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

DISCLAIMER
Please note that all substrates described in this brochure, except 13C-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.
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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 $\delta$‰ (IRIS-Doc: 0.4 $\delta$‰) 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.
13C-Urea Breath Test – Diabact® UBT

13C-Urea

<table>
<thead>
<tr>
<th>Test principle</th>
</tr>
</thead>
<tbody>
<tr>
<td>Isotopically labelled urea is metabolized 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.</td>
</tr>
</tbody>
</table>

Application of Diabact 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.

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.

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

$<1.5\% \delta$ value = Negative $H. pylori$ status
$>1.5\% \delta$ value = Positive $H. pylori$ status

The cut-off when using IRIS-3 is $1.5\% \pm 0.2$.
The cut-off when using IRIS-Doc is $1.5\% \pm 0.4$.

References
13C-Aminopyrine Breath Test

13C-Aminopyrine

Metabolic principle
13C-Aminopyrine undergoes a two-step N-demethylation by cytochrome P-450 monooxygenases including CYP2C19, CYP1A2 and CYP3A4, yielding formaldehyde and amino-antipyrine. The formaldehyde is further oxidized to bicarbonate and exhaled as 13CO2, or deposited in the bicarbonate pool. 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. 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. For example, it can be used to quantify progression of the disease in Hepatitis C patients.

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. 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 NDIRS.

Test Performance Procedure (see IRIS® 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. Analyze all 10 breath samples with IRIS®-3.

Table 1: 13C-Aminopyrine Breath Test Sample Collection

<table>
<thead>
<tr>
<th>#1 Bag</th>
<th>#2 Bag</th>
<th>#3 Bag</th>
<th>#4 Bag</th>
<th>#5 Bag</th>
<th>#6 Bag</th>
<th>#7 Bag</th>
<th>#8 Bag</th>
<th>#9 Bag</th>
<th>#10 Bag</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 min</td>
<td>10 min</td>
<td>20 min</td>
<td>30 min</td>
<td>40 min</td>
<td>50 min</td>
<td>60 min</td>
<td>80 min</td>
<td>100 min</td>
<td>120 min</td>
</tr>
</tbody>
</table>
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).

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

Table 2: Cut-off values for $^{13}$C-Aminopyrine Breath Test $^{11}$

References

**13C-Methacetin Breath Test**

**13C-Methacetin**

\[
\text{O} \quad \text{N} \quad \text{O}^{13}\text{CH}_3
\]

**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 \(^{13}\text{CO}_2\).

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 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 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 \(^{13}\text{CO}_2\) 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 13C-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.

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

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

<table>
<thead>
<tr>
<th>Bag</th>
<th>Duration</th>
</tr>
</thead>
<tbody>
<tr>
<td>#1 Bag</td>
<td>0 min</td>
</tr>
<tr>
<td>#2 Bag</td>
<td>10 min</td>
</tr>
<tr>
<td>#3 Bag</td>
<td>20 min</td>
</tr>
<tr>
<td>#4 Bag</td>
<td>30 min</td>
</tr>
<tr>
<td>#5 Bag</td>
<td>40 min</td>
</tr>
<tr>
<td>#6 Bag</td>
<td>50 min</td>
</tr>
<tr>
<td>#7 Bag</td>
<td>60 min</td>
</tr>
<tr>
<td>#8 Bag</td>
<td>80 min</td>
</tr>
<tr>
<td>#9 Bag</td>
<td>100 min</td>
</tr>
<tr>
<td>#10 Bag</td>
<td>120 min</td>
</tr>
</tbody>
</table>

**Table 2: 13C-Methacetin Breath Test Sample Collection**
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. 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).

