

^{13}C -Breath Tests for the
Assessment of Specific
Enzymatic and Metabolic
Functions *in vivo*





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Introduction

Founded by a management team of clinical scientists, physicians, laboratory experts and business development specialists, Seahorse Laboratories Ltd is a privately owned and academically centred Diagnostics and Distribution services company. We specialise in the provision of turnkey solutions for bio-pharmaceutical and medical device companies including warehousing, distribution, service development, training, sales and marketing.

Seahorse Laboratories is a licensed wholesale distributor, and a member of the Seahorse Group of companies.

Seahorse Laboratories are the exclusive UK distributor for Kibion AB, the leading provider of complete solutions of both diagnostic isotope breath tests and instruments, and has attained a leading position in the testing of *H. pylori*. The tests and instruments are cost effective, reliable and easy to use in settings including the hospital, laboratory and doctor's office.

Metabolic breath tests

Non-invasive breath tests can serve as valuable diagnostic tools in medicine as they can determine particular enzymatic and metabolic functions in vivo. This has wide applications in the fields of gastroenterology, oncology, hepatology and nutrition control. A $^{13}\text{CO}_2$ breath test measures increased levels of $^{13}\text{CO}_2$ in exhaled breath after ingestion of a stable ^{13}C isotope labelled substance and its subsequent metabolism with a specific function or enzyme as a rate limiting step. Breath samples are collected and measured, for example, with a Kibion® instrument, measuring the stage between ingestion by the patient of the labelled substance and its appearance in the exhaled breath.

This brochure describes the principles and general test procedures based on information in published literature for a number of tests, which are the most common in today's clinical research.

In the UK, these substrates are available directly from Seahorse Laboratories.

Kibion® Dynamic

The Kibion® Dynamic is the latest offer in the Kibion instrument family based on the IRIS technology, and a foremost instrument for quantitative diagnosis of breath tests. Kibion® Dynamic employs detectors of a non-radioactive ^{13}C -labelled stable isotope based on infra-red technology.

The Kibion® Dynamic Infra Red Isotope analyser measures the $^{13}\text{CO}_2$ and $^{12}\text{CO}_2$ concentrations from sequences of breath samples and relates their ratios to the PDB- ^{13}C stable isotope standard. The reproducibility is in optimal conditions better than 0.2 ‰ over a wide range of $^{13}\text{C}/^{12}\text{C}$ stable isotope ratios, and over a wide range of CO_2 concentrations in breath.

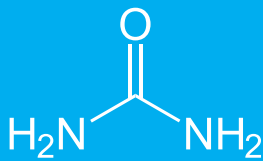
Measurements are made on breath samples as they come from the breath sample bags or tubes. No separation of water or isolation of CO_2 is required prior to analysis.

The Kibion® Dynamic can be up-scaled by using an extension device – Kibion® Dynamic Pro – for increased throughput based on the needs of the laboratory, hospital or doctor's office and can be connected to a multisampler for high throughput testing.



¹³C-Urea Breath Test – Diabact® UBT

¹³C-Urea



Molecular weight:	61.05 g/mol
Enrichment:	99 %
Labeled C-atoms:	1
Dosage:	50 mg

Test principle

Isotopically labelled urea is metabolised into carbon dioxide and ammonia by the enzyme urease which is produced by the bacteria, *Helicobacter pylori*. The available ¹³C isotope, now in the form of ¹³CO₂ diffuses into the blood to be transported to the lungs, where it is exhaled in the breath to be captured during sampling. An increased ratio of ¹³C is conclusive proof of the presence of *Helicobacter pylori* in the patient's stomach.

Application of UBT -¹³C Urea Breath Test

Helicobacter pylori is extremely common in humans, infecting around 50 % of the world's population. It is recognised as the main etiological factor for chronic gastritis, peptic ulcer and possibly also gastric malignancies. Much suffering and even death related to ulcers can be easily prevented through accurate diagnosis and appropriate treatment with antibiotics.

The current challenge is to prevent a chronic *Helicobacter pylori* infection and its development to gastric cancer, as well as to understand the role of *Helicobacter pylori* in extra-gastric diseases.

Diabact UBT -¹³C Urea Breath Test

Diabact UBT is a proprietary formulation from Kibion, with several advantages in a clinical setting, including no need for a test meal, no mixing of solution, only 10 minutes wait and a high specificity and sensitivity (respectively 100% and 99%).

Test Performance Procedure

Patient preparation

The patient should have fasted for 6 hours prior to the test and not have taken PPI for 2 weeks before the test is performed. Antibiotic treatment should have been discontinued one month before testing. Please also refer to the SPC.

No test meal needed

With Diabact® UBT no test meal is necessary. Citric acid is included in the tablet and there is no need for mixing of solution; simply swallow a tablet.

