C-liver function breath tests (13 C-LFBT), and other 13 C-breath tests. Statements and recommendations were developed based on these results, relevant consensus documents including those of participating societies (published after the year 2000), 2 and on pertinent literature known to members of the core group. Statements reflect key aspects and definitions but give no direct instructions on how to act, whereas recommendations advise when and how to perform individual 13 C-breath tests and how to report on breath test results. The wording used to indicate the strength of recommendation is detailed in Table 1 . 1

Of note, we aimed to develop recommendations on the specific indications for 13 C-breath tests, whereas general indications for H. pylori testing, gastric emptying testing and monitoring of pancreatic exocrine or liver function were cited from current national and international guidelines.

Statements and recommendations were distributed among all reviewers via email for voting and commenting. All reviewers voted on all statements and recommendations according to the 6-point Likert scale given in Table 2 1 and gave comments in case of disagreement. "Agreement" was established if $\geq 80\%$ of reviewers voted A+/A AND $< 10\%$ D+/D.

Statements and recommendations, which did not receive agreement during the first Delphi round, were modified according to comments as previously described, 1 and all modified statements and recommendations underwent further rounds of the Delphi process. Statements that achieved "agreement" after three Delphi rounds were accepted. Percentages for agreement and disagreement are given for all recommendations and statements. Recommendations additionally include quality of evidence (Q) and strength of recommendation (S). 1

The guideline is organized in a way that information, which will enable the reader to perform clinical tests, is summarized in statements, recommendations and respective tables. Background information detailed in the comments explains the choice of test protocols and further methodological aspects.

Since their introduction into clinical practice in the 1970s, ^{13}C breath tests have gained considerable importance and have been recommended for distinct diagnostic purposes by national and international guidelines and expert consensus papers. 4, 5, 6, 7 This is due to the fact that they are not only reliable, but also noninvasive, relatively simple and safe diagnostic tools. ^{13}C is a stable, nonradioactive carbon isotope with a natural abundance of about 1% of carbon isotopes. The use of nonradioactive stable isotope tracers in biomedical experiments and diagnosis is generally considered ethically acceptable in humans at all ages. Toxicity of ^{13}C has been examined in animals given amounts far in excess of those employed in the clinic. Up to sixty percent enrichment with ^{13}C was achieved over prolonged periods of time without negative effects in adult animals and without signs of teratogenicity or embryotoxicity. 8, 9, 10 Thus, the small requirements of ^{13}C as a tracer in most clinical studies, particularly relative to its naturally high abundance, precludes any discernible risk of toxicity. Accordingly, ^{13}C enrichment of marker substances does not affect their tolerability. Depending on pharmacological properties of the marker substance itself, most clinically established ^{13}C breath tests can also be performed (repeatedly) in young children and pregnant women. This chapter gives important background information on methodological aspects and delineates general recommendations on test performance.

Statement 1.1 ^{13}C breath tests are used for investigation of a variety of gastrointestinal and liver functions and for diagnosis of *Helicobacter pylori* infection (100%, 0%) .

In addition to the clinically established indications extensively discussed in this consensus report, several other tests have been developed for gastroenterological and other purposes (compare “Other tests”).

Statement 1.2 The general principle requires that the digestive/metabolic process under investigation represents the rate-limiting step in the sequence of events leading to occurrence of $^{13}\text{CO}_2$ in the exhaled air (100%, 0%) .

For instance, within the time frame used for testing, the presence of bacterial urease in the stomach determines whether ^{13}C urea is metabolized leading to a selective increase in $^{13}\text{CO}_2$ exhalation in *H. pylori* positive patients.

Statement 1.3 Most ^{13}C breath tests require sample collection and measurement over several hours. For selected indications (e.g., ^{13}C urea breath test in adults) measurements at two time points can be sufficient (100%, 0%) .

Analysis of ^{13}C breath tests is frequently based on kinetic data of the $^{13}\text{CO}_2$ exhalation characteristics (e.g., gastric emptying tests) or on quantitative analysis of the whole metabolic process, that is, by analysis of cumulative $^{13}\text{CO}_2$ exhalation (e.g., pancreatic or liver function tests). Accordingly, several breath samples have to be collected at predefined intervals. Depending on the metabolic and whole body distribution pathways of each ^{13}C labeled substrate and the process under investigation, breath sampling over several hours may be necessary. For instance, protocols for pancreatic function testing usually require 4 to 9 h of sample collection at 15 or 30 min intervals. 11, 12, 13, 14 On the other hand, ^{13}C UBT in adults are usually based on two point measurements. 15, 16 However, even for *H. pylori* testing, some experts calculate cumulative ^{13}C exhalation over 1 h based on breath samples collected at 15 min intervals. The major advantage is that the patient's anthropometry is taken into account: a small and light person has a much higher measurement of δ over baseline value (DOB) than a very tall and heavy person for the same cumulative percentage of administered dose. In this way, the test can also be applied in children.

Statement 1.4 Isotope ratio mass spectrometry is the reference method for measurement of the $^{13}\text{CO}_2$ concentration in the exhaled air (100%, 0%) .

Statement 1.5 Isotope selective nondispersive infrared spectroscopy can be used alternatively (91%, 0%) .

At present, mass spectrometry is the most accurate and efficient method for measuring carbon isotope ratios in exhaled breath (IRMS), but its application is restricted by the high cost of the equipment and operational complexity. Nondispersive infrared spectroscopy (NDIRS) is the most widely used alternative method. Apart from lower costs, the devices are smaller, easier to handle and can be used on site, for example, in outpatient facilities. 17 On the other hand, NDIRS measurements usually require higher sample volumes. Samples are frequently collected in aluminum bags (200–1300 ml) instead of 10 ml glass tubes as used for IRMS, which limits its use in large laboratories to which samples are delivered from distant sites.

Studies comparing the results of IRMS and NDIRS measurements of identical samples have shown comparable results for both methods. Most of these studies have investigated samples from ^{13}C UBT. 17 , 18 , 19 , 20 , 21 Data show correlation coefficients of up to 0.999 for both analytical methods. A meta-analysis assessing the diagnostic accuracy of ^{13}C UBT in adult patients with dyspepsia showed no significant difference for studies reporting NDIRS or IRMS results. 22

Similarly, results of ^{13}C octanoic acid breath tests (^{13}C OABT), which measure gastric emptying, have been compared using IRMS and NDIRS. 23 As expected, precision and repeatability of ^{13}C measurements with NDIRS were inferior to IRMS. However, correlation coefficients for ^{13}C exhalation and all gastric emptying parameters as computed on the basis of IRMS and NDIRS measurements were >0.98 . Mean gastric emptying half time calculated using nonlinear regression (NLR) analysis was almost identical (87 ± 39 min vs. 90 ± 39 min). For the ^{13}C methacetin breath test, a dynamic liver function test, molecular correlations spectroscopy, a method similar to NDIRS, showed comparable results to IRMS in Bland-Altman and correlation analysis. 24 Breath samples were collected continuously via a nasal cannula with one sample analyzed about every 3 min.

Theoretically, these results should be transferable to other ^{13}C breath tests since $^{13}\text{CO}_2$ is the ultimate metabolic product, which is analyzed in all of these tests. However, caution may be necessary when applying tests, that only result in small increases in ^{13}C exhalation, for example, ^{13}C pancreatic function tests, for which highly accurate measuring devices are required. 25

Statement 1.6 The δ value (‰) is the measuring parameter and is defined as the $^{13}\text{CO}_2 / ^{12}\text{CO}_2$ ratio in a given sample in comparison to the $^{13}\text{CO}_2 / ^{12}\text{CO}_2$ ratio in a reference material (97%, 0%) .

Statement 1.7 Differences between δ values obtained after application of a marker substance and the baseline δ value (δ over baseline, DOB) are used for calculation of outcome parameters (97%, 0%) .