![Figure 1-2: 13C-Methacetin Breath Test, Dose/h curve and % Cum Dose, healthy [normal] subject]

<table>
<thead>
<tr>
<th>Liver Cirrhosis</th>
<th>13C-Methacetin Breath Test</th>
<th>Fibroindex</th>
<th>Cut-off</th>
<th>Sensitivity</th>
<th>Specificity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal Max</td>
<td>&lt; 14.6 %</td>
<td>92.6 %</td>
<td>84.1 %</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Healthy</td>
<td>1.82</td>
<td>70.4 %</td>
<td>91.3 %</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Advanced Fibrosis</th>
<th>13C-Methacetin Breath Test</th>
<th>Fibroindex</th>
<th>Cut-off</th>
<th>Sensitivity</th>
<th>Specificity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal Max</td>
<td>&lt; 21 %</td>
<td>75.4 %</td>
<td>79.5 %</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Healthy</td>
<td>&gt; 1.35</td>
<td>66.7 %</td>
<td>84.6 %</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

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

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

**References**

13C-L-Methionine Breath Test

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 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 oxidized to 13CO2 and expired. Since the oxidation of sarcosine occurs in the mitochondria of the liver, 13C-methionine can be used to evaluate the oxidative capacity of the liver. This test is therefore a good measure of the hepatic metabolic capacity.

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, ethanol-induced liver oxidative stress, impaired hepatic mitochondrial oxidation in liver steatosis such as non-alcoholic fatty liver disease (NAFLD) or cirrhosis.

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. 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 NDIRS.

Test Performance Procedure (see IRIS® 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. Analyze all 10 breath samples with IRIS®-3 or IRIS®-Doc.

<table>
<thead>
<tr>
<th>Bag</th>
<th>Time</th>
</tr>
</thead>
<tbody>
<tr>
<td>#1</td>
<td>0 min</td>
</tr>
<tr>
<td>#2</td>
<td>10 min</td>
</tr>
<tr>
<td>#3</td>
<td>15 min</td>
</tr>
<tr>
<td>#4</td>
<td>20 min</td>
</tr>
<tr>
<td>#5</td>
<td>25 min</td>
</tr>
<tr>
<td>#6</td>
<td>30 min</td>
</tr>
<tr>
<td>#7</td>
<td>40 min</td>
</tr>
<tr>
<td>#8</td>
<td>60 min</td>
</tr>
<tr>
<td>#9</td>
<td>90 min</td>
</tr>
<tr>
<td>#10</td>
<td>120 min</td>
</tr>
</tbody>
</table>

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

In healthy subjects, a peak in the exhaled Dose/h of labeled CO₂ 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% 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., 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.

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

<table>
<thead>
<tr>
<th>Condition</th>
<th>Cut-off</th>
</tr>
</thead>
<tbody>
<tr>
<td>Non-alcoholic steatohepatitis (NASH) vs. non-NASH</td>
<td>&lt; 4.20%</td>
</tr>
<tr>
<td>Fibrosis stage 0-1 vs. Fibrosis stage 2-3 (within NAFLD cohort)</td>
<td>&lt; 3.65%</td>
</tr>
</tbody>
</table>

References

**13C-Sodium-Acetate Breath Test**

### 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 metabolized in the liver. Whilst some of the labeled carbon is incorporated in different metabolic pathways, about 50% enters the body’s bicarbonate pool and is exhaled. As the rate-limiting step in this process is the stomach-emptying rate, this test is a reliable application to assess liquid gastric emptying.

### 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 neuropathy. Gastroparesis has also been shown to be associated with functional gastrointestinal and inflammatory disorders of the gastrointestinal tract.

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 NDIRS.

### 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 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).
5. Analyze all 13 breath samples with IRIS®-3 or IRIS®-Doc.

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

<table>
<thead>
<tr>
<th>#1 Bag</th>
<th>#2 Bag</th>
<th>#3 Bag</th>
<th>#4 Bag</th>
<th>#5 Bag</th>
<th>#6 Bag</th>
<th>#7 Bag</th>
<th>#8 Bag</th>
<th>#9 Bag</th>
<th>#10 Bag</th>
<th>#11 Bag</th>
<th>#12 Bag</th>
<th>#13 Bag</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 min</td>
<td>15 min</td>
<td>30 min</td>
<td>45 min</td>
<td>60 min</td>
<td>75 min</td>
<td>90 min</td>
<td>105 min</td>
<td>120 min</td>
<td>150 min</td>
<td>180 min</td>
<td>210 min</td>
<td>240 min</td>
</tr>
</tbody>
</table>
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. 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. Another study by Braden et al. resulted in half-emptying times of 90 minutes as cut-off value in children. 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.

