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REVIEW ARTICLE

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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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Abstract

Introduction: ^{13}C-breath 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.

Methods: This consensus-based clinical practice guideline defines the clinical indications, performance, and interpretation of ^{13}C-breath 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.

Results: 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 *Helicobacter 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, patient preparation, and reporting of test results.

Conclusion: 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}C-breath tests.

KEYWORDS
breathtest, diagnosis, gastroenterology, gastroparesis, helicobacter pylori, liver cirrhosis, motility, pancreatic exocrine insufficiency, pancreatitis

INTRODUCTION

Breath tests are valuable, noninvasive diagnostic tests that are widely applied for the assessment of gastroenterological symptoms and diseases. ^{13}C-breath 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 non-gastroenterological 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.

METHODS

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:

1. 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?
2. What are the general technical requirements and operating procedures for performance of ^{13}C-breath tests, including preparation, dosage, breath sampling, technical analysis?
3. What are the reporting requirements?
4. 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 ≥ 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.

GENERAL ^{13}C-BREATH TEST METHODOLOGY

Since their introduction into clinical practice in the 1970s,[3] stable isotope ^{13}C-breath tests have gained considerable importance and

TABLE 2 Six-point Likert-scale[1]

Point	Description
A+	Agree strongly
A	Agree with minor reservation
A–	Agree with major reservation
D-	Disagree with major reservation
D	Disagree with minor reservation
D+	Disagree strongly

TABLE 1 Descriptors of grading[1]

Descriptor	Meaning	Wording
A—Strength high	Evidence or general accord that the procedure or statement is useful or effective. Further research is very unlikely to change our confidence in the estimate of effect	…has to be… …is to be… …shall…
B—Strength moderate	Conflicting evidence or discordant opinions that the procedure or statement is useful or effective. The weight of evidence/opinion is in favor of utility. Further research is likely to have an important impact on our confidence in the estimate of effect and may change the estimate	…should… …can…
C—Strength low	Conflicting evidence or discordant opinions that the procedure or statement is useful or effective. Further research is very likely to have an important impact on our confidence in the estimate of effect and is likely to change the estimate	…could…
D—Strength very low	Any estimate of effect is very uncertain	…may…

have been recommended for distinct diagnostic purposes by national and international guidelines and expert consensus papers.[4–7] 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 non-radioactive 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.[8–10] 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 $^{13}CO_2$ 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 $^{13}CO_2$-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 $^{13}CO_2$ exhalation characteristics (e.g., gastric emptying tests) or on quantitative analysis of the whole metabolic process, that is, by analysis of cumulative $^{13}CO_2$ 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.[11–14] 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 $^{13}CO_2$ 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 spectrometry (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.[17–21] 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 $^{13}CO_2$ 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 $^{13}CO_2/^{12}CO_2$ ratio in a given sample in comparison to the $^{13}CO_2/^{12}CO_2$ 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 Belemnitella 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 $^{13}C/^{12}C$ 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 *$^{13}CO_2$ 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 *$^{13}CO_2$ 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 $^{13}CO_2$, 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.[30–40] 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.

What are the general prerequisites for performance of ^{13}C-breath tests for clinical or research purposes?

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.