Conventionally, ^{13}C content of breath samples is expressed as δ ‰ PDB units, that is, relative to the international standard, which originally was the calcium carbonate of the fossil Belemnite of the Pee Dee formation (PDB) in South Carolina, USA. Zero δ ‰ corresponds to 1.112372‰ ^{13}C atoms within CaCO_3 . Thus, if a sample of carbon dioxide has a $^{13}\text{C} / ^{12}\text{C}$ ratio which is less than that of the standard by 5 per mil, it is said to have a δ value of -5 ‰. 26 , 27 DOB values are the differences between δ values obtained before (baseline) and after application of the marker substance. They reflect the increase in ^{13}C exhalation, which is the basis for calculation of test parameters. For most established ^{13}C UBT protocols, the DOB value at 30 min is the relevant outcome parameter (compare below). For most other tests, based on DOB values and the assumption of a stable CO_2 production rate of 300 mmol per square meter of body surface per

hour, the quantity of ^{13}C appearing in breath per unit time is calculated. These data are usually expressed as percentage of ^{13}C dose administered.

Statement 1.8 $^{13}\text{CO}_2$ concentrations in samples collected using breath tubes for isotope ratio mass spectrometry (IRMS) remain stable for at least 4 weeks so that measurement of breath samples can be delayed by this period (84%, 0%) .

Statement 1.9 $^{13}\text{CO}_2$ concentrations in samples collected using aluminum bags for nondispersive infrared spectroscopy (NDIRS) remain stable for at least 72 h so that measurement of breath samples can be delayed by this period (93%, 0%) .

For IRMS, stability of samples for a minimum period of 4 weeks has been demonstrated. A brief report even suggests that with 10 ml samples stored in glass tubes at room temperature in the absence of light, ^{13}C concentrations are stable for 8 months. 28 For NDIRS there are hardly any published data on sample stability. Mana et al. showed that samples are stable for 72 h. 18 Personal experience of the authors suggests longer stability (1–2 weeks by additional sealing of the rubber tubes of aluminum bags with gas-tight tapes).

Statement 1.10 Digestive and metabolic processes can be influenced by several factors including demographic parameters, fasting or fed state, composition and size of a test meal, physical activity, pre-existing diseases and drug intake (100%, 0%) .

^{13}C exhalation from marker substances depends not only on the process under investigation (e.g., gastric emptying), but also on absorption of the marker substance and/or its metabolites, further (mostly hepatic) metabolism leading to production of $^{13}\text{CO}_2$, its transport to the lung and pulmonary excretion and distribution to other body compartments leading to a relevant loss of label, for example, into muscles or bone. 29 All of these functions as well as the process under investigation can be influenced by demographic and physiological parameters, concomitant diseases and drug intake. 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40 A study investigating the influence of clinical parameters on the results of ^{13}C OABT in more than 1200 patients has shown that ^{13}C exhalation was increased in women and correlated with age. Diabetes mellitus and inflammatory bowel disease were associated with decreased, and bacterial overgrowth and malignant disease with increased ^{13}C exhalation. 39

Figure 1 summarizes the general principle of ^{13}C breath testing.

Recommendation 1.1 ^{13}C breath tests performed for clinical reasons have to adhere strictly to standardized study protocols adequately validated in a representative patient population (100%, 0%; Q:C S:A) .

Recommendation 1.2 For research projects, test parameters such as composition of the test meal or duration of breath sampling can be varied to evaluate the impact of the variation on test results (89%, 3%; Q:D S:B) .

As discussed above, ^{13}C breath tests are indirect tests depending on several digestive and metabolic processes, which ultimately lead to exhalation of ^{13}C enriched breath. All intermediary steps as well as the process under investigation can be influenced by demographic and physiological parameters, concomitant diseases and drug intake. Moreover, alterations of marker substance, dose, or other components of the test meal/solution can markedly influence test results. Accordingly, it is of pivotal importance that tests are validated in representative patient populations and that clinicians adhere to strictly standardized study protocols. Research projects are required to better delineate the impact of variations on test results in order to optimize test procedures.

Recommendation 1.3 ^{13}C rich food, ingested before the test (e.g., corn, pineapple, broccoli, sugarcane) can increase the baseline δ value and thereby compromise the measurements.

Accordingly, they should be avoided at least 48 h before ^{13}C -breath testing (85%, 0%; Q:C S:B) .

Isotopic fractionation—change in isotopic ratios between materials, due to the different rates at which various isotopes undergo chemical reactions—is a well-established phenomenon. ²⁷ Carbon isotopes are strongly fractionated during photosynthesis, when plants metabolize carbon dioxide. Three types of photosynthesis occur in the plant world, commonly referred to as the C₃, C₄, and CAM pathways. ²⁷ While most plants traditionally consumed in European diets perform C₃ photosynthesis, leading to comparably lower ^{13}C content, other plants such as corn, pineapple, broccoli, and sugarcane are C₄ plants with relatively higher ^{13}C abundance. Their consumption prior to a ^{13}C -breath test increases basal ^{13}C exhalation, and further metabolism may influence ^{13}C exhalation over time. Avoidance for 48 h before breath testing is deemed satisfactory by most experts, while some recommend 72 h.

Recommendation 1.4 Drugs with potential influence on test results should be avoided before the test, unless essential long-term medication is concerned or the effect of the drug on the digestive/metabolic process is to be determined (92% 0%; Q:D S:B) .

Recommendation 1.5 Dialysis solutions and glucose infusions mostly contain glucose that originates from hydrolysis of maize starch naturally enriched in ^{13}C and should therefore be avoided during ^{13}C -breath testing (94%, 0%; Q:D S:B) .

Drugs can influence test results by altering GI transit, absorption or (postabsorptive) metabolism of the ^{13}C -labeled substrate. Accordingly, reliable performance of ^{13}C -breath tests may require avoidance of specific drugs as discussed for the individual tests below. However, if long-term treatment is mandatory, drug avoidance is not always reasonable because it confounds the normal clinical situation of the patient. Moreover, ^{13}C -breath tests can be used for monitoring drug effects. For instance, the ^{13}C -mixed triglyceride breath test (^{13}C -MTGBT) has been used to monitor improvement of lipid absorption with enzyme replacement therapy in pancreatic exocrine insufficiency (PEI). ¹¹

Dialysis solutions and glucose infusions mostly contain glucose that originates from hydrolysis of maize starch, so that they are naturally enriched in ^{13}C . Accordingly, they may confound test results and should also be avoided. ³⁷

Recommendation 1.6 Physical activity alters gastrointestinal transit of orally administered substrates and markedly increases CO₂ production. Therefore, physical activity has to be avoided during ^{13}C -breath testing (100%, 0%; Q:C S:A) .

Even moderate physical activity such as walking roughly doubles energy expenditure compared with sedentary subjects and has corresponding effects on endogenous CO₂ production. ⁴¹ , ⁴² In addition, exercise leads to a shift toward oxidation of nonlipid components. This increases ^{13}C exhalation because the lipid molecules in the body contain substantially lower concentrations of ^{13}C than the nonlipid molecules, due to fractionation processes during lipid synthesis. ³⁵ Moreover, ^{13}C -breath tests using orally applied marker substances depend on GI transit which is accelerated by moderate exercise, while strenuous exercise has opposite effects. ⁴³ Accordingly, it has been shown that physical activity during ^{13}C -OABT markedly alters $^{13}\text{CO}_2$ exhalation in healthy volunteers as well as respective normal values. ³⁶

In summary, physical activity has profound and complex effects on ^{13}C exhalation and breath test results. For standardization purposes under clinical conditions, patients must be asked to strictly avoid physical activity during tests.

Recommendation 1.7 Disturbances of gastrointestinal motor and secretory function, hepatic and pulmonary function can generally affect the time course and/or amount of ^{13}C exhalation. This has to be taken into account for performance and interpretation of ^{13}C -breath tests (100%, 0%;

Q:B S:A) .

