Protocols - $^{13}$C Breath Tests - LIVER
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Introduction

13C Breath Test protocols
This folder contains a set of protocols describing the principles and general test procedures for today’s most relevant 13C Breath Tests to study specific functions of the liver, pancreas, stomach and intestine. The list will be updated regularly adding additional tests or additional information on the already described tests.

The information is meant as a start to enter the field of stable isotope 13C Breath testing initiated by the interest in a specific test. To actually be able to introduce a test in your hospital you must familiarize yourself with basic knowledge of breath testing with 13C substrates and the existing knowledge on the particular application of interest. There is no such thing as a standard protocol for all tests.

13C Breath Testing: principle and requirements
A 13C Breath Test consists of the administration to a patient of a 13C labeled substrate that is metabolized by a specific enzyme system resulting in 13CO2 as the end product. To monitor the enzyme response 13C enrichment is measured in breath CO2.

The total procedure of 13C Breath testing includes the definition of the preparation of the patient before the test, administration of the 13C labeled substrate, collection of breath samples, measurement of 13C enrichment in breath CO2 and the calculation of an end result.

Preparation of the patient
In general, tests will be performed in the fasting state and the patient should be at a low and stable level of natural 13C abundance. Therefore, the patient must be instructed to avoid eating 13C enriched foods such as corn products, cane sugar, pineapple and tequila the last days before the test and to come to the clinic fasted. In certain cases (13C Lactose-Ureide breath test) the patient must be pretreated with unlabeled substrate to stimulate the involved enzyme system.

Administration of 13C labeled substrate
The test substrate may be administered as a simple solution in water with or without a standardized test meal. Sometimes it needs to be incorporated into a specific ingredient of the meal. The test meal and the dose of substrate may be different for adults and children.

Collection of breath samples
Every protocol has its own time schedule of breath collections. The number of samples may be as small as 2 or more than 20. To define the 13C enrichment in breath CO2, it is also necessary to obtain at least two breath samples before the ingestion of the 13C substrate to determine the natural background of 13C abundance. The methodology of collecting breath samples is dependent on the technology to determine the 13C enrichment. The protocols are based on Continuous Flow Isotope Ratio Mass Spectrometry as the analytical technique. In this case breath samples are simply blown through a straw into special 10 ml gas collection tubes that directly fit into the sample tray of the instruments. In case of Infrared technology special bags provided by the instrument manufacturer must be used.

Measurement of 13C enrichment
To determine the 13C abundance in breath CO2 you need the availability of Isotope Ratio Mass Spectrometry (IRMS) or specialized Infrared instrumentation. The protocols are based on Isotope Ratio Mass Spectrometry as the analytical technique. For a number of tests (Aminopyrine, Methacetin, Urea) Infrared Spectroscopy has proven to be a valid alternative analytical technique. For other tests Infrared technology has not yet been validated so far. In principle the test substrate is not a determinant of the validity of the analytical technique. It is the level of 13C enrichment that determines the analytical requirement. Validation of Infrared analysis for other application is recommended, as it is recommended to validate any breath test in your own clinical laboratory. You may have instrumentation available or contact a service center for the analyses.

Calculation of the end result
For some tests the only calculation needed is the subtraction of the natural background value from the measured value at a defined time. In other cases it is necessary to calculate the amount of 13C that is recovered in breath during the experimental period. In a third type of application the time course of the enrichment appearance is of importance requiring calculation of the rate of appearance.
Applications
In the present update the following tests have been described:

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Literature
Included is a list of literature references that will introduce you to the most important articles describing aspects of the different tests described in the protocols.

Note
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Please contact us for technical and price information.
1. Microsomal Liver Function
   
   **13C-Aminopyrine Breath Test**

   - **Principle**
     
     13C₂-Aminopyrine contains two methyl groups both labeled with the non-radioactive isotope 13C. After oral administration, 13C₂-Aminopyrine is completely absorbed and cleared by the liver. 13C₂-Aminopyrine is demethylated by the microsomal cytochrome P450 dependent N-demethylase enzyme. By this reaction 13C-Formaldehyde is formed of which 50% is oxidized to bicarbonate. The degree of appearance of 13C in breath CO₂ reflects the degree of demethylation.

   - **Applicability of 13C-Aminopyrine Breath Test**
     
     13C-Aminopyrine Breath Test has so far been applied to adults, children and even neonates.