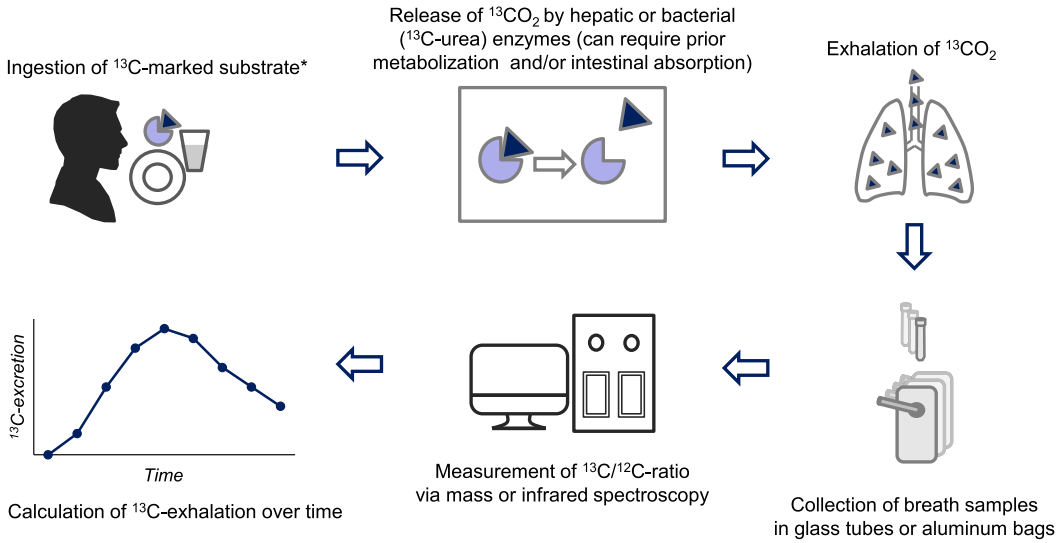

FIGURE 1 General principle of ^{13}C-breath testing. The ^{13}C-marked substrate is ingested, frequently with a specific test meal or test solution. Hepatic or bacterial enzymes release $^{13}CO_2$, this requires prior intraluminal metabolization and/or intestinal absorption for most tests. $^{13}CO_2$ 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 $^{13}C/^{12}C$-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

Which dietary restrictions need to be observed before ^{13}C-breath testing?

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 metabolization 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.

Which drugs and medical interventions need to be avoided before and during ^{13}C-breath testing?

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]

Is physical activity allowed during ^{13}C-breath testing?

Recommendation 1.6 *Physical activity alters gastrointestinal transit of orally administered substrates and markedly increases CO_2*

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 CO_2 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 $^{13}CO_2$-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.

Which concomitant diseases may influence ^{13}C-breath test results?

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[44–46] and the ^{13}C-OABT correlated well with scintigraphy in patients who were critically ill.[47]

Disturbances of $^{13}CO_2$-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, $^{13}CO_2$-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]

^{13}C-UREA BREATH TEST

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]

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

TABLE 3 Indications for *H. pylori* testing in adults and children[4,5]

Grade of recommendation		
Adults	High	Suspicion/evidence of peptic ulcer disease, atrophic gastritis, gastric adenocarcinoma, MALT (mucosa-associated lymphoid tissue) lymphoma
		Test-and-treat strategy for uninvestigated dyspepsia
		Exclusion of *H. pylori* gastritis before reliable diagnosis of functional dyspepsia
	Moderate	Aspirin and NSAIDs users with a history of peptic ulcer
	Low	Unexplained iron deficiency anemia, idiopathic thrombocytopenic purpura, vitamin B12 deficiency
Children	High	Suspicion/evidence of peptic ulcer disease
		Monitoring of outcome of eradication therapy
	Low	Chronic immune thrombocytopenic purpura

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 (±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 $^{13}CO_2$, 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]

When should ^{13}C-UBT be utilized?

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 ≥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).

How should ^{13}C-UBT be performed?

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.

How should patients prepare for the 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).*

TABLE 4 Established test protocol for ^{13}C-UBT in adults and children

Reference		Test solution	Breath sampling	Cut off	Validity	Remarks
Leodolter 1999[15]	Adults	**75 mg ^{13}C-urea** dissolved in 200 ml 0.1 mol/L citric acid solution (~4 g/200 ml water) with two tablets artificial sweetener	Before and 30 min after ingestion	≥4‰	SENS 95% SPEC 98% ACC 97%	Equal performance as test with citric acid solution 10 min before marker ingestion; With 200 ml orange juice instead of citric acid lower SENS (88%) with equally high SPEC (100%)[54]; with semiliquid meals longer test duration required[55]
Elitsur et al. 2009[56]	Children	**75 mg ^{13}C-urea** and 2 g citric acid dissolved in 4 ounces (~120 ml) potable water	Baseline and 15 min after ingestion	≥2.4‰	SENS 97.9% SPEC 96.1% PPV 90.4% NPV 99.2%	Test performed best in children aged >6 years; in ages 2–5 calculation of urea hydrolysis rate can lead to higher SENS and SPEC compared with DOB values

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

Recommendation 2.6 *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 $^{13}CO_2$ 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]

How should test results be reported?