Concomitant diseases affecting digestive and metabolic processes which ultimately lead to exhalation of ^{13}C -enriched breath may confound breath test results. In particular, major disturbances of GI transit and absorption, hepatic and lung function have to be considered. However, the influence of concomitant diseases on many ^{13}C -breath tests appears to be small:

Even in patients with nonalcoholic steatohepatitis or liver cirrhosis (~50% Child–Pugh score C), ^{13}C -octanoic acid metabolism was found to be normal 44 , 45 , 46 and the ^{13}C -OABT correlated well with scintigraphy in patients who were critically ill. 47

Disturbances of $^{13}\text{CO}_2$ -exhalation in patients with lung diseases are only expected in patients with very severe disease and reduced CO_2 -diffusion capacity. Data on potential impairment of ^{13}C -breath tests in such cases are not available in the literature.

Since digestion of dietary lipids by pancreatic lipase cannot occur before the meal has entered the duodenum, results of the ^{13}C -MTGT are influenced by and correlate with gastric emptying parameters. 14 This predominantly applies to early postprandial ^{13}C -exhalation rates (1–3 h), which have insufficient specificity for detection of PEI. However, this problem can be overcome by prolongation of the breath sampling period so that gastric emptying is of minor importance for ^{13}C -exhalation.

Vice versa, $^{13}\text{CO}_2$ -excretion following administration of ^{13}C -octanoic acid was similar in patients with and without PEI, including those with severe exocrine insufficiency characterized by overt steatorrhea. 39 , 48 , 49

H. pylori is a common bacterial pathogen responsible for substantial GI morbidity worldwide. In addition to causing inflammatory gastroduodenal alterations, *H. pylori* is the major risk factor for gastric cancer development and is associated with various other, partly non-GI diseases. Table 3 summarizes important indications for *H. pylori* testing in adults and children recommended by current European guidelines. 4 , 5

Indications for *H. pylori* testing in adults and children 4 , 5

There are differences in the approach to *H. pylori* infection between adults and children. Thus, the European Society of Pediatric Gastroenterology, Hepatology, and Nutrition (ESPGHAN) explicitly recommends against a test and treat strategy in children, as well as against *H. pylori* testing as part of the initial investigation in children with iron deficiency or as part of the investigations of causes of short stature. Moreover, these guidelines recommend against *H. pylori* testing in children with functional abdominal pain disorders. 5

For the diagnosis of *H. pylori* infection, histology ($\pm$ culture) from biopsy samples is regarded as the reference standard. 50 , 51 Several other tests are available, including endoscopic biopsy followed by rapid urease testing and noninvasive methods like serology, fecal antigen tests and the ^{13}C -UBT. The principle of the ^{13}C -UBT relies upon the capacity of *H. pylori* , when present in the stomach, to hydrolyze orally administered ^{13}C -urea to produce $^{13}\text{CO}_2$, which diffuses into the blood, is transported to the lungs and exhaled, so that it can be detected in breath samples. ^{13}C -urea is innocuous and can be safely administered repeatedly, including in children and pregnant women.

According to the Maastricht V/Florence Consensus report, ^{13}C -UBT is regarded as the best approach to the diagnosis of *H. pylori* infection in the context of a “test-and-treat strategy” in adults because of its high sensitivity and specificity, and excellent performance. 4 This is confirmed by a recent Cochrane review showing superior diagnostic accuracy compared with other non-invasive tests. 50

Stool antigen tests may be less acceptable in some societies but also have a high sensitivity and specificity, provided a monoclonal antibody-based ELISA is used. 4 Some serology tests have high sensitivity and specificity, but these tests may perform differently in different geographic locations according to the antigenic composition of the circulating strains. Thus, the Maastricht V/Florence Consensus recommends that only locally validated tests should be used. 4 Moreover, due to the slow decrease of serum antibody levels, they are inadequate for assessment of *H. pylori* eradication after treatment.

In clinical practice when there is an indication for endoscopy, and there is no contraindication for biopsy, the rapid urease test (RUT) is recommended as a first-line diagnostic test. In the case of a positive test, it allows immediate treatment. 4

Recommendation 2.1 The 13 C-urea breath test is to be considered as a noninvasive alternative for all indications for *Helicobacter pylori* testing if endoscopy is not required or if biopsies are contraindicated (92%, 0%; Q:A S:A) .

Recommendation 2.2 The 13 C-urea breath test is a preferred option for confirmation of *Helicobacter pylori* eradication in adults and children. It has to be performed at least 4 weeks after completion of therapy (89%, 0%; Q:A S:A) .

Meta-analyses confirm that 13 C-UBT achieves $\geq 95\%$ sensitivity and specificity in both adults and children. 50 , 52 Accordingly, it is a highly accurate test and generally appropriate for all indications for *H. pylori* testing. However, in clinical practice, when there is an indication for endoscopy, and there is no contraindication for biopsy, the RUT is recommended as a first-line diagnostic test. 4 Some national guidelines suggest two positive tests for reliable diagnosis, except in cases with a very high a priori likelihood of *H. pylori* infection, for example, duodenal ulcer, 53 and 13 C-UBT can be readily performed. Moreover, 13 C-UBT is regarded as the best option for confirmation of *H. pylori* eradication, 4 , 50 with stool antigen tests being an alternative. Testing has to be delayed for at least 4 weeks after the end of therapy, otherwise it may lead to false negative results (compare patient preparation).

Recommendation 2.3 If commercially available kits are used for 13 C-urea breath test, manufacturers' instructions regarding preparation of test solution and test performance have to be followed (97%, 0%; Q:B S:A) .

Recommendation 2.4 If the test is prepared on site, investigators have to adhere to well established test protocols (for examples compare Table 4) (97, 0%, 0%; Q:B S:A) .

As discussed above, alterations of marker substance, dose or other components of the test meal/solution can markedly influence test results. Hence, it is of pivotal importance that tests are validated in representative patient populations and that clinicians adhere to strictly standardized study protocols. Since the original description of the 13 C-UBT by Graham et al., 57 several changes of the test protocol have been proposed affecting the dose of 13 C-urea, type of test meal/solution, time of breath collection, cut-off values, and measuring device. 58 Logan et al. first introduced a modified version with only one breath sample to be analyzed, however this was pooled from several samples collected over 30 min at 5 min intervals. 59 Currently, most studies report DOB values of a single sample collected 30 min after application of 13 C-urea with a threshold of $>4\%$ for diagnosis of *H. pylori* infection. At this threshold the summary sensitivity (95% confidence interval [CI]) and specificity (95% CI) from 10 studies (958 participants) were 0.95 (95% CI: 0.79–0.99) and 0.95 (95% CI: 0.87–0.98). 50 A minority of studies used sampling periods of 10 or 20 min and thresholds between DOB $>3\%$ and DOB $>6\%$. 50 13 C-urea was usually applied with a citric acid solution; however, orange juice 54 or semiliquid meals have also been used. 55 Examples for well-established test protocols are given in Table 4 .

Established test protocol for ^{13}C ■UBT in adults and children

Abbreviations: ACC, accuracy; DOB, δ over baseline; NPV, negative predictive value; PPV, positive predictive value; SENS, sensitivity; SPEC, specificity; UBT, urea breath test.

Recommendation 2.5 Ideally, adult patients should have fasted overnight. If this is not feasible, a fasting period of 4 to 6 h is sufficient (97%, 0%; Q:C S:B) .

Recommendation 2.6 Children of all ages should have fasted for 4 h (100%, 0%; Q:B S:B) .

Theoretically, food in the stomach may dilute the marker substance and thereby impair the contact between the infected mucosa and the substrate leading to lower $^{13}\text{CO}_2$ production and decreased sensitivity of the test. Although this aspect is still controversial, some studies suggest that fasting before the ^{13}C ■UBT indeed improves accuracy of test results. 18 , 60 Accordingly, it appears to be prudent to perform the ^{13}C ■UBT in fasting conditions. 60 In adults, fasting overnight appears reasonable. If this is not feasible, a fasting period of 4–6 h is deemed sufficient. In young children, long fasting periods may be problematic. However, sensitivity of the ^{13}C ■UBT was markedly reduced to about 50% when children were fed a meal immediately before the test. 61 Therefore, a fasting period of 4 h is suggested, including in young children.