Test procedure

1. Patient exhales into basal sample tubes (0-tubes).
2. Patient swallows a Diabact® UBT tablet with a glass of water.
3. After a 10-minute wait, patient exhales into sample tubes.
4. Samples are analysed with Kibion® Dynamic

Results and Interpretation

Diabact® UBT for diagnosis of *Helicobacter pylori* is a qualitative test. The result will show if the patient is infected or not infected.

The established cut-off using mass spectrometry is

<1.5 ‰ δ value = Negative *H.pylori* status

>1.5 ‰ δ value = Positive *H.pylori* status



1. Breathe into base line tubes



2. Swallow Diabact® UBT tablet



3. After a 10-minute wait, breathe into sample tubes



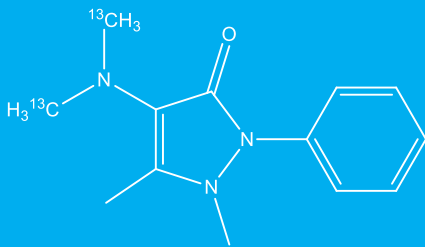
4. Send the tubes for analysis.

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¹³C-Aminopyrine Breath Test

¹³C-Aminopyrine



Molecular weight:	233.29 g/mol
Enrichment:	99 %
Labeled C-atoms:	2
Dosage:	75 mg

Metabolic principle

¹³C-Aminopyrine undergoes a two-step N-demethylation by cytochrome P-450 monooxygenases including CYP2C19, CYP1A2 and CYP3A4, yielding formaldehyde and amino-antipyrine¹. The formaldehyde is further oxidized to bicarbonate and exhaled as ¹³CO₂, or deposited in the bicarbonate pool². As N-demethylation occurs exclusively in the liver with a low extraction rate, this parameter is an overall reflection of the efficiency of aminopyrine metabolism³. It is therefore a good measure of hepatic metabolic capacity, i.e. the "functional hepatic mass".

Applications of ¹³C-Aminopyrine Breath Test

The ¹³C-Aminopyrine Breath Test is very useful for quantitative assessment of liver function in conditions such as established chronic hepatitis and cirrhosis^{4,5}. For example, it can be used to quantify progression of the disease in Hepatitis C patients⁶.

The patient should have fasted for 8 hours prior to the test. Smoking should also be avoided at least one hour prior to the test⁷. The patient should not drink carbonated water or soft drinks prior to the test since that might interfere with the results. In addition, oxygen supplementation should be avoided because increased oxygen content in exhaled breath can influence ¹³CO₂ measurement by NDIRS⁸.

Test Performance Procedure (see Operating Manual for additional information).

1. Collect zero (basal) breath sample as described in manual.
2. Patient takes ¹³C-Aminopyrine (75 mg) dissolved in warm water (100 ml).
3. Collect additional breath samples as shown below (Table 1).
4. Analyse all 10 breath samples with a Kibion instrument.

#1 Bag	#2 Bag	#3 Bag	#4 Bag	#5 Bag	#6 Bag	#7 Bag	#8 Bag	#9 Bag	#10 Bag
0 min	10 min	20 min	30 min	40 min	50 min	60 min	80 min	100 min	120 min

Table 1: ¹³C-Aminopyrine Breath Test Sample Collection

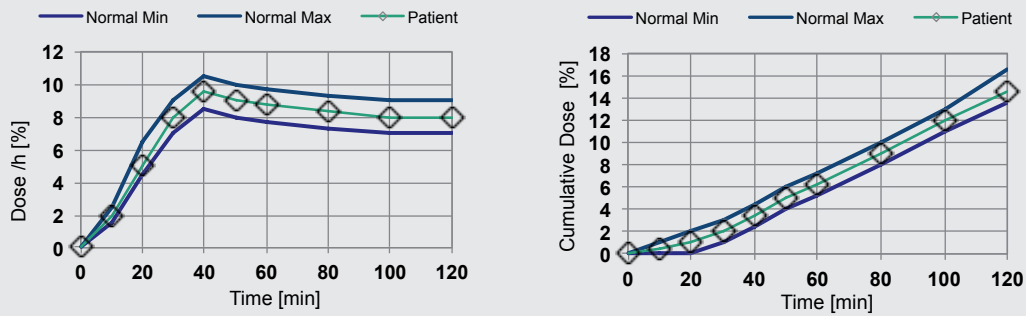


Fig. 1,2: ¹³C-Aminopyrine Breath Test, Dose/h curve and % Cum Dose curve, healthy (normal) subject¹¹

