kibion

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

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DISCLAIMER

Please note that all substrates described in this brochure, except ^{13}C-urea – Diabact® UBT 50 mg (registered pharmaceutical), are laboratory chemicals (also called extempore or special medicine preparations –human use). Please contact your local pharmacy for more information about how to order substrates in your region.

It is also advisable to contact the relevant medical product agency (MPA) for more precise information about the use of substrates and its accompanying responsibilities.

NOTE

The information in this brochure is based on literature references, which are believed to be correct. The possibility of mistakes or errors cannot be excluded completely. Therefore Kibion AB does not accept any legal or other liability with respect to incorrect details and their consequences.

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Introduction

Kibion is a dynamic, world-leading supplier of simple and reliable breath tests for diagnosing the stomach ulcer bacterium *Helicobacter pylori*.

A subsidiary of the Swedish pharmaceutical company Orexo AB, Kibion was founded in 2005 to create a dedicated platform for commercializing breakthrough discoveries in the diagnosis of *Helicobacter pylori.*

Kibion together with its subsidiary in Bremen, Germany, is the present day provider of complete solutions of both diagnostic 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.

Quality
Kibion provides customers with high quality products and services.

The quality of our processes, products and services are continuously optimized and improved to meet customers' demands and needs.

Kibion AB is certified based on EN ISO 13485 – Medical Devices – Quality Management Systems – Requirements for regulatory purposes. The scope of the certificate includes development, production and distribution of IVD medical devices. The Certification was carried out by TÜV SÜD Product Service GmbH which is a globally recognized Certification Body.

The EN ISO 13485 certification allows Kibion AB to further strengthen and develop its leading position as provider of breath tests for detection of *Helicobacter pylori* worldwide.

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 $^{13}CO_2$ breath test measures increased levels of $^{13}CO_2$ 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 an IRIS® instrument, measuring the stage between ingestion by the patient of the labeled 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.

IRIS

IRIS® is a foremost instrument for quantitative diagnosis of breath tests. IRIS® employs detectors of non-radioactive ^{13}C-labelled stable isotope based on infra-red technology.

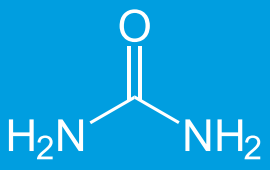
The IRIS® Infra Red Isotope analyzer measures the $^{13}CO_2$ and $^{12}CO_2$ concentrations from sequences of breath samples and relates their ratios to the PDB-^{13}C stable isotope standard. The reproducibility is in optimal conditions better than 0.2 δ ‰ (IRIS-Doc: 0.4 δ ‰) over a wide range of $^{13}C/^{12}C$ 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. Standard breath bags have a volume of 120 ml breath gas, which allows for two measurements per sample.

The IRIS® instrument is available in two different models, IRIS®-3 and IRIS®-Doc and can be connected to the IRIS®-Multisampler for high throughput testing.

^{13}C-Urea Breath Test – Diabact® UBT

^{13}C-Urea



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

Test principle

Isotopically labelled urea is metabolized into carbon dioxide and ammonia by the enzyme urease which is produced by the bacteria, *Helicobacter pylori*. The available ^{13}C isotope, now in the form of $^{13}CO_2$ 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 ^{13}C is conclusive proof of the presence of *Helicobacter pylori* in the patient's stomach.

Application of Diabact UBT -^{13}C 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.

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.

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 IRIS®-Doc or IRIS®-3.

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

The cut-off when using IRIS-3 is 1.5 ‰ ± 0.2.
The cut-off when using IRIS-Doc is 1.5‰ ± 0.4.

1. Breath 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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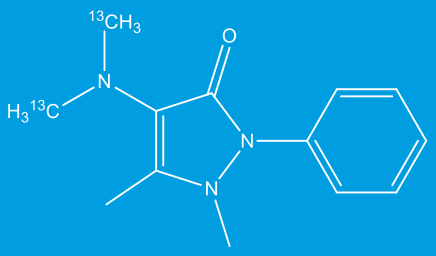
^{13}C-Aminopyrine Breath Test

^{13}C-Aminopyrine



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

Metabolic principle

^{13}C-Aminopyrine undergoes a two-step N-demethylation by cytochrome P-450 monooxygenases including CYP2C19, CYP1A2 and CYP3A4, yielding formaldehyde and amino-antipyrine[1]. The formaldehyde is further oxidized to bicarbonate and exhaled as $^{13}CO_2$, or deposited in the bicarbonate pool[2]. As N-demethylation occurs exclusively in the liver with a low extraction rate, this parameter is an overall reflection of the efficiency of aminopyrine metabolism[3]. It is therefore a good measure of hepatic metabolic capacity, i.e. the "functional hepatic mass".