Recommendation 2.7 Prior to the ^{13}C ■urea breath test patients have to abstain from: proton pump inhibitor therapy for ≥ 2 weeks; antibiotic therapy (including *Helicobacter pylori* eradication) for ≥ 4 weeks (97%, 0%; Q:A/B S:A) .

Recommendation 2.8 Antacids can be allowed before the ^{13}C ■urea breath (86%, 0%; Q:B S:B) .

Proton pump inhibitors (PPI) need to be discontinued because they decrease the load of *H. pylori* leading to false■negative results on several tests including ^{13}C ■UBT. 4 , 62 , 63 A 7■day withdrawal has been shown to be sufficient in most patients; however, as a precaution, 14 days are recommended. 4 H 2 ■receptor antagonists slightly decrease sensitivity of ^{13}C ■UBT for up to 2 weeks. 64 Topical antacids do not affect sensitivity 62 so that they can be allowed before testing. By contrast, antibiotics, including those used for eradication therapy, and bismuth compounds need to be discontinued for 4 weeks to allow an increase of a detectable bacterial load. 4

Recommendation 2.9 To allow for reliable interpretation of test results, the following parameters should be reported: marker dose and test solution; test result including normal values and interpretation (*Helicobacter pylori* negative/positive) (95%, 0%; Q:D S:B) .

Recommendation 2.10 The test report could be complemented by including clinical characteristics of the patient, last use of proton pump inhibitor and the exact test protocol including equipment used for breath sampling and analysis (84%, 0%; Q:D S:C) .

Experts agree that a minimum of information on methodology (marker dose and test solution) and test results (e.g., DOB■value) including normal values and interpretation (*H. pylori* positive/negative) are required for medical personnel not involved in the testing to reliably interpret individual findings. Clinical characteristics of the patient including last use of PPI and further methodological information may further facilitate assessment of reliability of test results and choice of clinical consequences.

Gastric dysmotility can manifest as rapid gastric emptying with dumping syndrome (even in the absence of upper GI surgery) or delayed gastric emptying with symptoms of gastroparesis. The latter applies to the majority of affected patients and is typically associated with nausea, vomiting, early satiety, postprandial fullness, upper abdominal pain, and bloating in adults and children. 6 , 65 , 66 , 67 , 68 , 69 Anorexia and weight loss are further frequent symptoms. Children with gastroparesis experience more vomiting while adolescents with gastroparesis report more nausea

and abdominal pain. 68 , 70 There is general consensus that the diagnosis of gastroparesis requires objective evidence of clearly delayed gastric emptying in symptomatic patients. 6 , 66 However, prior to gastric emptying testing, the exclusion of mucosal or structural disorders such as inflammatory or malignant diseases as the underlying cause of symptoms is required. 6 Specific indications for gastric emptying testing in adults as suggested by international guidelines and expert consensus papers are given in Table 5 . 6 , 65 , 71 For pediatric patients, there are no generally accepted guideline recommendations on gastric emptying testing. The recommendations given in Table 5 are derived from a recent review of the literature, which shows that nausea, vomiting, and abdominal pain are the most common symptoms in children, while early satiety, postprandial fullness, bloating, and weight loss occur less frequently in pediatric gastroparesis. 72

Indications for gastric emptying testing in adults and children 6 , 7 , 65 , 71 , 72

Recommendation 3.1 13 C■gastric emptying breath tests are to be regarded as an established alternative to scintigraphy for measurement of gastric emptying velocity (92%, 0%; Q:A S:A) .

Scintigraphy is the reference standard for measurement of gastric emptying. However, while there is a consensus report recommending a standardized protocol in adults in the United States, 73 no European consensus exists on the type of test meal and duration of data acquisition. Likewise, no consensus exists for a standard gastric emptying scintigraphy in pediatrics. However, recent studies have provided confirmation that extending studies from 2 to 4 h increases the diagnostic yield and should be the standard in children and adolescents as it is in adults for measurement of solid gastric emptying. 73 , 74 , 75 For liquids, 2 h are probably sufficient and early gastric emptying has to be accounted for. 76

Several protocols for 13 C■based gastric emptying tests have been successfully validated in comparison with scintigraphy. The medium■chain fatty acid, 13 C■octanoic acid, 77 , 78 or the edible blue■green algae, 13 C■Spirulina platensis 79 are typically used to label solids; 13 C■acetate is used for liquids. 80 , 81 On delivery to the duodenum, the 13 C■containing substrate is either absorbed directly (octanoic acid, acetate) or digested and then absorbed (Spirulina) . Subsequently, it is metabolized in the liver, and finally excreted by the lungs as 13 CO₂ . 6

The first use of the 13 C■OABT in adults was published by Ghoo et al. in 1993. 77 Gastric half emptying time (T_{1/2}) was assessed by NLR analysis and corrected for the expected delay caused by postgastric processes (absorption, metabolism, exhalation). The authors observed an excellent correlation between 13 C■OABT parameters and parameters obtained from simultaneous scintigraphy (R = 0.89 for T_{1/2} scintigraphy vs. breath test). Sensitivity and specificity, positive and negative predictive values of breath test parameters for delayed gastric emptying were ≥94%. 77 Results of the 13 C■OABT also closely correlate with those of scintigraphy in other studies in adults and children. 78 , 82 , 83 , 84 Following this initial study, all kinds of variation of the test meal and the test protocol have been described, depending on cultural differences and practical considerations (e.g., muffins, pancakes, rolls). In addition, different mathematical analysis methods have been proposed 77 , 80 , 85 , 86 , 87 , 88 (also compare Table 6). Pancakes marked with 13 C■octanoic acid are an acceptable and palatable solid test meal for children but cannot be used in case of allergy to egg, milk or wheat, in coeliac disease or very young children. 78 , 88 , 89

Established test protocols for 13 C■GEBT in adults and children a

Normal values: Percentiles according to age

Delayed gastric emptying defined as T_{1/2} > P90

Rapid gastric emptying defined as T_{1/2} < P10

T_{1/2} 13 C■ABT and T_{1/2} SCINTI: R = 0.604 (p = 0.0006)

Reproducibility tested in 21 healthy children: CV $T_{1/2}$ (median, range) = 8.3%, 1.6%–16.2%

Normal values: Percentiles according to age:

Delayed gastric emptying defined as $T_{1/2} > P90$

Rapid gastric emptying defined as $T_{1/2} < P10$

$T_{1/2}$ ■ 13 C ■ OABT and $T_{1/2}$ ■ SCINTI: $R = 0.748$ ($p < 0.0001$)

Reproducibility tested in 19 healthy children: CV $T_{1/2}$ (median, range) = 13.3%, 2.56%–29.6%

Abbreviations: 13 C ■ ABT, 13 C ■ acetate breath test; 13 C ■ OABT, 13 C ■ octanoic acid breath test; CV, coefficient of variance; GE, gastric emptying; GEBT, gastric emptying breath test; GI, gastrointestinal; HC, healthy controls; kPCD, percent dose excreted $\times 1000$; NLR, nonlinear regression model; NPV, negative predictive value; P10, 10th percentile; P90, 90th percentile; pp, postprandial; PPV, positive predictive value; SCINTI, scintigraphy; SENS, sensitivity; SPEC, specificity; $T_{1/2}$, gastric half emptying time.

Calculated breath test data not corrected for scintigraphy.

Scintigraphic equivalent values or breath test data corrected for scintigraphy according to Ghoo et al. 1993. 77

[Corrections added on June 28, 2021 after first online publication: Typos have been corrected in Table 6.]