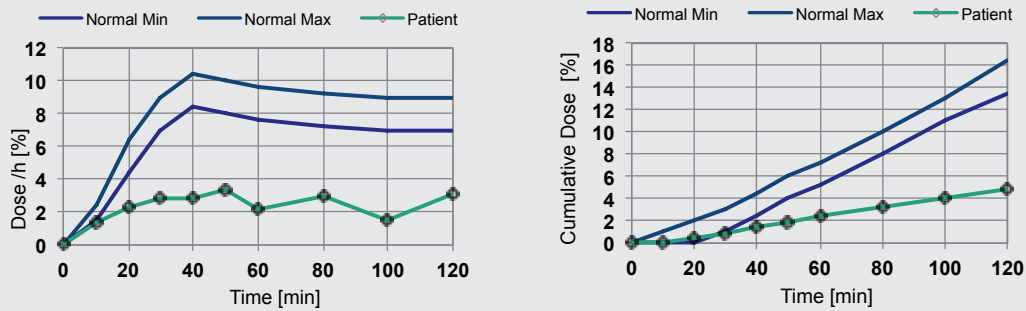


Fig. 3,4: ¹³C-Aminopyrine Breath Test, Dose/h curve and % Cum Dose curve, subject with liver disease¹¹

Results and interpretation

Typical results for the ¹³C-Aminopyrine Breath Test are presented in Figures 1 to 4. The ¹³C-Aminopyrine test is very sensitive and precise, as can be seen from the very narrow “normal” range. This makes it even possible to detect patients with early stage liver disease^{6,9,10}.

For the ¹³C-Aminopyrine Breath Test, cut-off values have been established in a study with 135 patients¹¹ (see table below).

Condition	dose/hr (‰) at 30 min	% cum. dose at 120 min
Fibrosis stages 0/1/2	6.62 - 7.10 ± 2.9	9.21 - 10.06 ± 3.8
Fibrosis stages 3 / 4	2.48 - 3.13 ± 1.2	3.62 - 4.56 ± 2.0
Cirrhosis, not established	6.77 ± 2.7	9.63 ± 3.6
Cirrhosis, established	2.48 ± 1.2	3.68 ± 1.9

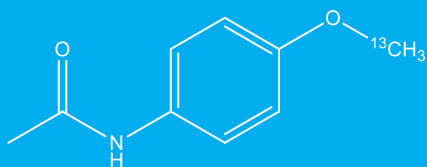
Table 2: Cut-off values for ¹³C-Aminopyrine Breath Test ¹¹

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¹³C-Methacetin Breath Test

¹³C-Methacetin



Molecular weight:	166.19 g/mol
Enrichment:	99 %
Labeled C-atoms:	1
Dosage:	75 mg

Metabolic principle

Methacetin is metabolised rapidly in normal subjects, being highly extracted by the liver¹, implying that the metabolism of methacetin is mainly dependent on hepatic blood flow, the latter being generally decreased in cirrhotic patients². Methacetin undergoes dealkylation by hepatic CYP1A2 to acetaminophen³ with the methoxy group being eliminated as ¹³CO₂.

Published data of previous studies suggest that the Methacetin Breath Test is a rapid and precise quantitative liver function test without any evidence of toxicities due to the small doses used, in contrast to other substrates⁴⁻⁷.

Applications of ¹³C-Methacetin Breath Test

The liver status of patients who have been diagnosed with liver disease can be assessed or monitored non-invasively using the ¹³C-Methacetin Breath Test:

The patient should have fasted for 8 hours prior to the test.

Smoking should also be avoided at least one hour prior to the test¹³. The patient should not drink carbonated water or soft drinks prior to the test since this might interfere with the results. In addition, oxygen supplementation should be avoided because increased oxygen content in exhaled breath can influence ¹³CO₂ measurement by NDIRS¹⁴.

Test Performance Procedure (see Operating Manual for additional information).

1. Collect zero (basal) breath sample as described in the manual.
2. Patient takes ¹³C-Methacetin (75 mg) dissolved in water (100 ml).
3. Collect additional breath samples as shown below (Table 2).
4. Analyse all 10 breath samples with a Kibion Instrument.

Condition	Assessment
Non-alcoholic steatohepatitis (NASH) or alcoholic steatohepatitis (ASH), Fibrosis or Cirrhosis	State of evolution (correlation with Child-Pugh Score) ^{8,9}
Fibrosis or Cirrhosis	State of evolution (correlation with Child-Pugh Score) ^{8,9}
Liver tumor	Hepatic reserve
Hepatitis B or C	Hepatic reserve ¹⁰
Long-term medication e.g. anticonvulsants	Monitor hepatotoxicity
Liver transplant	Liver status of both donor and recipient ^{11,12}

Table 1: Liver diseases assessed by ¹³C-Methacetin Breath Test

#1 Bag	#2 Bag	#3 Bag	#4 Bag	#5 Bag	#6 Bag	#7 Bag	#8 Bag	#9 Bag	#10 Bag
0 min	10 min	20 min	30 min	40 min	50 min	60 min	80 min	100 min	120 min