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.

^{13}C-GASTRIC EMPTYING BREATH TESTS

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.[6,65–69] 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]

When should ^{13}C-GEBT be utilized?

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.[73–75] 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 $^{13}CO_2$.[6]

The first use of the ^{13}C-OABT in adults was published by Ghoos et al. in 1993.[77] Gastric half emptying time (T½) was assessed by NLR analysis and corrected for the expected delay caused by postgastric processes (absorption, metabolization, exhalation). The authors observed an excellent correlation between ^{13}C-OABT parameters and parameters obtained from simultaneous scintigraphy ($R = 0.89$ for T½ 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.[78,82–84] 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[77,80,85–88] (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]

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

TABLE 5 Indications for gastric emptying testing in adults and children[6,7,65,71,72]

Grade of recommendation		
Adults	High/moderate	Symptoms suggestive of gastroparesis* without evidence of mucosal or structural disease explaining these symptoms (*nausea, vomiting, early satiety, postprandial fullness, bloating, upper abdominal pain)
		Unexplained impairment of blood glucose control in patients with diabetes mellitus, even in the absence of abdominal symptoms (because of the central role of gastric emptying for regulation of postprandial glycemia)
	Moderate	To support the diagnosis of dumping syndrome
	Low	Severe gastroesophageal reflux disease unresponsive to acid suppressants (particularly before fundoplication); systemic sclerosis; after lung transplantation; Parkinson disease; generalized GI motility disorders; patients under consideration for intestinal or colonic surgery or transplantation because of motility disorders
Children	High/moderate	Most common GI symptoms located in the upper GI tract suggestive for gastroparesis: Nausea, vomiting and abdominal pain
	Low	Less frequent GI symptoms located in the upper GI tract suggestive for gastroparesis: Early satiety, postprandial fullness and bloating

Abbreviation: GI, gastrointestinal.

TABLE 6 Established test protocols for ^{13}C-GEBT in adults and children[a]

Reference	Age	Estimated parameter	Test meal	Breath sampling	Endpoints and normal values	Validity	Remarks
Ghoos 1993[77]	Adults	Solid GE	Two slices of white bread, 5 g butter, 200 ml water, omelet made from one egg, yolk doped with **91 µg (= 100 µl) ^{13}C-octanoic acid**	Samples at baseline (preferentially taken as duplicate), further samples at 15 min intervals up to 4 h pp	T ½ (mean ± 2SD): 28–116 min[c]	T ½: SENS 95% SPEC 94% PPV 94% NPV 94%	Evaluated against SCINTI in HC (N = 16) and patients with dyspepsia (N = 20), normal values from 42 HC (NLR model), no test kit commercially available, other groups report slightly different T ½ normal values using same protocol: 50–150 min[99]
Szarka 2008[90]	Adults	Solid GE	Freeze-dried scrambled eggs mix containing **100 mg ^{13}C-*Spirulina platensis***, six saltine crackers, and 180 ml of water	Breath samples at baseline, on completion of the meal and at 45, 90, 120, 150, 180, and 240 min pp	kPCD values at 45, 150, and 180 min provide strongest concordance with scintigraphy for accelerated and delayed GE	Delayed GE: SENS 89%, SPEC 80% Accelerated GE: SENS 93%, SPEC 80%	38 HC and 129 patients with clinically suspected delayed GE, normal T ½ according to SCINTI (10th–90th percentile): 52–86 min,FDA approved, CE marked, commercially available in the United States, only
Bertram 2014[100]	Adults	Liquid GE	**150 mg ^{13}C-acetate** dissolved in 200 ml water with 10 g lacutose	Breath samples at baseline, at 5 min intervals for first hour, at 15 min intervals for second hour pp	Time of maximal ^{13}C-exhalation[b]: (P10–P90): 15–40 min	Time of maximal ^{13}C-exhalation and T ½ SCINTI: R = 0.88, $p < 0.005$ in validation study by Chew 2003[87]	22 HC, lactulose used for simultaneous measurement of liquid gastric emptying and small bowel transit by H_2-breath test, time to maximal ^{13}C-exhalation in HC identical with validation study[87] in 10 HC which used 15 g glucose instead of lactulose
Van Den Driessche 1999[101]	29 healthy premature and term infants gestational age 27––41 weaks, postnatal age 7–74 days	Liquid GE	Group 1: 50 ml expressed breast milk, Group 2: 50 ml infant formula (Nutrilon premium®) (33.5 kcal), each with **50 µl ^{13}C-octanoic acid**	Breath sample at baseline, further samples at 5 min intervals for 30 min, then at 10 min intervals up to 4 h pp	T ½ (mean, range): group 1 = 47, 16–86 min[c]; Group 2 = 65, 27–98 min[c]	-	-