The lack of standardization is of concern because it makes comparison of values between different laboratories difficult. On the other hand, it allows flexibility in research projects to measure gastric emptying of very different test meals and evaluate the impact of composition on gastric emptying.

Results of the 13 C ■ Spirulina ■ GEBT also show high concordance ($R = 0.86$) with scintigraphic data. 90 The protocol is exactly defined and has been validated in a large group of healthy volunteers and patients. 90 The test was approved by the FDA for evaluation of gastric emptying in 2015 (<https://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfpma/pma.cfm?id=P110015>), and it is also CE marked according to the producer's information (<https://serconinstruments.com/wp-content/uploads/2017/06/019gebt.pdf>). However, it has not been marketed in Europe, so far.

13 C ■ acetate has been used as a marker for acaloric and caloric liquids such as formula diets. 81 , 87 , 91 , 92 , 93 In young children or children dependent on gastrostomy feeding, the test meal often consists of milk or milk ■ based formulas. 94 Apple juice has also been used. 32 Results of the test closely correlate with those of scintigraphy in adults and children. 81 , 87 , 91 , 92 , 93 Delayed gastric emptying of solids usually precedes disturbances in gastric emptying of liquids. 95 Therefore, tests of solid gastric emptying are supposed to have a higher sensitivity. However, gastric emptying of liquids can be abnormal in patients with normal gastric emptying of solids. 96 Moreover, liquid test meals are obviously more useful in young children 81 , 94 and probably also for confirmation of rapid gastric emptying in patients with suspected dumping syndrome. 6 , 97

Intra ■ individual and inter ■ individual variabilities of all 13 C ■ GEBTs are high in adults and children, but similar to variations observed with scintigraphy 85 , 88 , 89 , 90 , 94 , 98 and, therefore, reflect day ■ to ■ day physiological variability in gastric emptying. 6

Recommendation 3.2 ^{13}C -GEBT (gastric emptying breath test) performed for clinical reasons have to adhere strictly to standardized study protocols adequately validated in a representative patient population. This refers to preparation of the test meal as well as test performance and evaluation of test results (for examples compare Table 6) (97%, 0%; Q:C S:A) .

As discussed above, ^{13}C -GEBT are indirect tests that involve multiple steps and are prone to influences caused by demographic, physiological, and other parameters. 39 , 99 , 104 It has been hypothesized that ^{13}C -GEBT might be inaccurate in conditions associated with substantial malabsorption, liver, or lung diseases, though this is not substantiated by clinical studies. 39 , 44 , 47 However, it is still important that tests are validated not only in healthy adults and children, but also in the relevant patient population. 77 , 78 , 79 , 81 , 84

Moreover, alterations of marker substance, dose or other components of the test meal markedly influence test results. For instance, a larger labelled test meal will result in higher normal ranges for $T_{1/2}$ and gastric lag time. 39 , 82 , 105 For ^{13}C -acetate an interaction has been demonstrated between the rate of ^{13}C -delivery to the duodenum and ^{13}C -recovery in breath. 33

Different mathematical models have been developed for analysis of gastric emptying curves derived from breath tests, in particular the NLR model, the generalized linear regression (GLR) model and the Wagner–Nelson method. 77 , 79 , 106 Cumulative $^{13}\text{CO}_2$ excretion over time is inversely related but analogous to the scintigraphic gastric emptying curve. However, $^{13}\text{CO}_2$ excretion does not only depend on gastric emptying velocity but also on postgastric absorption and metabolism of the substrate and CO_2 exhalation rates. For this reason, Ghoo et al. developed the original NLR model. 77 According to this model, $T_{1/2}$ indicates the time at which half of the $^{13}\text{CO}_2$ is excreted, relative to the cumulative excretion when time is infinite. Accordingly, results are determined by the shape of the exhalation curve, independent of absolute $^{13}\text{CO}_2$ excretion. Measurements are usually performed over 4 h with breath samples at 15 min intervals.

The GLR model published by Lee et al. proposed a minimum number ($N = 3$) of breath samples at pre-specified times during the 3 h postprandial period to mathematically predict the gastric emptying endpoints measured by simultaneous scintigraphy. 79 Results reflect absolute $^{13}\text{CO}_2$ excretion. A similar model with breath samples obtained upon completion of the meal and then at 45, 90, 120, 150, 180, and 240 min postprandially was suggested by the same group 90 and is used for analysis of the test commercially available in the USA.

The Wagner–Nelson method has been suggested for analysis of ^{13}C -GEBT by Sanaka et al. 106 This method has been developed to describe the entrance of ingested drugs into the venous system based on its urinary excretion data. When applied on breath tests, it describes the manner in which $^{13}\text{CO}_2$ appears in the venous system based on pulmonary $^{13}\text{CO}_2$ excretion data. 106 , 107 It is used less frequently, and, similar to the NLR model, the $^{13}\text{CO}_2$ exhalation curve must exhibit the decreasing portion during the sampling period for correct estimation of gastric emptying parameters. 108 Accordingly, breath sampling has to be routinely performed for 4 h and potentially longer in gastroparetic patients.

Given that in Europe there is no standardized, well-validated test kit commercially available, and that tests are usually prepared on site, it is of pivotal importance that clinicians adhere strictly to standardized study protocols including established analysis methods. Especially in children, a large variety of test meals have been explored in accordance with the variable requirements of different age groups. 78 , 101 , 102 , 103 , 109 , 110 , 111 , 112 , 113 , 114 However, several of these studies were performed in small patient groups. Examples of well validated ^{13}C -GEBT protocols in adults and children (studies with $N \geq 20$) are given in Table 6 .

Recommendation 3.3 Before and during the test, precautions as described in General Methodology (avoidance of ^{13}C -rich food, avoidance of physical activity, and ^{13}C -rich infusions during test) have to be observed (100%, 0%; Q:C S:A) .

Recommendation 3.4 Drugs with potential influence on gastrointestinal transit should be avoided before the test, unless essential long term medication is concerned or the test is performed to monitor the drug effect on gastric emptying (100%, 0%; Q:C S:B) .

Recommendation 3.5 Adult patients, older children, and adolescents have to be fasted overnight (94%, 0%; Q:C S:A) .

Recommendation 3.6 A shorter fasting period can be sufficient in very young children (92%, 0%; Q:C S:B) .

Dietary and other restrictions, which generally apply before and during ¹³C-■breath testing, have been explained in General Methodology. Physical activity, in particular, has to be avoided during ¹³C-■GEBT, not only to standardize CO₂ ■production but also because physical activity influences gastric emptying velocity. ⁴³ , ¹¹⁵ Tests should preferentially be performed in the sitting position since the supine position may be associated with slower gastric emptying. ¹¹⁶ Experts agree that solid test meals should be consumed within 10–15 min, liquids within 5–10 min.

Adults, adolescents and older children are required to fast overnight prior to breath testing ⁷⁷ , ⁷⁹ , ⁸⁸ ; more precisely, a fasting period of ≥12 h is recommended by experts in adults as questionable results have been obtained in patients eating large meals very late. Fasting duration varies between 8 and 12 h in children and 3–4 h in infants less than 12 months old, depending on the clinical scenario. ⁸¹

Drugs which influence gastric motor function should be avoided before the test. This includes established prokinetics as well as drugs with anticholinergic properties (e.g., tricyclic antidepressants), smooth muscle relaxants, and opioids. The duration of withdrawal depends on the half-life of the drug, 48–72 h are usually sufficient. However, in a patient with dyspeptic symptoms, who requires long-term medication with, for example, amitriptyline, it is not reasonable to alter the normal clinical situation by discontinuation of the drug before the test.

As ¹³C-■GEBT are harmless, they can be performed repeatedly and have been used successfully to monitor drug effects in clinical studies and individual patients. ¹¹⁷ , ¹¹⁸ , ¹¹⁹ , ¹²⁰

Recommendation 3.7 To allow for reliable interpretation of test results the following parameters should be reported: assessment of gastric emptying of solids versus liquids; marker substance; caloric content of the test meal; duration of breath sampling period; test result including normal values; and interpretation (accelerated, normal, delayed gastric emptying) with T_{1/2} being the best established and most widely used parameter (94%, 0%; Q:D S:B) .