Table 2: ¹³C-Methacetin Breath Test Sample Collection

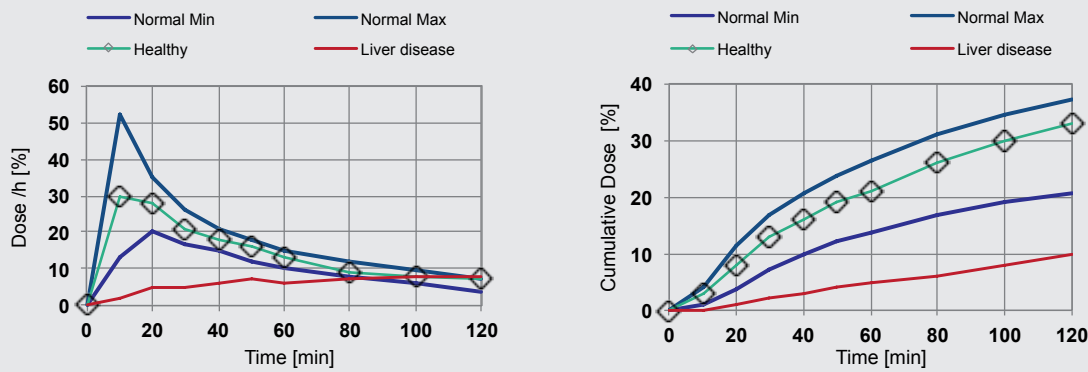


Fig. 1-2: ¹³C-Methacetin Breath Test, Dose/h curve and % Cum Dose, healthy (normal) subject¹⁶

Results and interpretation

In healthy subjects a peak in the exhaled Dose/h of labeled CO₂ is to be expected after 10 to 20 minutes (see Figure 1). About 30% of the administered dose is recovered as ¹³CO₂ after 120 minutes (see Figure 2). In general, the more severe the liver disease, the lower the % cum dose after 120 minutes.^{8,10,15}

The value of the maximum metabolic rate (dose/h) has been shown to be a good quantitative predictor of

cirrhosis and fibrosis in chronic hepatitis C (Table 3). The % cumulative dose at 120 minutes has been shown to correlate with different stages of liver disease (Table 4).

% Cumulative Dose, 120 min	Indication/ Correlation
31.0 (25.9 – 38.7)	Normal
13.6 (5.7 – 22.3)	Cirrhosis, Child-Pugh Class A
3.1 (1.1 – 16.5)	Cirrhosis, Child-Pugh Class B
0.6 (-1.1 – 3.5)	Cirrhosis, Child-Pugh Class C

Table 4: Correlation of ¹³C-Methacetin Breath Test (% cum dose) with stage of liver disease⁸

		Cut-off	Sensitivity	Specificity
Liver Cirrhosis	¹³ C-Methacetin Breath Test	< 14.6 %	92.6 %	84.1 %
	Fibroindex	> 1.82	70.4 %	91.3 %
Advanced Fibrosis	¹³ C-Methacetin Breath Test	< 21 ‰	75.4 %	79.5 %
	Fibroindex	> 1.35	66.7 %	84.6 %

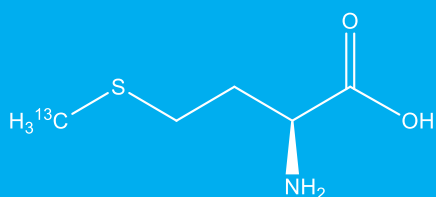
Table 3: Comparison of ¹³C-Methacetin Breath Test and FibroIndex as predictors of cirrhosis and fibrosis. (Adapted from Dinesen et al.¹⁷)

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¹³C-L-Methionine Breath Test

¹³C-L-methionine



Molecular weight:	150.2 g/mol
Enrichment:	99 %
Labeled C-atoms:	1
Dosage:	75 mg

Metabolic principle

Methionine is an essential amino acid, metabolised in the liver through two major pathways: transamination and transmethylation. Transmethylation is the predominating metabolic pathway by which methionine is normally converted to S-adenosyl-L-methionine (SAM) and which is used as a cofactor by methyltransferases to transfer the ¹³C-methyl group to different target molecules (methylation). However, the major pathway to remove excess methionine and for the transfer of its methyl group is via sarcosine production, which in this instance generates ¹³C-sarcosine. The labeled sarcosine is oxidized by sarcosine dehydrogenase to produce ¹³C-formaldehyde in the mitochondria which is further oxidised to ¹³CO₂ and expired. Since the oxidation of sarcosine occurs in the mitochondria of the liver¹, ¹³C-methionine can be used to evaluate the oxidative capacity of the liver². This test is therefore a good measure of the hepatic metabolic capacity.³⁻⁵

Applications of ¹³C-L-Methionine Breath Test

The ¹³C-L-Methionine Breath Test is a non-invasive diagnostic test to assess in vivo hepatic mitochondrial function. Dysfunction of hepatic mitochondria is associated with several chronic liver diseases and the

test can be applied to investigate drug-related acute liver toxicity^{6,7}, ethanol-induced liver oxidative stress⁸, impaired hepatic mitochondrial oxidation in liver steatosis such as non-alcoholic fatty liver disease (NAFLD) or cirrhosis^{4,9}.

