$^{13}$C-Breath Tests for the Assessment of Specific Enzymatic and Metabolic Functions in vivo
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 Seahorse Laboratories does not accept any legal or other liability with respect to incorrect details and their consequences.

Seahorse Laboratories would like to thank Dr. Marko Silvestric-Scheel for his expert contribution.

Please note that all substrates described in this brochure, except 13C-urea – Diabact® UBT 50 mg (registered pharmaceutical), are unlicensed for use, but available as specials in the UK and select geographies.

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.
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 $^{13}$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 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 $^{13}$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 ‰ 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.

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

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

\[ <1.5 \% \delta \text{ value} = \text{Negative } H. pylori \text{ status} \]
\[ >1.5 \% \delta \text{ value} = \text{Positive } H. pylori \text{ status} \]
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 monoxygenases 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 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.

<table>
<thead>
<tr>
<th>Bag</th>
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<td>60 min</td>
<td>80 min</td>
<td>100 min</td>
<td>120 min</td>
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Table 1: 13C-Aminopyrine Breath Test Sample Collection
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.

For the $^{13}$C-Aminopyrine Breath Test, cut-off values have been established in a study with 135 patients (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>
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<tr>
<td>Fibrosis stages 3 / 4</td>
<td>2.48 - 3.13 ± 1.2</td>
<td>3.62 - 4.56 ± 2.0</td>
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<tr>
<td>Cirrhosis, not established</td>
<td>6.77 ± 2.7</td>
<td>9.63 ± 3.6</td>
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<tr>
<td>Cirrhosis, established</td>
<td>2.48 ± 1.2</td>
<td>3.68 ± 1.9</td>
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Table 2: Cut-off values for $^{13}$C-Aminopyrine Breath Test

References

Metabolic principle

Methacetin is metabolised 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.

<table>
<thead>
<tr>
<th>Condition</th>
<th>Assessment</th>
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<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>
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<tr>
<td>Hepatitis B or C</td>
<td>Hepatic reserve(^\text{10})</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(^\text{11})(^\text{12})</td>
</tr>
</tbody>
</table>

Table 1: Liver diseases assessed by \(^{13}\)C-Methacetin Breath Test

<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(^\text{10})</td>
</tr>
<tr>
<td>Long-term medication e.g. anticonvulsants</td>
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</tr>
<tr>
<td>Liver transplant</td>
<td>Liver status of both donor and recipient(^\text{11})(^\text{12})</td>
</tr>
</tbody>
</table>

Table 1: Liver diseases assessed by \(^{13}\)C-Methacetin Breath Test

Test Performance Procedure (see 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. Analyse all 10 breath samples with a Kibion Instrument.

Table 2: \(^{13}\)C-Methacetin Breath Test Sample Collection

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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.⁶,¹⁰,¹⁵

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).

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

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

| Advanced Fibrosis | ¹³C-Methacetin Breath Test | <21 % | 75.4 % | 79.5 % |
|                   | Fibroindex                | >1.35 | 66.7 % | 84.6 % |

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

<table>
<thead>
<tr>
<th>% Cumulative Dose, 120 min</th>
<th>Indication/Correlation</th>
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</thead>
<tbody>
<tr>
<td>31.0 (25.9 – 38.7)</td>
<td>Normal</td>
</tr>
<tr>
<td>13.6 (6.7 – 22.3)</td>
<td>Cirrhosis, Child-Pugh Class A</td>
</tr>
<tr>
<td>3.1 (1.1 – 16.5)</td>
<td>Cirrhosis, Child-Pugh Class B</td>
</tr>
<tr>
<td>0.6 (-1.1 – 3.5)</td>
<td>Cirrhosis, Child-Pugh Class C</td>
</tr>
</tbody>
</table>

References

13C-L-Methionine Breath Test

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 capacity3-5.

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 toxicity6-7, ethanol-induced liver oxidative stress8, impaired hepatic mitochondrial oxidation in liver steatosis such as non-alcoholic fatty liver disease (NAFLD) or cirrhosis9-10.

The patient should have fasted for 8 hours prior to the test. Smoking should also be avoided at least one hour prior to the test11. 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.

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

<table>
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<tr>
<th>Bag</th>
<th>0 min</th>
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Molecular weight: 150.2 g/mol
Enrichment: 99%
Labeled C-atoms: 1
Dosage: 75 mg

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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., 2013). In general, the more severe the liver disease, the lower the % cumulative dose after 90 or 120 minutes.

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>
<thead>
<tr>
<th>Disease Comparison</th>
<th>Cut-off Value</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>
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</table>

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.
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 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 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).

<table>
<thead>
<tr>
<th>Bag</th>
<th>0 min</th>
<th>15 min</th>
<th>30 min</th>
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<th>90 min</th>
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Table 1: 13C-Sodium-Acetate Test Sample Collection
5. Analyse all 13 breath samples with a Kibion instrument.

Results and interpretation

Gastric emptying parameters are assessed by calculation of the half-emptying time ($T_{1/2}$), the lag phase ($T_{lag}$) 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 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 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 $T_{1/2}$ and 47 minutes for $T_{lag}$ 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 baking1,2. 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)
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 150ml of water (14 g of protein, 26 g of carbohydrate and 9 g of fat, 250 kcal)13.

2. Collect a zero (basal) breath sample as described in the manual.
3. Enter patient height and weight into the instrument software.
4. Allow patient to eat the prepared egg meal.
5. Collect breath samples as shown below (Table 1).
6. Analyse all 13 breath samples with a Kibion instrument.

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

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<td>0 min</td>
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<td>120 min</td>
<td>150 min</td>
<td>180 min</td>
<td>210 min</td>
<td>240 min</td>
</tr>
</tbody>
</table>

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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 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 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 hydrolysed 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 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.

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<thead>
<tr>
<th>#1 Bag</th>
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<th>#5 Bag</th>
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<tbody>
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<td>0 min</td>
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<td>60 min</td>
<td>90 min</td>
<td>120 min</td>
<td>150 min</td>
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<td>240 min</td>
<td>270 min</td>
<td>300 min</td>
<td>330 min</td>
<td>360 min</td>
</tr>
</tbody>
</table>

Table 1: 13C-Mixed Triglyceride Test Sample Collection
### References


### Results and interpretation

Pancreatic function is assessed by the 6 hour cumulative $^{13}$CO$_2$ 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 $35.6 \% \pm 2.8 \%$ ⁴. Another study by Swart et al. resulted in a normal value of $33.6 \% \pm 4.6 \%$ 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.