Applications of ^{13}C-Aminopyrine Breath Test

The ^{13}C-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 patients[6].

The patient should have fasted for 8 hours prior to the test. Smoking should also be avoided at least one hour prior to the test[7]. 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 $^{13}CO_2$ measurement by NDIRS[8].

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

1. Collect zero (basal) breath sample as described in manual.
2. Patient takes ^{13}C-Aminopyrine (75 mg) dissolved in warm water (100 ml).
3. Collect additional breath samples as shown below (Table 1).
4. Analyze all 10 breath samples with IRIS®-3.

#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: ^{13}C-Aminopyrine Breath Test Sample Collection

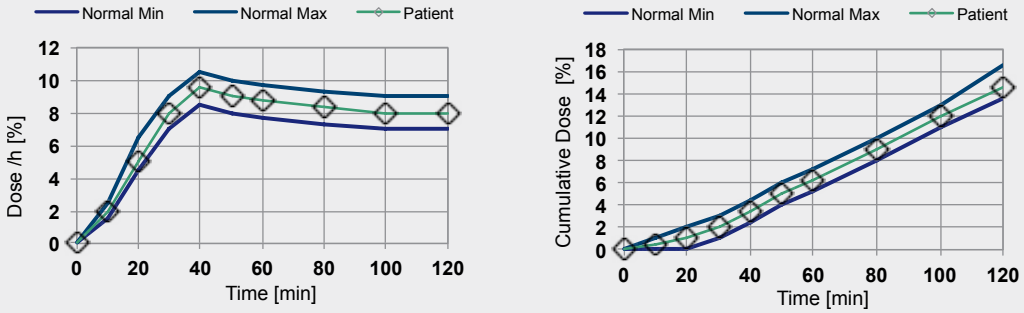


Fig. 1,2: ^{13}C-Aminopyrine Breath Test, Dose/h curve and % Cum Dose curve, healthy (normal) subject[11]

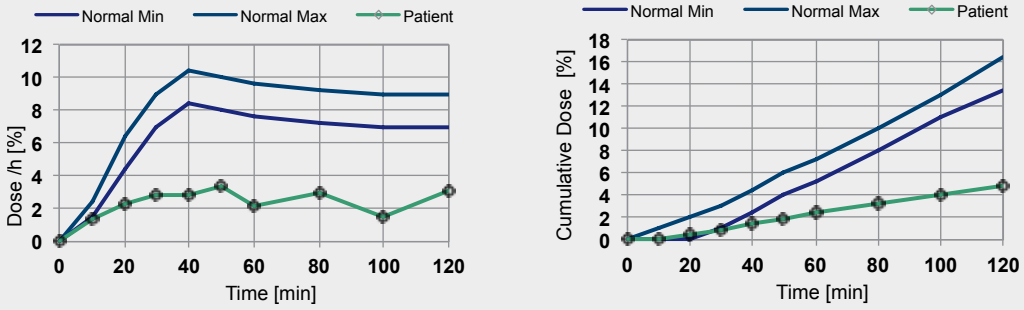


Fig. 3,4: ^{13}C-Aminopyrine Breath Test, Dose/h curve and % Cum Dose curve, subject with liver disease[11]

Results and interpretation

Typical results for the ^{13}C-Aminopyrine Breath Test are presented in Figures 1 to 4. The ^{13}C-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 ^{13}C-Aminopyrine Breath Test, cut-off values have been established in a study with 135 patients[11] (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 ^{13}C-Aminopyrine Breath Test [11]

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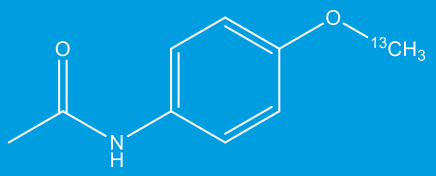
^{13}C-Methacetin Breath Test

^{13}C-Methacetin



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

Metabolic principle

Methacetin is metabolized rapidly in normal subjects, being highly extracted by the liver[1], implying that the metabolism of methacetin is mainly dependent on hepatic blood flow, the latter being generally decreased in cirrhotic patients[2]. Methacetin undergoes dealkylation by hepatic CYP1A2 to acetaminophen[3] with the methoxy group being eliminated as $^{13}CO_2$.