The patient should have fasted for 8 hours prior to the test. Smoking should also be avoided at least one hour prior to the test¹⁰. The patient should not drink carbonated water or soft drinks prior to the test since that might interfere with the results. In addition, oxygen supplementation should be avoided because increased oxygen content in exhaled breath can influence ¹³CO₂ measurement by NDIRS¹¹.

Test Performance Procedure (see Operating Manual for additional information)

1. Collect zero (basal) breath sample as described in the manual.
2. Patient takes ¹³C-L-Methionine (75 mg) dissolved in water (100 ml).
3. Collect additional breath samples as shown below (Table 2).
4. Analyse all 10 breath samples with a Kibion instrument.

#1 Bag	#2 Bag	#3 Bag	#4 Bag	#5 Bag	#6 Bag	#7 Bag	#8 Bag	#9 Bag	#10 Bag
0 min	10 min	15 min	20 min	25 min	30 min	40 min	60 min	90 min	120 min

Table 1: ¹³C-L-Methionine Breath Test Sample Collection

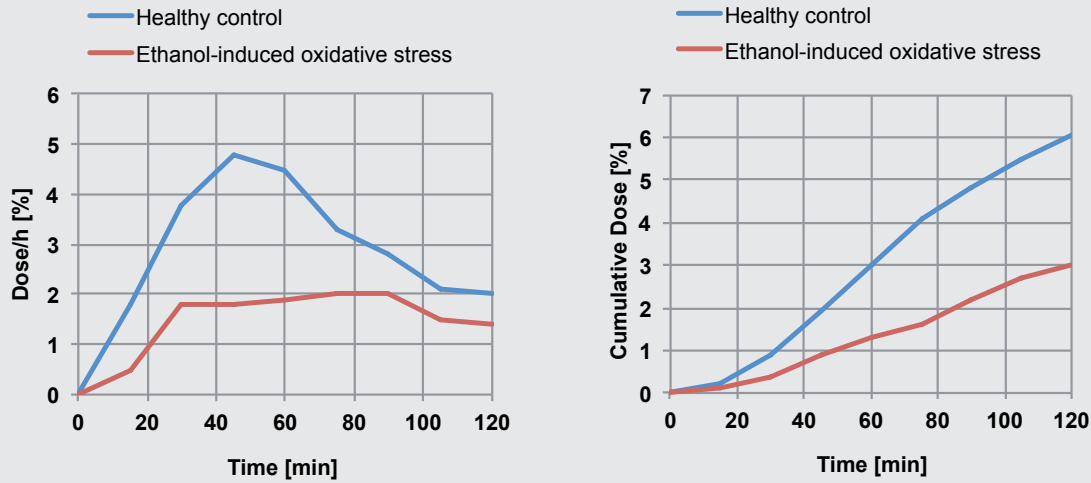


Fig. 1,2: Example of ^{13}C -Methionine Breath Test, Dose/h curve and % Cum Dose, (Armuzzi et al., 2000⁸)

Results and interpretation

In healthy subjects, a peak in the exhaled Dose/h of labeled CO_2 is to be expected after 30 to 60 minutes (see Figure 1). According to published values by Armuzzi et al.⁷ the cumulative dose in healthy controls after 120 minutes reaches $6.07 \pm 0.46\%$ ⁸ whereas control groups in the following studies also showed slightly increased values (e.g. cumulative dose after 90 minutes: $7.16\% \pm 1.91\%$; see Stüwe et al., 2013¹²). In general, the more severe the liver disease, the lower the % cumulative dose after 90 or 120 minutes.^{4,7,8}

In another study by Banasch et al. specific cut-off values for the cumulative dose at 90 minutes to assess non-alcoholic steatohepatitis and fibrosis stage 0-1 versus fibrosis stage 2-3 in a NAFLD cohort have been calculated.

	Cut-off
non-alcoholic steatohepatitis (NASH) vs. non-NASH	< 4.20 %
Fibrosis stage 0-1 vs. Fibrosis stage 2-3 (within NAFLD cohort)	< 3.65 %

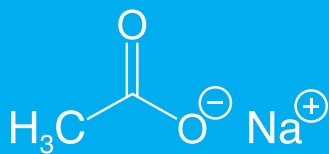
Table 3: Cut-off values for non-alcoholic steatohepatitis (NASH) and mild vs. severe fibrosis in a NAFLD cohort according to Banasch et al., 2011⁴

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¹³C-Sodium-Acetate Breath Test

¹³C-Sodium-Acetate



Molecular weight:	82,03 g/mol
Enrichment:	99 %
Labeled C-atoms:	1
Dosage:	75 mg

Metabolic principle

¹³C-Sodium-Acetate is administered together with a liquid or semi-solid test meal. After passing through the stomach, where it is not absorbable, it is absorbed in the small intestine and metabolised in the liver¹. Whilst some of the labeled carbon is incorporated in different metabolic pathways, about 50 % enters the body's bicarbonate pool and is exhaled². As the rate-limiting step in this process is the stomach-emptying rate, this test is a reliable application to assess liquid gastric emptying^{3,4}.