Results can be calculated from breath test data alone, not corrected for scintigraphy ¹⁵ , ²⁰ or given as scintigraphic equivalent values (for T_{1/2}) according to Ghoo et al. ¹³

Recommendation 3.8 The test report could be complemented by including composition and preparation of the test meal, breath sampling intervals, methods used for analysis, and additional gastric emptying parameters such as T lag and GEC (gastric emptying coefficient) with normal values (86%, 0%; Q:D S:C) .

These recommendations are based on the expert consensus that a minimum of information on methodology and test results including normal ranges and interpretation (accelerated/normal/delayed gastric emptying of solids/liquids) is required for reliable interpretation. Further information on methodological aspects and inclusion of several outcome parameters could improve diagnostic gain.

In adults, chronic pancreatitis, pancreatic cancer, and surgical procedures are the most common causes of PEI, whereas in children, cystic fibrosis is of particular relevance. In these diseases,

inflammatory destruction, pancreatic atrophy, ductal obstruction, or resection of pancreatic tissue lead to decreased exocrine secretion. Furthermore, PEI can be caused by regulatory imbalances in the presence of a normal pancreas, such as reduced hormonal and vagal stimulation of pancreatic secretion or inactivation of pancreatic enzymes in the intestinal lumen due to hyperchlorhydria (e.g., Zollinger–Ellison syndrome). 49

Due to the large reserve capacity of the pancreas, mild to moderate exocrine insufficiency is frequently not associated with clinical symptoms, and overt steatorrhea is not expected unless the secretion of pancreatic lipase is reduced to less than 10% of normal (severe PEI). However, patients with “compensated” PEI also have an increased risk of nutritional deficiencies, in particular, of lipid-soluble vitamins with respective clinical consequences. 7

Indications for pancreatic function testing in adults and children as recommended by international guidelines are given in Table 7. 7, 121, 122, 123, 124

Indications for pancreatic function testing in adults and children 7, 121, 122, 123, 124, 125, 126

only applicable for indirect tests measuring digestion/absorption.

Established test protocols for 13 C-MTG-BT in adults and children

For detection of PEI (decreased lipase output):

NPV 95% (control pts with nonpancreatic steatorrhea included)

29 pts with pancreatic disease, controls: 25 healthy subjects + 22 pts with nonpancreatic steatorrhea

comparison with both, stimulated duodenal lipase output (reference standard for pancreatic secretion) and quantitative fecal fat (reference standard for steatorrhea)

Effect of PERT demonstrated in subgroup of pancreatic pts

40 g bread, 20 g butter 200 ml water, 13 C-MTG spread on butter (total fat content 16 g) plus 250 mg 13 C-MTG

10 mg metoclopramide 20 min before meal ingestion

ACC 92% (4h-test is associated with slightly lower diagnostic ACC.: SENS 91%, SPEC 89%)

Cumulative 6h 13 CO₂ excretion (% of dose administered) mean ± SD: Premature infants: 23.9 ± 5.2%

Abbreviations: 13 C-GEBT, 13 C-gastric emptying breath test; 13 C-MTG, mixed triglycerides; 13 C-MTG-BT, 13 C-mixed triglyceride breath test; ACC, accuracy; HC, healthy controls; PEI, pancreatic exocrine insufficiency; PERT, pancreas enzyme replacement therapy; pp, postprandially; SENS, sensitivity; SPEC, specificity.

[Corrections added on June 28, 2021 after first online publication: Typos have been corrected in Table 8].

Recommendation 4.1 The 13 C-mixed triglyceride breath test is to be regarded as an established alternative to other non-invasive and invasive pancreatic function tests (97%, 0%; Q:A S:A) .

Several breath tests using ^{13}C -labeled lipids, proteins, or complex carbohydrates have been developed that indirectly assess pancreatic lipase, protease, and amylase activities. 11, 12, 14, 100, 127, 128, 129, 130, 131, 132, 133, 134, 135, 136 Tests investigating lipid digestion and absorption are preferred because, from a clinical point of view, steatorrhea is by far the most important digestive malfunction in PEI: It is generally more severe and develops several years prior to malabsorption of protein or starch 49 and can be associated with decreased absorption of lipid-soluble vitamins, as mentioned above. Indeed, a ^{13}C -breath test using naturally enriched maize starch lacks sensitivity and specificity for diagnosis of PEI. 136

Tests with various ^{13}C -labeled lipids have been investigated, in which triolein, trioctanoin, tripalmitin, cholesteryl-octanoate, and mixed triglycerides (MTG) are used to generate marker substances. 128 Currently, the original 127 or modified versions 11, 12, 14, 133 of the ^{13}C -MTGBT developed by Vantrappen et al. are almost exclusively used in the clinic because of practical advantages (e.g., shorter labeled fatty acid allowing for shorter breath sampling period). It is based on the principle that intestinal triglyceride absorption requires prior hydrolysis by pancreatic lipase to produce free fatty acids and mono-acylglycerol. These metabolites are incorporated into micelles, absorbed, resynthesized, and transported to the liver. Hepatic enzymes subsequently release fatty acids, including ^{13}C -octanoic acid, that is specifically bound to the Sn-2 position of ^{13}C -mixed triglycerides (^{13}C -MTG: 1,3 distearyl 2[^{13}C -octanoyl] glycerol). ^{13}C -octanoic acid then undergoes β -oxidation, which results in the formation of $^{13}\text{CO}_2$, which is absorbed into the bloodstream, transported to the lung, and exhaled. The increase in $^{13}\text{CO}_2$ concentration in breath thus correlates with pancreatic lipase secretion.

Direct comparison with the reference standard (determination of pancreatic enzyme and/or bicarbonate output in duodenal aspirates following exogenous stimulation with secretin $\pm$ cerulein) demonstrates high sensitivity for severe exocrine insufficiency (90%–100%) with specificity ranging between 80% and 90% in adults. 121, 137 A modified test using comparably high lipid loads in subjects explicitly avoiding physical activity during testing reached high sensitivity and specificity rates even in mild to moderate PEI (100% and 92%, respectively). 12

However, as evident from the test principle, the ^{13}C -MTGBT is a test of lipid digestion and absorption. Therefore, the ^{13}C -MTGBT is accepted as an appropriate alternative to the coefficient of fat absorption, both for the diagnosis of PEI and for evaluating the efficacy of pancreatic enzyme replacement therapy (PERT) in clinical practice. 7, 11, 132, 133, 138 On the other hand, $^{13}\text{CO}_2$ exhalation is not only decreased by lipase deficiency but also by other causes of lipid malabsorption, for example, celiac disease, short bowel syndrome, or postcibal asynchrony following gastric resection. 139 Thus, the specificity of the test for the differential diagnosis of chronic diarrhea is limited.

Unfortunately, fecal elastase, the other noninvasive test which is predominantly used for pancreatic function testing in the clinic, also has limited specificity in these cases. 137 Direct comparisons between ^{13}C -MTGBT and fecal elastase favor the ^{13}C -MTGBT for diagnosis of steatorrhea, 140 and generally in patients with chronic pancreatitis after pancreatic resections. 141

In infants with cystic fibrosis, sensitivity of the ^{13}C -MTGBT for diagnosis of steatorrhea was high, but specificity was low. 142 Thus, the test has been mainly, though not exclusively, 143, 144 used to evaluate the efficacy of PERT in children with cystic fibrosis. 145

Recommendation 4.2 ^{13}C -MTGBT performed for clinical reasons have to adhere strictly to standardized study protocols adequately validated in a representative patient population. This refers to preparation of the test meal as well as test performance and evaluation of test results (for examples compare Table 8) (100%, 0%; Q:C S:A).