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 substrates[4–7].

Applications of ^{13}C-Methacetin Breath Test

The liver status of patients who have been diagnosed with liver disease can be assessed or monitored non-invasively using the ^{13}C-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 test[13]. 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 $^{13}CO_2$ measurement by NDIRS[14].

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

1. Collect zero (basal) breath sample as described in the manual.
2. Patient takes ^{13}C-Methacetin (75 mg) dissolved in water (100 ml).
3. Collect additional breath samples as shown below (Table 2).
4. Analyze all 10 breath samples with IRIS®-3 or IRIS®-Doc.

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 ^{13}C-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: ^{13}C-Methacetin Breath Test Sample Collection

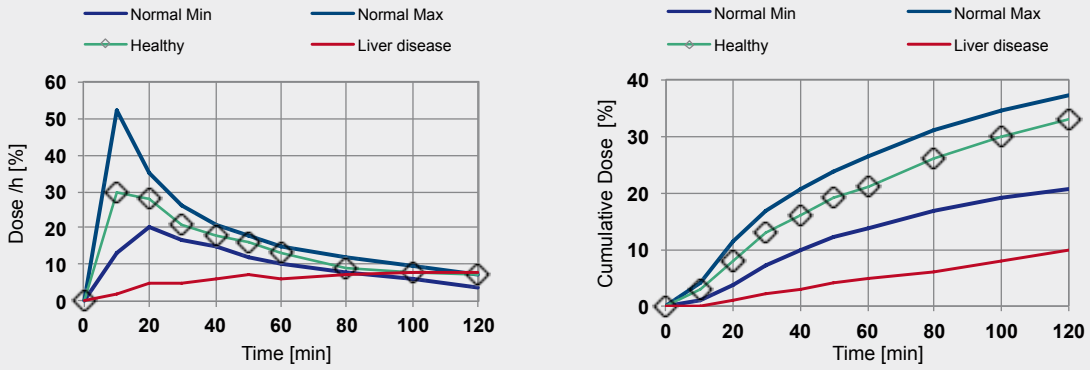


Fig. 1-2: ^{13}C-Methacetin Breath Test, Dose/h curve and % Cum Dose, healthy (normal) subject[16]

Results and interpretation

In healthy subjects a peak in the exhaled Dose/h of labeled CO_2 is to be expected after 10 to 20 minutes (see Figure 1). About 30% of the administered dose is recovered as $^{13}CO_2$ 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 ^{13}C-Methacetin Breath Test (% cum dose) with stage of liver disease[8]

		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 ^{13}C-Methacetin Breath Test and FibroIndex as predictors of cirrhosis and fibrosis. (Adapted from Dinesen *et al.*[17])

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14. Riecke, B., Neuhaus, P. & Stockmann, M. Major influence of oxygen supply on $^{13}CO_2$:$^{12}CO_2$ ratio measurement by nondispersive isotope-selective infrared spectroscopy. Helicobacter 10, 620–622 (2005).
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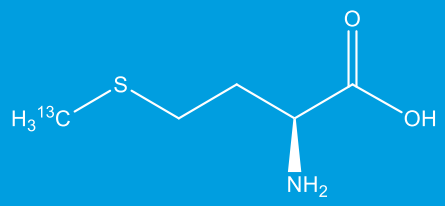
^{13}C-L-Methionine Breath Test

^{13}C-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, metabolized 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 ^{13}C-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 ^{13}C-sarcosine. The labeled sarcosine is oxidized by sarcosine dehydrogenase to produce ^{13}C-formaldehyde in the mitochondria which is further oxidized to $^{13}CO_2$ and expired. Since the oxidation of sarcosine occurs in the mitochondria of the liver[1], ^{13}C-methionine can be used to evaluate the oxidative capacity of the liver[2]. This test is therefore a good measure of the hepatic metabolic capacity.[3–5]

Applications of ^{13}C-L-Methionine Breath Test

The ^{13}C-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 stress[8], 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 test[10]. 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 $^{13}CO_2$ measurement by NDIRS[11].