   - **Applications**
     
     13C-Aminopyrine Breath Test is used to detect diminished microsomal liver function by comparison with a control range. The result reflects microsomal mass and is independent of liver blood flow. The 13C-Aminopyrine Breath Test, most useful in cirrhotic patients, is correlated with severity of liver disease and promoted as a reliable method for the optimal timing for liver transplantation. Also P450 dependent enzyme induction by therapeutic means can be monitored by 13C-Aminopyrine Breath Test.

   - **Protocol**
     
     **Adults:** The 13C-Aminopyrine Breath Test is performed after an overnight fast. A dose of 2 mg/kg body weight 13C₂-Aminopyrine is administered orally after dissolution in about 100 ml water. Breath samples are collected before (2x) and every 30 minutes for 120 minutes (2 h) after the ingestion of the 13C₂-Aminopyrine. 13C enrichment in breath CO₂ is determined by Isotope Ratio Mass Spectrometry (IRMS). The cumulative percentage of 13C recovered in breath during the 120 minutes, collection period is used as the diagnostic parameter.

     **Children:** A dose of 2-5 mg/kg body weight is used for children. The same time schedule for breath collections can be used.

   - **Interpretation of test results**
     
     It is advised to obtain your own internal control values. Generally a cut-off value of 7% for the 120 minutes cumulative recovery may serve as a starting point in the case of adults and older children. Values below 7% are indicate diminished microsomal function. Within the first year of life the microsomal mass develops and the test results are therefore age dependent.

   - **Precautions**
     
     Microsomal cytochrome P450 enzyme activity may be induced by alcohol or drugs (spironolactone, glutethimide, diphenylhydantoin) or depressed (cimetidine) which affects the outcome of the test. In exceptional cases chronic dosing of aminopyrine has been associated with aminopyrine-induced agranulocytosis.

   - **Summary**
     
     | Dose | Samples |
     |------|---------|
     | Adults 2 mg/kg body weight 13C₂-Aminopyrine | 2 Before administration |
     | | 4 Every 30 minutes for 120 minutes after administration (2 hours) |
     | Children 2-5 mg/kg body weight 13C₂-Aminopyrine | 2 Before administration |
     | | 4 Every 30 minutes for 120 minutes after administration (2 hours) |
2. Microsomal Liver Function

13C-Methacetin Breath Test

- **Principle**
  13C-Methacetin contains one methyl group labeled with the non-radioactive isotope 13C. After oral administration 13C-Methacetin is completely absorbed and cleared by the liver. 13C-Methacetin is demethylated by the microsomal cytochrome P450 dependent N-demethylase enzyme. By this reaction 13C-formaldehyde is formed of which 50% is oxidized to bicarbonate. The degree of appearance of 13C in breath CO2 reflects the degree of demethylation.

- **Applicability of 13C-Methacetin Breath Test**
  13C-Methacetin Breath Test has so far been applied to adults and children.

- **Applications**
  13C-Methacetin Breath Test is used to detect diminished microsomal liver function by comparison with a control range. The result reflects microsomal mass and is independent of liver blood flow. The 13C-Methacetin Breath Test, most useful in cirrhotic patients, is correlated with severity of liver disease and promoted as a reliable method for the optimal timing for liver transplantation. Also P450 dependent enzyme induction by therapeutic means can be monitored by the 13C-Methacetin Breath Test.

- **Protocol**
  **Adults**: The 13C-Methacetin Breath Test is performed after an overnight fast. A dose of 2 mg/kg body weight 13C-Methacetin is administered orally after dissolution in about 100 ml water. Breath samples are collected before (2x) and every 10 minutes for 30 minutes after ingestion of the 13C-Methacetin. 13C enrichment in breath CO2 is determined by Isotope Ratio Mass Spectrometry (IRMS). The cumulative percentage of 13C recovered in breath during the 30 minutes collection period is used as the diagnostic parameter.

- **Interpretation of test results**
  It is advised to obtain your own internal control values. Generally a cut-off value of 8% for the 30 minutes cumulative recovery may serve as a starting point.

- **Precautions**
  Microsomal P450 enzyme activity may be induced by alcohol or drugs (spironolactone, glutethimide, diphenylhydantoin) or depressed (cimetidine), which affects the outcome of the test.

- **Summary**

<table>
<thead>
<tr>
<th>Dose</th>
<th>Samples</th>
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<tbody>
<tr>
<td>2 mg/kg body weight 13C-Methacetin</td>
<td>2 Before administration</td>
</tr>
<tr>
<td></td>
<td>3 Every 10 minutes for 30 minutes after administration (0.5 h)</td>
</tr>
</tbody>
</table>
3. Mitochondrial Liver Funktion
13C-Ketoisocaproic Acid Breath Test (13C-KICA)

■ Principle
Ketoisocaproic Acid (KICA) is the result of branched chain amino acid aminotransferase degradation of leucine. 13C-Ketoisocaproic acid (13C-KICA) is labeled with the non-radioactive isotope 13C. After oral administration, 13C-KICA is completely absorbed and cleared by the liver. 13C-KICA is decarboxylated almost exclusively in the hepatic mitochondria producing 13CO2. The degree of appearance of 13C in breath CO2 reflects the degree of mitochondrial decarboxylation.