Applications of ¹³C-Sodium-Acetate Breath Test

The ¹³C-Sodium-Acetate Breath Test is very useful for the investigation of functional dyspepsia and autonomic diabetic neuropathy⁵. Gastroparesis has also been shown to be associated with functional gastrointestinal^{6,7} and inflammatory disorders of the gastrointestinal tract⁸.

The patient should have fasted for 10 hours prior to the test. The patient should not drink carbonated water or soft drinks prior to the test since that might interfere with the results. In addition, oxygen supplementation should be avoided because increased oxygen content in exhaled breath can influence ¹³CO₂ measurement by NDIRS⁹.

Test Performance Procedure (see Operating Manual for additional information)

1. Collect zero (basal) breath sample as described in manual.
2. Enter patient height and weight into the instrument software.
3. Patient takes ¹³C-Sodium-Acetate (75 mg) dissolved in a liquid or semi-solid test-meal with about 250 kcal (e.g. 200 ml Fresubin®, Fresenius Kabi AG, Switzerland)
4. Collect breath samples as shown below (Table 1).

#1 Bag	#2 Bag	#3 Bag	#4 Bag	#5 Bag	#6 Bag	#7 Bag	#8 Bag	#9 Bag	#10 Bag	#11 Bag	#12 Bag	#13 Bag
0 min	15 min	30 min	45 min	60 min	75 min	90 min	105 min	120 min	150 min	180 min	210 min	240 min

Table 1: ¹³C-Sodium-Acetate Test Sample Collection

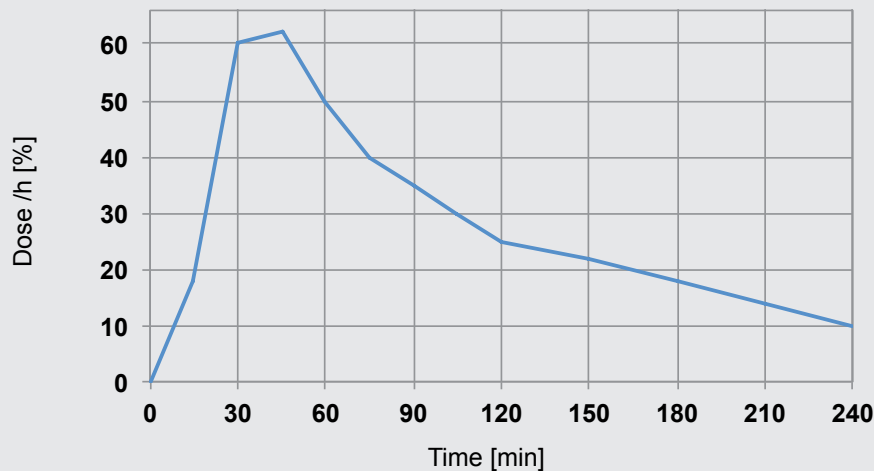


Fig. 1: Example of ^{13}C -Sodium-Acetate gastric emptying breath test, Dose/h curve

- Analyse all 13 breath samples with a Kibion instrument.

Results and interpretation

Gastric emptying parameters are assessed by calculation of the half-emptying time ($T_{1/2B}$), the lag phase (T_{lagB}) and the gastric emptying coefficient (GEC), which have been introduced and validated against scintigraphy by Ghooos et al¹⁰. This method is still the most frequently applied method, although different analytical methods are currently under validation. These parameters are estimated by non-linear regression analysis directly with the instrument software (please refer to the manual).

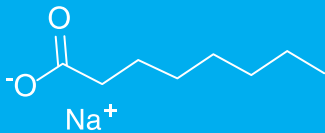
As the results are dependent on the test meal, it is strongly recommended that each laboratory establishes its own reference values. For semi-solid test meals, Braden et al. found cut-off values of 106 minutes (mean + 2 SD) for the half-emptying time and 55 minutes (mean + 2 SD) for the peak excretion in 20 healthy patients³. Another study by Braden et al. resulted in half-emptying times of 90 minutes as cut-off value in children¹¹. In 2006, Hauser et al. found median values of 81 minutes for $T_{1/2B}$ and 47 minutes for T_{lagB} with a liquid test meal in children¹².

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¹³C-Sodium-Octanoate and ¹³C-Octanoic Acid Breath Test

¹³C-Sodium-Octanoate



Molecular weight:	167.2 g/mol
Enrichment:	99 %
Labeled C-atoms:	1
Dosage:	100 mg

¹³C-Octanoic Acid



Molecular weight:	145.21 g/mol
Enrichment:	99 %
Labeled C-atoms:	1
Dosage:	91 mg

Metabolic principle

¹³C-Sodium-octanoate or ¹³C-Octanoic acid is administered together with solid test meals to assess the gastric emptying. Labeled octanoic acid is most commonly administered in egg yolk, into which it can be injected before baking^{1,2}. After passing through the stomach, it is absorbed in the small intestine and catabolised in the liver³. Whilst some of the labeled carbon is incorporated into different metabolic pathways, about 50 % enters the body's bicarbonate pool and is exhaled⁴. As the rate-limiting step in this process is the stomach-emptying rate, this test is a reliable application to assess solid gastric emptying⁵⁻⁷. Whether ¹³C-sodium-octanoate or ¹³C-octanoic acid is used is a matter of feasibility.