Test Performance Procedure (see IRIS® Operating Manual for additional information)

1. Collect zero (basal) breath sample as described in the manual.
2. Patient takes ^{13}C-L-Methionine (75 mg) dissolved in water (100 ml).
3. Collect additional breath samples as shown below (Table 2).
4. Analyze all 10 breath samples with IRIS®-3 or IRIS®-Doc.

#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: ^{13}C-L-Methionine Breath Test Sample Collection

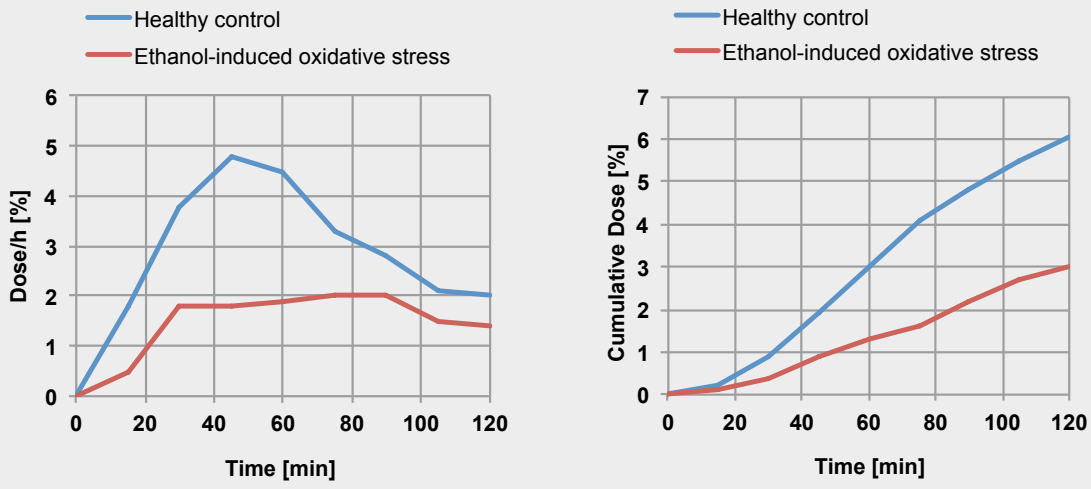


Fig. 1,2: Example of ^{13}C-Methionine Breath Test, Dose/h curve and % Cum Dose, (Armuzzi *et al.*, 2000[8])

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.*' the cumulative dose in healthy controls after 120 minutes reaches 6.07±0.46%[8] whereas control groups in the following studies also showed slightly increased values (e.g. cumulative dose after 90 minutes: 7.16% ± 1.91%; see Stüwe et al., 2013[12]). 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.*, 2011[4]

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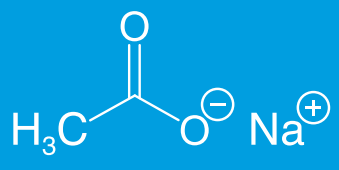
^{13}C-Sodium-Acetate Breath Test

^{13}C-Sodium-Acetate



Molecular weight:	145.21 g/mol
Enrichment:	99 %
Labeled C-atoms:	1
Dosage:	75 mg

Metabolic principle

^{13}C-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 metabolized in the liver[1]. Whilst some of the labeled carbon is incorporated in different metabolic pathways, about 50 % enters the body´s bicarbonate pool and is exhaled[2]. 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 ^{13}C-Sodium-Acetate Breath Test

The ^{13}C-Sodium-Acetate Breath Test is very useful for the investigation of functional dyspepsia and autonomic diabetic neuropathy[5]. Gastroparesis has also been shown to be associated with functional gastrointestinal[6,7] and inflammatory disorders of the gastrointestinal tract[8].

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 $^{13}CO_2$ measurement by NDIRS[9].

Test Performance Procedure (see IRIS® Operating Manual for additional information)

1. Collect zero (basal) breath sample as described in manual.
2. Enter patient height and weight into the IRIS®-3 or IRIS®-Doc Software.
3. Patient takes ^{13}C-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).
5. Analyze all 13 breath samples with IRIS®-3 or IRIS®-Doc.