(Continues)

TABLE 6 (Continued)

Reference	Age	Estimated parameter	Test meal	Breath sampling	Endpoints and normal values	Validity	Remarks
Hauser 2016[102]	133 healthy children mean 9 years, range 1–17 years	Liquid GE	200 ml INZA® milk-drink (skimmed milk) (112 kcal) with 50 mg (body weight 10–30 kg) or **100 mg (>30 kg) ^{13}C-acetate**	Breath samples at baseline and at 5 min intervals for 40 min, then at 10 min intervals up to 3 h pp	T ½ (mean ±2SD): 55–109 min[b] Normal values: Percentiles according to age Delayed gastric emptying defined as T ½ > P90 Rapid gastric emptying defined as T ½ < P10	T ½ ^{13}C-ABT and T ½ SCINTI: $R = 0.604$ ($p = 0.0006$) Reproducibility tested in 21 healthy children: CV T ½ (median, range) = 8.3, 1.6%–16.2%	Comparison with scintigraphy in 21 children with upper GI symptoms
Eradi 2006[103]	25 healthy children mean 7.8 years, SD 0.3 years, range 5–10 years	Solid GE	30 g chocolate crispy cake (147 kcal) with **100 mg ^{13}C-octanoic acid**	Breath samples at baseline, further samples at 15 min intervals up to 4 h pp	T ½ (mean ± 2SD): 44–155 min[b]	Comparison ^{13}C-OABT and gastric emptying scintigraphy:T ½-OBT and T ½-SCINTI: $R = 0.69$ ($p < 0.01$)	-
Hauser 2016[78]	120 healthy children mean 9 years, SD 4 years, range 1–17 years	Solid GE	One pancake (17 g wheat flour, 7 g sugar, one egg white, one egg yolk, 40 ml semi-skimmed milk, 5 g margarine) + 5 g sugar + 100 ml water (230 kcal) with **50 µl ^{13}C-octanoic acid**	Breath sample at baseline further samples at 15 min intervals up to 4 h pp	T ½ (mean ± 2SD): 50–266 min[b] Normal values: Percentiles according to age: Delayed gastric emptying defined as T ½ > P90 Rapid gastric emptying defined as T ½ < P10	T ½-^{13}C-OABT and T ½-SCINTI: $R = 0.748$ ($p < 0.0001$) Reproducibility tested in 19 healthy children: CV T ½ (median, range) = 13.3, 2.56%–29.6%	Comparison with scintigraphy in 19 dyspeptic children

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 × 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; T½, gastric half emptying time.

[a]Only studies with $N \geq 20$.

[b]Calculated breath test data not corrected for scintigraphy.

[c]Scintigraphic equivalent values or breath test data corrected for scintigraphy according to Ghoos et al. 1993.[77]

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://sercon-instruments.com/wp-content/uploads/2017/06/019-gebt.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.[81,87,91–93] 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.[81,87,91–93] 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[85,88–90,94,98] and, therefore, reflect day-to-day physiological variability in gastric emptying.[6]

How should ^{13}C-GEBT be performed?

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.[77–79,81,84]

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 T½ 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 $^{13}CO_2$-excretion over time is inversely related but analogous to the scintigraphic gastric emptying curve. However, $^{13}CO_2$-excretion does not only depend on gastric emptying velocity but also on postgastric absorption and metabolisation of the substrate and CO_2-exhalation rates. For this reason, Ghoos et al. developed the original NLR model.[77] According to this model, T½ indicates the time at which half of the $^{13}CO_2$ 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 $^{13}CO_2$-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 $^{13}CO_2$-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 $^{13}CO_2$ appears in the venous system based on pulmonary $^{13}CO_2$ excretion data.[106,107] It is used less frequently, and, similar to the NLR model, the $^{13}CO_2$-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.[78,101–103,109–114] 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.