Applications of ¹³C-Sodium-Octanoate Breath Test

The ¹³C-Sodium-Octanoate Breath Test is very useful for the investigation of functional dyspepsia and autonomic diabetic neuropathy⁸. Gastroparesis has also been shown to be related to irritable bowel syndrome (IBS)^{9,10} and inflammation of the distal gastrointestinal tract¹¹.

The patient should have fasted for 10 hours prior to the test. The patient should not drink carbonated water or soft drinks prior to the test since that might interfere with the results. In addition, oxygen supplementation should be avoided because increased oxygen content in exhaled breath can influence ¹³CO₂ measurement by NDIRS¹².

Test Performance Procedure (see Operating Manual for additional information)

- Mix an egg with 100 mg of ¹³C-sodium-octanoate or inject 91 mg of ¹³C-octanoic acid into an egg yolk, mix it with egg white and bake. Serve it with 60 g of white bread, 5 g of margarine and 150ml of water (14 g of protein, 26 g of carbohydrate and 9 g of fat, 250 kcal)¹³.
- Collect a zero (basal) breath sample as described in the manual.
- Enter patient height and weight into the instrument software.
- Allow patient to eat the prepared egg meal.
- Collect breath samples as shown below (Table 1).
- Analyse all 13 breath samples with a Kibion instrument.

#1 Bag	#2 Bag	#3 Bag	#4 Bag	#5 Bag	#6 Bag	#7 Bag	#8 Bag	#9 Bag	#10 Bag	#11 Bag	#12 Bag	#13 Bag
0 min	15 min	30 min	45 min	60 min	75 min	90 min	105 min	120 min	150 min	180 min	210 min	240 min

Table 1: ¹³C-Sodium-Octanoate Test Sample Collection

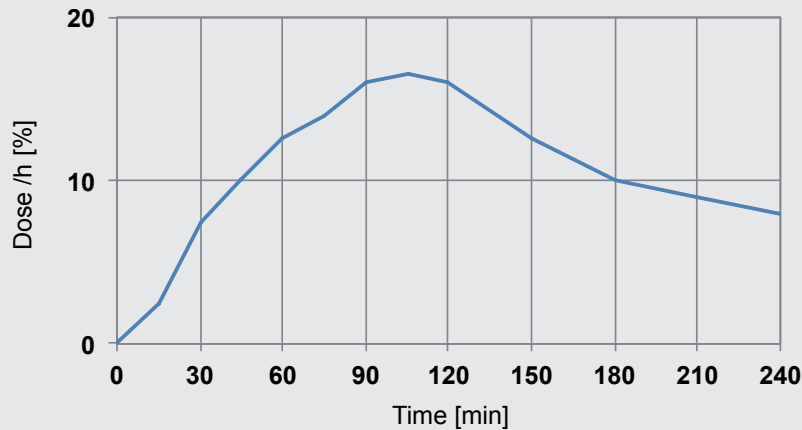


Fig. 1: Example of ¹³C-Sodium-Octanoate gastric emptying breath test, Dose/h curve

Results and interpretation

Gastric emptying parameters are assessed by calculation of the half-emptying time ($T_{1/2B}$), the lag phase (T_{lagB}) and the gastric emptying coefficient (GEC), which have been introduced and validated against scintigraphy by Ghooos et al¹³. This method is still the most frequently applied method, although different analytical methods are currently under validation. These parameters are estimated by non-linear regression analysis directly with the instrument software (please refer to the manual).

As the results are dependent on the test set-up – especially the calories of the provided meal - and the

population, it is strongly recommended that each laboratory establishes its own reference values. For solid test meals, Delbende et al. found a cut-off value for $T_{1/2B}$ of 124 minutes compared to scintigraphy for diagnosis of delayed gastric emptying⁶. Normal values calculated and corrected with scintigraphy by Ghooos et al. are for $T_{1/2B} = 72 \pm 22$ minutes and $T_{lagB} = 32 \pm 20$ minutes for a test meal of 250 kcal.¹³ Delbende and Ghooos adjusted to the scintigraphy by subtraction of 67 minutes and 66 minutes, respectively. Recommended cut-off values for the breath test result are 130 minutes for T_{lagB} and 200 minutes for $T_{1/2B}$ ¹⁴.

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¹³C-Mixed Triglyceride Breath Test

¹³C-Mixed Triglyceride

¹³C-Mixed Triglyceride consists of a Triglyceride containing two Stearic Acid molecules and one Octanoic Acid molecule. The Octanoic Acid molecule is labeled with ¹³C at the carboxyl carbon.