#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: ^{13}C-Sodium-Acetate Test Sample Collection

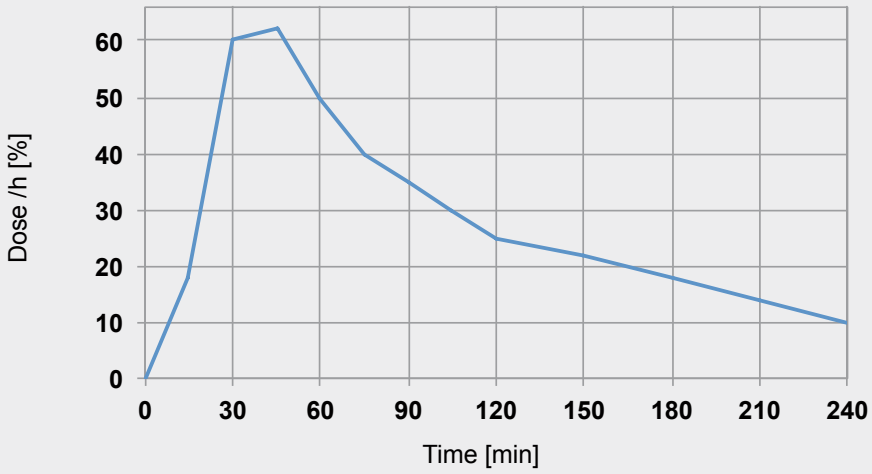


Fig. 1: Example of ^{13}C-Sodium-Acetate gastric emptying breath test, Dose/h curve

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 Ghoos *et al*[10]. 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 IRIS®-3 or IRIS®-Doc 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 patients[3]. Another study by Braden *et al.* resulted in half-emptying times of 90 minutes as cut-off value in children[11]. 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 children[12].

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^{13}C-Sodium-Octanoate and ^{13}C-Octanoic Acid Breath Test

^{13}C-Sodium-Octanoate

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

^{13}C-Octanoic Acid

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

Metabolic principle

^{13}C-Sodium-octanoate or ^{13}C-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 catabolized in the liver[3]. Whilst some of the labeled carbon is incorporated into different metabolic pathways, about 50 % enters the body´s bicarbonate pool and is exhaled[4]. As the rate-limiting step in this process is the stomach-emptying rate, this test is a reliable application to assess solid gastric emptying[5–7]. Whether ^{13}C-sodium-octanoate or ^{13}C-octanoic acid is used is a matter of feasibility.

Applications of ^{13}C-Sodium-Octanoate Breath Test

The ^{13}C-Sodium-Octanoate Breath Test is very useful for the investigation of functional dyspepsia and autonomic diabetic neuropathy[8]. Gastroparesis has also been shown to be related to irritable bowel syndrome (IBS)[9,10] and inflammation of the distal gastrointestinal tract[11].

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 $^{13}CO_2$ measurement by NDIRS[12].

Test Performance Procedure (see IRIS® Operating Manual for additional information)

1. Mix an egg with 100 mg of ^{13}C-sodium-octanoate or inject 91 mg of ^{13}C-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].
2. Collect zero (basal) breath sample as described in manual.
3. Enter patient height and weight into the IRIS®-3 or IRIS®-Doc Software.
4. Allow patient to eat the prepared egg meal.
5. Collect breath samples as shown below (Table 1).
6. Analyze all 13 breath samples with IRIS®-3 or IRIS®-Doc.

#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: ^{13}C-Sodium-Octanoate Test Sample Collection

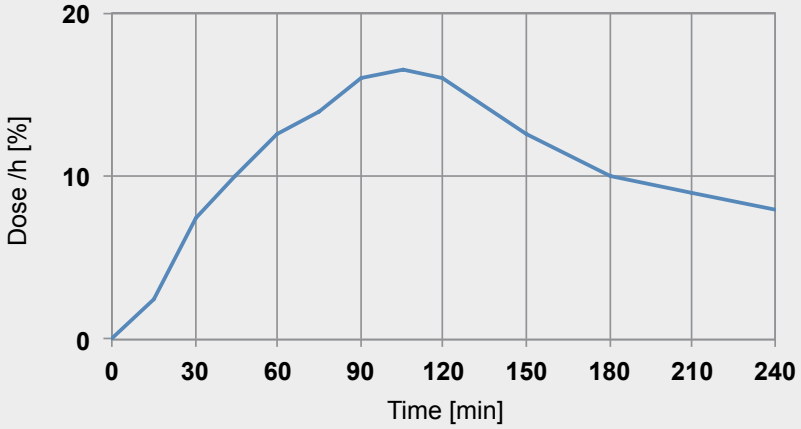

Fig. 1: Example of ^{13}C-Sodium-Octanoate gastric emptying breath test, Dose/h curve