How should patients prepare for the test?

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 ^{13}C-breath testing, have been explained in General Methodology. Physical activity, in particular, has to be avoided during ^{13}C-GEBT, not only to standardize CO_2-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 ^{13}C-GEBT are harmless, they can be performed repeatedly and have been used successfully to monitor drug effects in clinical studies and individual patients.[117–120]

How should test results be reported?

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 T½ 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 T½) according to Ghoos 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.

^{13}C-PANCREATIC FUNCTION TESTS

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.[7,121–124]

When should ^{13}C-PFBT be utilized?

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.[11,12,14,100,127–136] 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]

TABLE 7 Indications for pancreatic function testing in adults and children[7,121–126]

Grade of recommendation		
Adults	High	Suspected pancreatic exocrine insufficiency in patients with pancreatic disease/after pancreatic resection
		Patients with chronic pancreatitis at the time of diagnosis (and annually thereafter if not tested positive for pancreatic exocrine insufficiency)
	Moderate	Monitoring of pancreatic enzyme replacement therapy in patients with an inadequate therapeutic response[a]
		Suspected pancreatic exocrine insufficiency without evidence of pancreatic disease
		Differential diagnosis of chronic diarrhea
Children	High	Screening for pancreatic exocrine insufficiency of children with chronic pancreatitis every 6–12 months
		Newly diagnosed CF
		Every 3–12 months (age dependent) in CF patients with pancreatic sufficiency at time of diagnosis
		In CF patient with subnormal weight development

Abbreviation: CF, cystic fibrosis.

[a]only applicable for indirect tests measuring digestion/absorption.

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-acyl-glycerol. 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 $^{13}CO_2$, which is absorbed into the bloodstream, transported to the lung, and exhaled. The increase in $^{13}CO_2$-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 ± 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, $^{13}CO_2$ 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]

How should ^{13}C-PFBT be performed?

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).*

TABLE 8 Established test protocols for ^{13}C-MTGBT in adults and children

	Age	Test meal	Breath sampling	Endpoints and normal values	Validity	Remarks
Vantrappen et al. 1989[127]	Adults	100 g of toast with 0.25 g of butter per kilogram of body weight, plus **16 mg ^{13}C -MTG per gram of butter**	At baseline and at 30 min intervals for 6 h pp	Cumulative ^{13}C-recovery, normal (estimated from fig 4: Lowest value obtained in HC): >23% of dose	For detection of PEI (decreased lipase output): SENS 89%, SPEC 81% PPV 63% 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
Dominguez-Munoz et al. 2015[132]	Adults	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	At baseline and at 30 min intervals for 6 h pp	Cumulative ^{13}C-recovery, normal >29% of dose (>19% for 4 h test duration)	SENS 93%, SPEC 92% ACC 92% (4h-test is associated with slightly lower diagnostic ACC.: SENS 91%, SPEC 89%)	Developed using quantitative fecal fat (reference standard for steatorrhea) for comparison in healty volunteers (N = 10) and chronic pancreatitis patients with (N = 16) or without (N = 4) PEI, validated in 78 pts with advanced CP, also shown to be of value for monitoring of PERT efficacy, and to correlate with the nutritional status and the severity of chronic pancreatitis
Keller et al. 2011[12]	Adults	Two slices of white bread, 20 g butter, 30 g chocolate cream (31 g fat/100 g) carefully mixed with **250 mg ^{13}C-MTG** (total fat content 26 g)	At baseline and at 30 min intervals for 6 h pp	Cumulative ^{13}C-recovery, normal >26.8% of dose	SENS 100%, SPEC 92% verses secretin-test	Validated using secretin test (reference standard for pancreatic secretion) for comparison in HC and patients with pancreatic disease (N = 19), also detects mild and moderate PEI
Keller et al. 2014[14]	Adults	Two slices of white bread, 20 g butter, 30 g chocolate cream (31 g fat/100 g) carefully mixed with **250 mg ^{13}C-MTG** (total fat content 26 g)	At baseline and at 30 min intervals for 4 h pp	Cumulative ^{13}C-recovery, normal >13.8% of dose	SENS 88% SPEC 94%, versus 6 h test version	Evaluated in 200 pts undergoing both, ^{13}C-MTGT and ^{13}C-GEBT. More convenient, but decreasing duration of the test associated with lower diagnostic accuracy. Tests with less than 4 h duration are markedly influenced by gastric emptying time