Recommendation 4.3 Adult patients, adolescents and older children have to be fasted overnight (97%, 0%; Q:C S:A).

Recommendation 4.4 A shorter fasting period can be sufficient in very young children (95%, 0%; Q:C S:B) .

Recommendation 4.5 Before and during the test, precautions as described in general methodology have to be taken into account (avoidance of ^{13}C -rich food, avoidance of physical activity and ^{13}C -rich infusions during test) (100%, 0%; Q:D S:C) .

Compared to ^{13}C -GEBT, ^{13}C -MTGBT uses significantly lower amounts of ^{13}C resulting in lower DOB values and lower cumulative ^{13}C -exhalation rates. Consequently, avoidance of confounders such as ^{13}C -rich food is of particular importance when conducting the ^{13}C -MTGBT. Test results were not affected by liver disease in children with cystic fibrosis. 147

While adults and older children are required to fast overnight prior to breath testing, 11 , 12 , 127 , 132 , 133 a shorter period may be sufficient in very young children. In analogy to gastric emptying studies, 3–4 h of fasting are recommended in infants less than 12 months old, depending on the clinical scenario. 81

A mathematical correction for altered CO_2 -production rates in physically active subjects using heart rate recordings improves diagnostic accuracy of the ^{13}C -MTGBT in non-resting subjects. 148 However, under clinical conditions, it is pragmatic to request that patients remain seated throughout the test.

Recommendation 4.6 Drugs with potential influence on gastrointestinal transit and/or lipid digestion and absorption should be avoided before the test. The duration of withdrawal depends on the half-life of the drug, 48–72 h are usually sufficient (91%, 3%; Q:C S:B) .

Recommendation 4.7 Pancreatic enzyme replacement therapy should be avoided 2 days before the test, unless the test is performed for monitoring the therapeutic efficacy of pancreatic enzyme replacement therapy (97%, 0%; Q:B S:B) .

Intraluminal lipolysis of the marker and lipid absorption require prior delivery of the test meal into the duodenum, adequate mixing of the marker, meal nutrients and digestive secretions and regulated transport of chyme through the small intestine. Therefore, drugs, which accelerate (e.g., prokinetics, potent laxatives) or markedly inhibit (e.g., opioids) GI transit, may alter test results and should be avoided before the test, unless essential long-term medication is concerned. Depending on the half-life of the drug, 48–72 h of withdrawal are usually sufficient. However, some test protocols deliberately include administration of metoclopramide in order to accelerate gastric emptying and to shorten the required breath sampling period. 11 , 132 , 149

If the ^{13}C -MTGBT is performed to assess the presence of PEI, it is obvious that subjects have to stop oral PERT prior to testing. To account for potentially slow GI transit, it is recommended that patients abstain from PERT for 2 days before the test.

On the other hand, ^{13}C -MTGBT is a suitable and established alternative to fecal fat measurement for monitoring the efficacy of PERT. 11 , 132 To answer this question, subjects are allowed to continue with their usual enzyme replacement therapy. Indeed, it has been shown that PERT can be optimized by repetitive testing with increasing PERT doses until normal ^{13}C -MTGBT results are achieved. By this, a significant increase of body weight was observed. 11

Recommendation 4.8 To allow for reliable interpretation of test results, the following parameters should be reported: marker substance, test results and interpretation including normal values. Cumulative ^{13}C -recovery rate (in % of dose administered) represents the established main outcome parameter (94%, 0%; Q:D S:B) .

Recommendation 4.9 The test report can be complemented by including the ^{13}C exhalation curve, composition and preparation of the test meal, breath sampling intervals, methods used for analysis and interpretation of the ^{13}C exhalation with respect to the clinical context (91%, 0%; Q:D S:B) .

These recommendations are based on the expert consensus that a minimum of information on test methodology and test results including normal ranges and interpretation (normal/decreased intestinal lipolysis compatible with PEI) is required for reliable interpretation. Further information regarding methodological aspects and interpretation of test results with respect to the individual clinical context could further improve the diagnostic validity.

Established parameters for the assessment of liver function under routine clinical conditions are measurement of bilirubin, albumin, liver enzymes and parameters of coagulation factor synthesis in serum or plasma, respectively. Clinical prognostic grading systems (e.g., Child–Pugh score, Model for End-stage Liver Disease score) combine several of these biochemical parameters including clinical symptoms of advanced liver cirrhosis. 150

In contrast to these “static” liver function tests, “dynamic” quantitative tests measure the elimination of a substance, which is cleared and/or metabolized almost exclusively by the liver via specific metabolic pathways in subcellular compartments, for example, by cytochromes for microsomal liver function or cytosolic or mitochondrial enzymes. 151 Accordingly, these tests constitute a more accurate measure of the specific aspects of liver function. Established dynamic quantitative liver function tests are the indocyanine green clearance test and the galactose elimination capacity test. 150 ^{13}C LFBT also represent dynamic tests with oral consumption or intravenous application of the marker substance and measurement of the end product of hepatic metabolism, that is exhaled $^{13}\text{CO}_2$. Some modifications have been shown to detect early changes in liver metabolic capacity in patients, prior to the presence of structural damage to the liver (i.e., inflammation, fibrosis), 152 though with limited sensitivities and specificities. 153 , 154 , 155 , 156 , 157 , 158 , 159 , 160 Still, the dynamic nature of ^{13}C LFBT, their possible versatility in terms of assessing a range of different liver functions, and the ease with which they can be repeated to follow relative changes in liver function with time, generally imply a marked potential for clinical application. 40

Recommendation 5.1 ^{13}C liver function breath tests could be used for measurement of various aspects of liver function in adults (94%, 0%; Q:A S:C) .

Recommendation 5.2 Presently, due to very limited evidence, performance of ^{13}C liver function breath tests for clinical reasons cannot be recommended in children (95%, 0%; Q:D S:B)

Different ^{13}C LFBT have been developed for assessment of hepatic mitochondrial (substrates: ^{13}C ketoisocaproate, [methyl- ^{13}C]methionine), microsomal (^{13}C methacetin, ^{13}C aminopyrine, [3-methyl- ^{13}C]caffeine) and cytosolic (^{13}C phenylalanine) function. 152 , 157 , 159 , 160 , 161 , 162 , 163 , 164 , 165 , 166 , 167 , 168 These have been mainly used in patients with liver fibrosis and cirrhosis due to nonalcoholic fatty liver disease, 152 , 157 , 159 , 160 , 161 , 163 , 164 , 169 , 170 chronic hepatitis C, 162 , 165 cirrhosis, 166 , 171 and hepatocellular carcinoma. 172 Additional potential fields of application are in steatohepatitis, 156 , 157 , 159 fatty liver, 160 , 163 , 169 and assessment of prognosis in chronic liver diseases, in general: In a 7-year prospective follow-up study in 132 patients with chronic HCV infection, the 4'-O- ^{13}C methacetin breath was not inferior to liver biopsy in predicting liver-related death and transplantation. 173 Although several studies have reported close correlations between ^{13}C LFBT and histological alterations or other established parameters in adults, they have still not entered the mainstream of clinical practice but are used exclusively by highly specialized centers. For instance, the effect of transarterial chemoembolization on liver function has been monitored by ^{13}C methacetin test, 174 and several studies suggest that this test could also be used for planning of hepatic resections. 175 , 176 , 177

Given that, as yet, few studies have been performed involving small groups of children with rare diseases, 178 , 179 , 180 , 181 the use of 13 C■LFBT for clinical purposes cannot be recommended in the pediatric population, so far.

Recommendation 5.3 13 C■liver function breath tests performed for clinical purposes have to adhere strictly to standardized and adequately validated study protocols. This refers to preparation of the patient, test meal/solution, test performance and evaluation of test results (for examples compare Table 9) (100%, 0%; Q:C S:A).