Molecular weight:	752.0 g/mol
Enrichment:	99 %
Labeled C-atoms:	1
Dosage:	150 mg

Metabolic principle

1,3-distearyl-2-{carboxyl-¹³C}octanoylglycerol, the so-called ¹³C-Mixed Triglyceride passes through the stomach and is digested by lipase activity in the duodenum¹. The two distearyl groups have to be hydrolysed by pancreatic lipase before absorption and metabolism of the ¹³C-octanoyl monoglyceride². Thus, the oxidation to ¹³CO₂ is dependent on the rate-limiting step of hydrolysis of the fatty acids in positions 1 and 3³.

Applications of ¹³C-Mixed Triglyceride Breath Test

The ¹³C-Mixed Triglyceride Breath Test assesses duodenal pancreatic lipase activity. It is therefore useful for the investigation of severe exocrine pancreatic insufficiency^{4,5}. If applied under strict conditions even mild to moderate forms can be assessed with high sensitivity and specificity⁶.

The patient should have fasted for 10 hours prior to the test. The patient must not drink carbonated water or soft

drinks prior to the test since that might interfere with the results. In addition oxygen supplementation should be avoided because increased oxygen content in exhaled breath can influence ¹³CO₂ measurement by NDIRS⁷.

Test Performance Procedure (see Operating Manual for additional information)

1. Mix 150 mg of ¹³C-Mixed Triglyceride with 0.25 g of butter per kg body weight and prepare it with 100 g of bread.
2. Collect a zero (basal) breath sample as described in the manual.
3. Enter patient height and weight into the instrument software.
4. Allow the patient to eat the prepared bread.
5. Collect breath samples as shown below (Table 1).
6. Analyse all 13 breath samples with a Kibion instrument.

#1 Bag	#2 Bag	#3 Bag	#4 Bag	#5 Bag	#6 Bag	#7 Bag	#8 Bag	#9 Bag	#10 Bag	#11 Bag	#12 Bag	#13 Bag
0 min	30 min	60 min	90 min	120 min	150 min	180 min	210 min	240 min	270 min	300 min	330 min	360 min

Table 1: ¹³C-Mixed Triglyceride Test Sample Collection

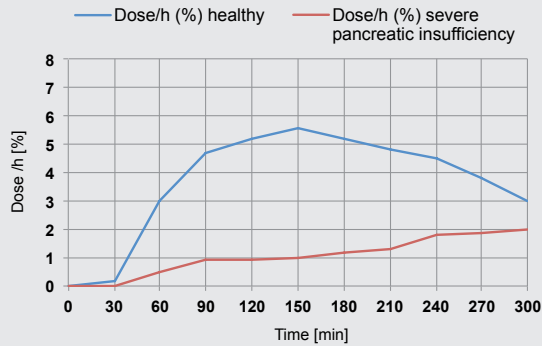


Fig. 1: Example of ¹³C-Mixed Triglyceride breath test, Dose/h (%) curve (see Löser et al.⁵)

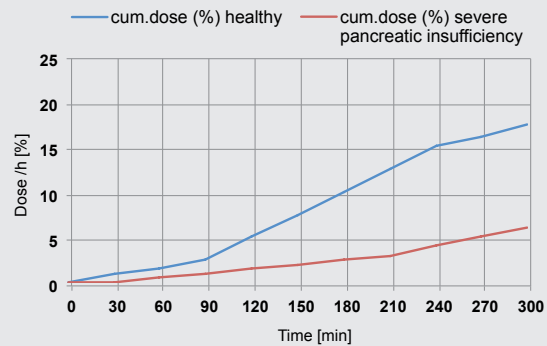


Fig. 2: Example of ¹³C-Mixed Triglyceride breath test, cum. dose (%) curve (see Löser et al.⁵)

Results and interpretation

Pancreatic function is assessed by the 6 hour cumulative ¹³CO₂ excretion. This can be calculated by the instrument software if the correct values for height and weight are entered. Vantrappen et al. found normal values to be at 35.6 % ± 2.8 %⁴. Another study by Swart et al. resulted in a normal value of 33.6 % ± 4.6 %¹. For detection of disease-diminished lipase output Vantrappen et al. suggested a cut-off value of 22 % cumulative CO₂ after 6 hours (sensitivity 0.89, specificity 0.81)⁴.

The two figures above show examples of curves for a 5-hour test set-up, taken from Löser et al.⁵

As the results are dependent on the test set-up and the population, it is strongly recommended that each laboratory establishes its own reference values.

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SEAHORSE
LABORATORIES

Seahorse Laboratories Ltd.

Tel: +44 20 8257 8412 · Fax: +44 20 8257 8417

21 Rhodesia Road, London, E11 4DF, United Kingdom

info@seahorse-labs.com · www.seahorse-labs.com