TABLE 8 (Continued)

	Age	Test meal	Breath sampling	Endpoints and normal values	Validity	Remarks
Van Dijk-van Aalst et al. 2001[146]	12 premature infants, 12 full-term infants (1–6 months), 20 children (3–10 years),20 teenagers (11–17 years)	Infants: Formula with low ^{13}C content (e.g., NAN1 (Nestlé), Pre-Aptamil (Milupa) with **100 mg ^{13}C-MTG** and 1 g polyethylene-glycol 3350 > 3 years: slice of white bread with 5 g butter and 15 g chocolate paste, mixed with **250 mg ^{13}C-MTG**, 100 ml whole-fat milk	Two samples at baseline, further samples at 15 min intervals for 6 h pp	Cumulative 6h-$^{13}CO_2$-excretion (% of dose administered) mean ± SD: Premature infants: 23.9 ± 5.2% Full-term infants: 31.9 ± 7.7% Children: 32.5 ± 5.3% Teenagers: 28.0 ± 5.4%	-	Mean value for healthy adults: 35.6%, lower limit of normal 22.8%

Abbreviations: ^{13}C-GEBT, ^{13}C-gastric emptying breath test; ^{13}C-MTG, mixed triglycerides; ^{13}C-MTGBT, ^{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.

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]

How should test results be reported?

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.

^{13}C-LIVER FUNCTION BREATH TESTS

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 $^{13}CO_2$. 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.[153–160] 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]

When should ^{13}C-LFBT be utilized?

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.[152,157,159–168] These have been mainly used in patients with liver fibrosis and cirrhosis due to nonalcoholic fatty liver disease,[152,157,159–161,163,164,169,170] 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.[175–177]

Given that, as yet, few studies have been performed involving small groups of children with rare diseases,[178–181] the use of ^{13}C-LFBT for clinical purposes cannot be recommended in the pediatric population, so far.

How should ^{13}C-LFBT be performed?

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).

TABLE 9 Examples of validated test protocols for ^{13}C-liver function breath tests in adults