For most validated test protocols, the marker substance is dissolved in up to 200 ml of water or unsweetened tea; breath samples are collected before drinking of the test solution and at regular intervals for up to 1.5 h postprandially. As shown in Table 9 , cumulative percentage of the marker dose exhaled at the end of the observation period is the primary outcome parameter of the vast majority of tests. The list of tests detailed in the table is incomplete but concentrates on examples for test protocols using different marker substances, which have been validated in an adequate patient population and have established normal or cut off values for specific diagnoses. Apart from tests with oral marker application, the LIMAx test 177 is commercially available in some European countries, which uses intravenous application of 4'■O■ 13 C■methacetin. For this test, the manufacturer's instructions have to be followed exactly.

Recommendation 5.4 Before and during the test precautions as described in general methodology have to be observed (avoidance of 13 C■rich food, of physical activity, and of 13 C■rich infusions) (100%, 0%; Q:C S:A) .

Recommendation 5.5 Drugs with potential influence on gastrointestinal transit and/or cytochrome p450 metabolism should be avoided before and during the test (96%, 0%; Q:C S:B) .

Recommendation 5.6 As far as essential long■term medication is concerned, it can be necessary to perform 13 C■liver function breath tests despite ongoing medication as long as these medications are not administered during the test period (93%, 0%; Q:B S:B) .

As discussed before, potential confounders of test results detailed in general methodology have to be avoided. For 13 C■LFBT that investigate cytochrome P 450 enzymes, drugs with potential influence on cytochrome P 450 metabolism are of particular relevance, 182 , 183 , 184 and it may be necessary to perform a test despite ongoing medication.

Especially for the most frequently applied test substrate 13 C■methacetin, strong CYP1A2 inhibitors (e.g., ciprofloxacin, fluvoxamin) will have an influence on hepatic methacetin metabolism, although no interaction studies have been systematically performed. Influences of weak CYP1A2 inhibitors (e.g., norfloxacin, propranolol) have not been reported. However, due to the rather short duration of the tests (no longer than 2 h for most protocols), administration during the test period can usually be circumvented, even if essential long■term medication is concerned.

Recommendation 5.7 To allow for reliable interpretation of test results the following parameters should be reported: marker substance; test results and interpretation including normal values (93%, 0%; Q:D S:B) .

Recommendation 5.8 The test report could be complemented by including the 13 C■exhalation curve, composition and preparation of the test meal/solution, breath sampling intervals, methods used for analysis, and interpretation of test results with respect to the clinical context (82%, 0%; Q:D S:C) .

As for other 13 C■breath tests, these recommendations are based on the expert consensus that a minimum of information on methodology and test results including normal ranges and interpretation is required for reliable interpretation. Further information regarding methodological aspects and

interpretation of test results with respect to the individual clinical context could further improve the diagnostic validity.

Examples of validated test protocols for ^{13}C liver function breath tests in adults

Abbreviations: HC, healthy controls; NAFLD, nonalcoholic Fatty Liver disease; NASH, nonalcoholic steatohepatitis; NPV, negative predictive value; PPV, positive predictive value; pp, postprandial; SENS, sensitivity; SPEC, specificity.

[Corrections added on June 28, 2021 after first online publication: In Table 9, typos have been corrected. In the 3rd column, 2nd row, “postprandially” has been deleted from “At baseline and at 10 min intervals postprandially for 60 min pp.”]

The ^{13}C breath tests discussed in the previous chapters have gastroenterological indications and are either clinically established or at least used regularly by specialized centers. Apart from these, there is a multitude of other test options at various developmental stages for gastroenterological indications and ^{13}C breath tests, which are used by other medical specialties. Examples for these are detailed in Table 10 and demonstrate the very broad potential applicability of ^{13}C breath test technology.

Congenital sucrase-isomaltase deficiency

Abbreviations: DOB, δ over baseline; OCTT, oro-cecal transit time; PEI, pancreatic exocrine insufficiency; PPI, proton pump inhibitor; SIBO, small intestinal bacterial overgrowth.

This consensus-based clinical practice guideline aims to assist physicians and provide them with the information required to perform high quality ^{13}C breath tests for patients with various GI symptoms and diseases. In a consensus process, in which representatives from all regions of Europe and specialists representing European scientific societies participated, the available evidence was evaluated, taking account of local facilities, diverse clinical practice, and health care environments. Patient involvement was not covered in our guideline because of its focus on diagnostic recommendations. However, after publication of the guideline, a qualitative and quantitative assessment of guideline adoption into clinical practice is planned, including patient and public involvement.

The guideline gives an overview over general methodology of ^{13}C breath testing and provides recommendations for the use of ^{13}C breath tests to diagnose *H. pylori* infection, measure gastric emptying time and monitor pancreatic exocrine and liver function in adult and pediatric patients. Other potential applications of ^{13}C breath testing are summarized briefly. The recommendations specifically detail when and how individual ^{13}C breath tests should be performed including examples for well-established test protocols.

^{13}C breath tests are indirect tests and mostly require several digestive and metabolic steps that ultimately lead to the exhalation of ^{13}C -enriched breath, including intestinal absorption, hepatic metabolism, and pulmonary excretion. Therefore, demographic parameters, concomitant diseases, and/or medication affecting these processes may confound breath test results. In fact, studies have shown that the influence of concomitant diseases on many ^{13}C breath tests is small or can be prevented by adaptation of the breath test protocol. Information on general ^{13}C breath test methodology as detailed in the guideline will help clinicians to optimize patient selection and preparation and to better understand and interpret breath test results for individual patients.

General principle of ^{13}C breath testing. The ^{13}C -marked substrate is ingested, frequently with a specific test meal or test solution. Hepatic or bacterial enzymes release $^{13}\text{CO}_2$, this requires prior intraluminal metabolism and/or intestinal absorption for most tests. $^{13}\text{CO}_2$ is absorbed into the blood stream, transported to the lung, and exhaled. Breath samples are collected in glass tubes

(mass spectroscopy) or aluminum bags (infrared spectroscopy) for measurement of $^{13}\text{C}/^{12}\text{C}$ ratio. Delta over basal values are used for calculation of ^{13}C excretion. * for specific liver function tests the ^{13}C marked substrate is applied intravenously

As depicted in Figure 2, ^{13}C UBT has excellent sensitivity and specificity for diagnosis of *H. pylori* infection, superior to most other tests, it is highly standardized and well established in the clinic. ^{13}C GEBT and ^{13}C PFBT are validated and accepted tests for diagnosis of gastric emptying disturbances and PEI, respectively. So far, their wide spread use has been hampered by the lack of standardization among centers, which we aim to improve by this guideline. Moreover, the tests are currently not commercially available in Europe, so that test meals need to be prepared on site. ^{13}C LFBT are currently used by highly specialized centers, only. They have the potential to measure various aspects of liver function, including assessment of prognosis in chronic liver disease. However, their clinical role, in general, and applicability in pediatric patients, in particular, still need to be established.

General principle, performance and clinical role of ^{13}C breath tests used in gastroenterology. ^{13}C urea used for detection of *H. pylori* (^{13}C UBT, marked in green) is metabolized by bacterial urease to produce $^{13}\text{CO}_2$, which is absorbed, transported to the lung (broken green arrow) and exhaled. For ^{13}C gastric emptying breath tests (^{13}C GEBT, marked in red), ^{13}C pancreatic function breath test (^{13}C PFBT, marked in brown), and ^{13}C liver function breath tests (^{13}C LFBT, marked in blue), orally applied substrates or their metabolites are absorbed in the small intestine. Subsequently, they are transported to the liver where they undergo further metabolism with production of $^{13}\text{CO}_2$, which is transported to the lung and exhaled [Corrections added on June 28, 2021 after first online publication: Figure 3 (image and caption) has been revised.]

Apart from identification of this and other areas of future research, the guideline should improve pan-European harmonization of diagnostic approaches to symptoms and disorders, which are very common in specialist and primary care gastroenterology practice, both in adult and pediatric patients.

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