References

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

### 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. After passing through the stomach, it is absorbed in the small intestine and catabolized in the liver. Whilst some of the labeled carbon is incorporated into different metabolic pathways, about 50% enters the body’s bicarbonate pool and is exhaled. As the rate-limiting step in this process is the stomach-emptying rate, this test is a reliable application to assess solid gastric emptying. 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 neuropathy. Gastroparesis has also been shown to be related to irritable bowel syndrome (IBS) and inflammation of the distal gastrointestinal tract.

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 NDIRS.

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

1. 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 150 ml of water (14 g of protein, 26 g of carbohydrate and 9 g of fat, 250 kcal).
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.

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

<table>
<thead>
<tr>
<th>Sample</th>
<th>#1 Bag</th>
<th>#2 Bag</th>
<th>#3 Bag</th>
<th>#4 Bag</th>
<th>#5 Bag</th>
<th>#6 Bag</th>
<th>#7 Bag</th>
<th>#8 Bag</th>
<th>#9 Bag</th>
<th>#10 Bag</th>
<th>#11 Bag</th>
<th>#12 Bag</th>
<th>#13 Bag</th>
</tr>
</thead>
<tbody>
<tr>
<td>Time</td>
<td>0 min</td>
<td>15 min</td>
<td>30 min</td>
<td>45 min</td>
<td>60 min</td>
<td>75 min</td>
<td>90 min</td>
<td>105 min</td>
<td>120 min</td>
<td>150 min</td>
<td>180 min</td>
<td>210 min</td>
<td>240 min</td>
</tr>
</tbody>
</table>

**13C-Sodium-Octanoate**

![Structure](image)

**13C-Octanoic Acid**

![Structure](image)

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

- **Molecular weight:** 145.21 g/mol
- **Enrichment:** 99 %
- **Labeled C-atoms:** 1
- **Dosage:** 91 mg
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. 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. Normal values calculated and corrected with scintigraphy by Ghoos 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. 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 $T_{1/2B}$.

References

**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.

**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 duodenum. The two distearyl groups have to be hydrolyzed by pancreatic lipase before absorption and metabolism of the 13C-octanoyl monoglyceride. Thus, the oxidation to 13CO2 is dependent on the rate-limiting step of hydrolysis of the fatty acids in positions 1 and 3.

**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. If applied under strict conditions even mild to moderate forms can be assessed with high sensitivity and specificity.

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 NDIRS.

**Test Performance Procedure (see IRIS® 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 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.

**Table 1: 13C-Mixed Triglyceride Test Sample Collection**

<table>
<thead>
<tr>
<th>#1 Bag</th>
<th>#2 Bag</th>
<th>#3 Bag</th>
<th>#4 Bag</th>
<th>#5 Bag</th>
<th>#6 Bag</th>
<th>#7 Bag</th>
<th>#8 Bag</th>
<th>#9 Bag</th>
<th>#10 Bag</th>
<th>#11 Bag</th>
<th>#12 Bag</th>
<th>#13 Bag</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 min</td>
<td>30 min</td>
<td>60 min</td>
<td>90 min</td>
<td>120 min</td>
<td>150 min</td>
<td>180 min</td>
<td>210 min</td>
<td>240 min</td>
<td>270 min</td>
<td>300 min</td>
<td>330 min</td>
<td>360 min</td>
</tr>
</tbody>
</table>

Molecular weight: 752.0 g/mol
Enrichment: 99 %
Labeled C-atoms: 1
Dosage: 150 mg
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 % $\pm$ 2.8 %. Another study by Swart et al. resulted in a normal value of 33.6 % $\pm$ 4.6 %. 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).

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

References