	Estimated parameter	Marker and test solution	Breath sampling	Endpoints and normal values	Validity	Remarks
Afolabi et al 2018[161]	Hepatic mitochondrial function	**1 mg/kg body weight of ^{13}C-ketoisocaproate** plus 20 mg/kg body weight L-leucine dissolved in 200 ml of water	At baseline and at 10 min intervals for 60 min pp	Cumulative ^{13}C-recovery, normal >21% of dose	-	Validated in 11 HC and 77 pts with NAFLD, SENS and SPEC to detect significant fibrosis was not determined
Portincasa et al 2006[157]	Hepatic mitochondrial function	**1 mg/kg body weight of ^{13}C-ketoisocaproate** plus 1 g of L-leucine dissolved in 200 ml of water	At baseline and at 10 min intervals postprandially for 60 min pp	Cumulative ^{13}C-recovery, normal >14% of dose	Diagnostic accuracy at identifying pts with NASH (cut-off value 9.6%):SENS 68%, SPEC 94%,PPV 90%, NPV 73%	Validated in 28 HC and 39 pts with NAFLDThe test was also able to discriminate fibrosis stages in patients with NASH
Banasch et al 2011[156]	Hepatic mitochondrial function	**2 mg/kg body weight [methyl-^{13}C]-methionine** dissolved in 100 ml of water, prior oral consumption of 200 ml of orange juice	At baseline and at 10 min intervals for 90 min pp	Cumulative ^{13}C-recovery, normal >6.1% of dose	Cut-off value <4.20% of dose for separation of pts with NASH from non-NASH, SENS 81%; SPEC 76%	Validated in 118 pts with NAFLD and 18 HCTest predicts higher stages of disease activity
Korkmaz et al. 2015[160]	Hepatic mitochondrial function	**2 mg/kg body weight [methyl-^{13}C]-methionine** dissolved in 100 ml water	At baseline and at 10 min intervals for 90 min pp	Cumulative ^{13}C-recovery, normal >6.2% of dose	Cut off value <3.71%: SENS 95% SPEC 88% for differentiating advanced liver fibrosis (F2–3) from mild (F0–1) fibrosis	Validated in 164 pts with NAFLD and 56 HC
Fierbinteanu-Braticevici et al.2013[163]	Hepatic microsomal function	Fixed dose of **75 mg ^{13}C-methacetin** dissolved in 200 ml of water	At baseline and at 10 min intervals for 60 min pp	Cumulative ^{13}C-recovery, normal >22% of dose	Cut off value <15.2%, SENS 91%, SPEC 82% at detecting significant fibrosis ($F \geq 2$)	Validated in 90 pts with NAFLD and 20 HC
Park et al 2003[167]	Hepatic microsomal function	**2 mg/kg body weight of [3-methyl-^{13}C]-caffeine** dissolved in 30 ml of water, followed by 40 ml water wash of the container	At baseline and at 10 min intervals for 60 min pp	Cumulative ^{13}C-recovery, normal >2.3% of dose	Cut off value <1.85%: 79% SENS 80% SPEC for detecting NASH	Validated in 48 pts with NAFLD, 48 patients with chronic hepatitis B and 24 HC subjects. Results reflect the extent of hepatic fibrosis

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

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,[182–184] 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.

How should test results be reported?

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.

OTHER ^{13}C-BREATH TESTS

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.

CONCLUSIONS AND FUTURE PERSPECTIVES

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 metabolization, and pulmonary excretion. Therefore, demographic

TABLE 10 Examples of other ^{13}C-breath tests

References	Estimated parameter	Remarks
(185–194)	Insulin resistance, metabolic syndrome	^{13}C-glucose + unmarked glucose (e.g., 75 g) dissolved in water, outcome parameters: Cumulative $^{13}CO_2$-exhalation, maximal DOB-value, ^{13}C-exhalation at specific time point
		^{13}C-exhalation is significantly decreased in patients with impaired insulin resistance/ metabolic syndrome, site of labeled carbon atom in glucose molecule is relevant for metabolic results
		Breath test described as valid surrogate index of clamp-derived measures of insulin resistance, with good accuracy and precision, but controversial findings regarding applicability as screening tool (insufficient accuracy) in minority of studies
		Non-invasive tool for early diagnosis and follow up of patients in high-risk groups
(195–198)	SIBO, OCTT	Lactose-[^{13}C]ureide, moderate sensitivity (66.7%), high specificity (100%) for SIBO in comparison with culture from jejunal aspirates, glucose-[^{13}C]ureide could be used alternatively
		Bacterial metabolism of marker is prerequisite for $^{13}CO_2$-exhalation, increase caused by SIBO or arrival of the marker substance in the colon
		Predosing with unlabelled ureide is frequently part of the protocol, induces enzyme induction, increases rate of $^{13}CO_2$-recovery but complicates procedure
(136,199–201)	PEI (amylase deficiency) Congenital sucrase-isomaltase deficiency	Maize starch is naturally enriched with ^{13}C, 50–100 g used as pancreatic function test, but limited sensitivity (up to 77%) and specificity (up to 74%) in comparison with "gold standard" (secretin cerulein test)
		$^{13}CO_2$ release following labelled starch or sucrose is decreased in patients with sucrase-isomaltase deficiency
(202–210)	Activity of drug metabolizing enzymes	^{13}C-erythromycin applied iv (or orally) → hepatic (and intestinal) cytochrome P450 3A4 and 3A5 (CYP3A4/5) activity in vivo, pilot studies
		^{13}C-pantoprazole → CYP2C19 activity, can predict the anti-platelet efficacy of clopidogrel (which is metabolized by CYP2C19 to its active metabolite) and high metabolization rate of PPI
		^{13}C-dextromethorphan → CYP2D6 activity (e.g., relevant for activation of the prodrug tamoxifen), [2-(13)C]uracil → dihydropyrimidine dehydrogenase (DPD) deficiency to predict 5-fluorouracil dose-related toxicity

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

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.