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 Ghoos *et al*[13]. 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 IRIS®-3 or IRIS®-Doc 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 emptying[6]. Normal values calculated and corrected with scintigraphy by Ghoos *et al.* are for $T_{1/2B}$ = 72 ± 22 minutes and T_{lagB} = 32 ± 20 minutes for a test meal of 250 kcal.[13]. Delbende and Ghoos 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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11. Keller, J., Beglinger, C., Holst, J. J., Andresen, V. & Layer, P. Mechanisms of gastric emptying disturbances in chronic and acute inflammation of the distal gastrointestinal tract. Am. J. Physiol. Gastrointest. Liver Physiol. 297, G861-868 (2009).
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^{13}C-Mixed Triglyceride Breath Test

^{13}C-Mixed Triglyceride

^{13}C-Mixed Triglyceride consists of a Triglyceride containing two Stearic Acid molecules and one Octanoic Acid molecule. The Octanoic Acid molecule is labeled with ^{13}C 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-^{13}C}octanoylglycerol, the so-called ^{13}C-Mixed Triglyceride passes through the stomach and is digested by lipase activity in the duodenum[1]. The two distearyl groups have to be hydrolyzed by pancreatic lipase before absorption and metabolism of the ^{13}C-octanoyl monoglyceride[2]. Thus, the oxidation to $^{13}CO_2$ is dependent on the rate-limiting step of hydrolysis of the fatty acids in positions 1 and 3[3].

Applications of ^{13}C-Mixed Triglyceride Breath Test

The ^{13}C-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 specificity[6].

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 $^{13}CO_2$ measurement by NDIRS[7].

Test Performance Procedure (see IRIS® Operating Manual for additional information)

1. Mix 150 mg of ^{13}C-Mixed Triglyceride with 0.25 g of butter per kg body weight and prepare it with 100 g of bread.
2. Collect zero (basal) breath sample as described in manual.
3. Enter patient height and weight into the IRIS®-3 or IRIS®-Doc Software.
4. Allow the patient to eat the prepared bread.
5. Collect breath samples as shown below (Table 1).
6. Analyze all 13 breath samples with IRIS®-3 or IRIS®-Doc.

#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: ^{13}C-Mixed Triglyceride Test Sample Collection

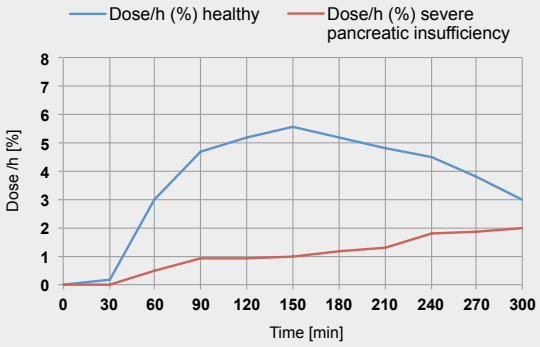

Fig. 1: Example of ^{13}C-Mixed Triglyceride breath test, Dose/h (%) curve (see Löser *et al.*[5])

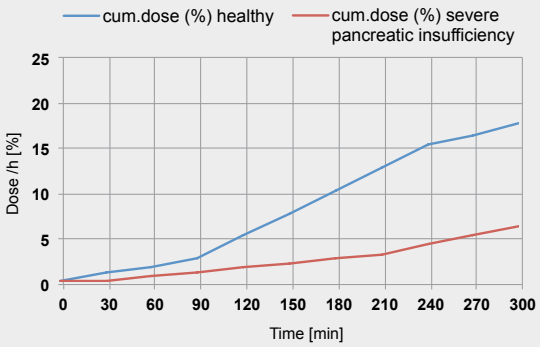

Fig. 2: Example of ^{13}C-Mixed Triglyceride breath test, cum.dose (%) curve (see Löser *et al.*[5])

Results and interpretation

Pancreatic function is assessed by the 6 hour cumulative $^{13}CO_2$ excretion. This can be calculated by the IRIS®-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 CO_2 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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