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.

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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CONFLICT OF INTEREST

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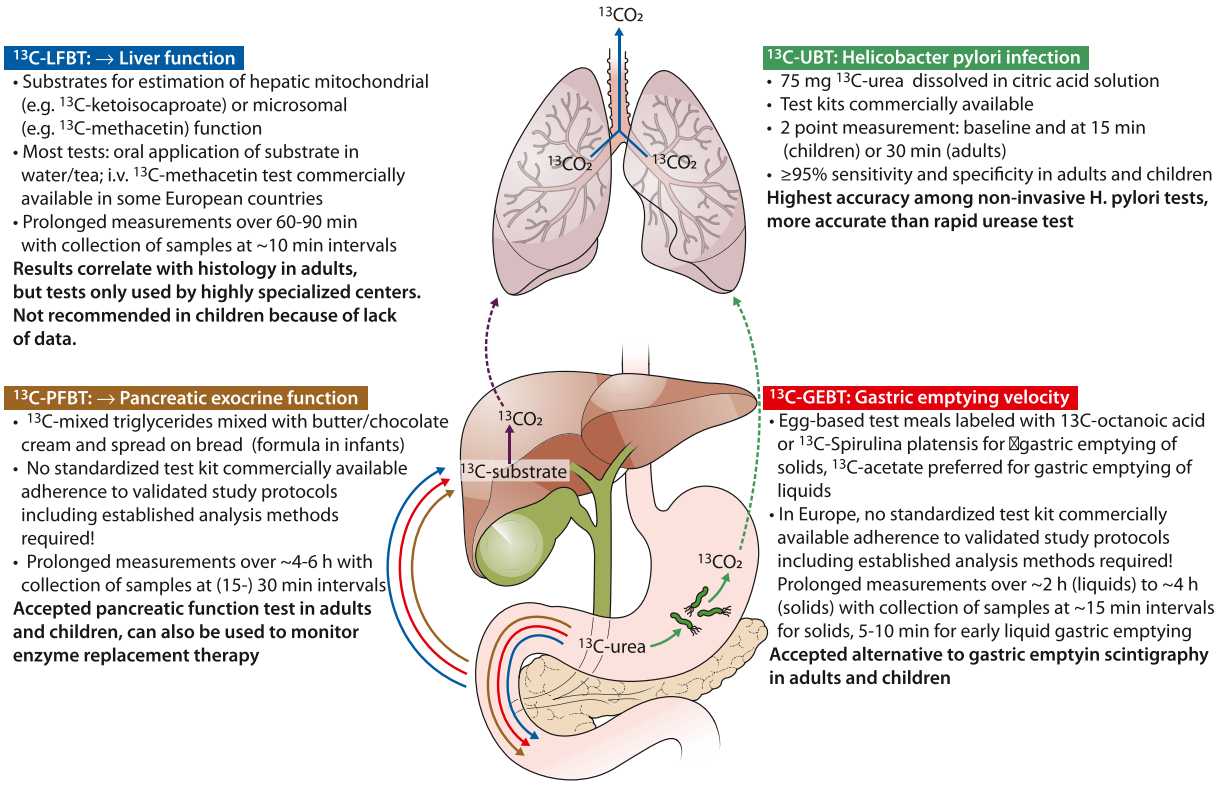

FIGURE 2 General principle, performance and clinical role of ^{13}C-breath tests used in gastroenterology. ^{13}C-urea used for detection of *H. pylori* (^{13}C-UBT, marked in green) is metabolized by bacterial urease to produce $^{13}CO_2$, which is absorbed, transported to the lung (broken green arrow) 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 metabolization with production of $^{13}CO_2$, which is transported to the lung and exhaled

DATA AVAILABILITY STATEMENT

Data sharing is not applicable to this article as no new data were created or analyzed in this study.

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