■ Applicability of 13C-KICA Breath Test
13C-KICA Breath Test has so far been applied to adults.

■ Applications
13C-KICA Breath Test is used to detect diminished mitochondrial liver function by comparison with a control range. The result reflects not a general but a specific ethanol-related impairment of mitochondrial function and is used to determine the involvement of ethanol abuse in liver disease.

■ Protocol
The 13C-KICA Breath Test is performed after an overnight fast. A dose of 1 mg/kg body weight 13C KICA is administered orally together with 20 mg/kg L-Leucine after dissolution in 200 ml 0.1N citric acid solution or 200 ml orange juice. The L-Leucine is added to increase the decarboxylation of KICA. Breath samples are collected before (2x) and 10, 15, 20, 25, 30 and 60 minutes after administration of the 13C-KICA. 13C enrichment in breath CO2 is determined by Isotope Ratio Mass Spectrometry (IRMS). The cumulative percentage of 13C recovered in breath during the 60 minutes collection period is used as the diagnostic parameter.

■ Interpretation of test results
It is advised to obtain your own internal control values. Generally a cut-off value of 7% for the 60 minutes cumulative recovery may serve as a starting point. Values below 7% indicate for impaired hepatic mitochondrial function.

■ Precautions
No contraindications or side effects have been described so far.

■ Summary

<table>
<thead>
<tr>
<th>Dose</th>
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<tr>
<td>1 mg/kg body weight 13C -KICA + 20 mg/kg body weight L-Leucine</td>
<td>2 Before administration</td>
</tr>
<tr>
<td>6</td>
<td>10, 15, 20, 25, 30 and 60 minutes after administration (1 h)</td>
</tr>
</tbody>
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4. Cytosolic Liver Funktion
\(^{13}\text{C}-\text{Galactose Breath Test}\)

■ Principle
Galactose is metabolized primarily in the liver resulting in glucose. The rate limiting step is the action of galactose kinase. After oral administration, \((1-{^{13}\text{C}})-\text{Galactose}\) is completely absorbed and cleared by the liver. \(^{13}\text{C}-\text{Galactose}\) is converted almost exclusively in the hepatic cytosol producing \(^{13}\text{C}-\text{Glucose}\). To a large extent \(^{13}\text{C}-\text{Glucose}\) is oxidized to \(^{13}\text{CO}_2\). The degree of appearance of \(^{13}\text{C}\) in breath \(^{13}\text{CO}_2\) reflects the degree of cytosolic conversion of galactose to galactose-1-phosphate.

■ Applicability of \(^{13}\text{C}-\text{Galactose Breath Test}\)
\(^{13}\text{C}-\text{Galactose Breath Test}\) has so far been applied to adults. Preliminary experiments have been performed in children.

■ Applications
\(^{13}\text{C}-\text{Galactose Breath Test}\) is used to detect diminished cytosolic liver function by comparison with a control range. The result reflects the severity of liver fibrosis in chronic hepatitis C and is proposed as a prognostic factor in the follow-up of chronic hepatitis C.

■ Protocol
The \(^{13}\text{C}-\text{Galactose Breath Test}\) is performed after an overnight fast. A dose of 5 mg/kg body weight \((1-{^{13}\text{C}})-\text{Galactose}\) is administered orally together with 495 mg/kg unlabeled galactose as a 25% solution in water. Breath samples are collected before (2x) and every 10 minutes for 1 hour after the administration of the \(^{13}\text{C}-\text{Galactose}\). \(^{13}\text{C}\) enrichment in breath \(^{13}\text{CO}_2\) is determined by Isotope Ratio Mass Spectrometry (IRMS). The cumulative percentage of \(^{13}\text{C}\) recovered in breath / hour during the 60 minutes collection period after ingestion of \((1-{^{13}\text{C}})-\text{Galactose}\), is used as the diagnostic parameter.

■ Interpretation of test results
It is advised to obtain own internal control values. Generally a cut-off value of 4% for the 60 minutes recovery of \(^{13}\text{C} / \text{hour}\) may serve as a starting point. Values below 4% indicate the presence of fibrosis.

■ Precautions
Alcohol consumption, galactosemia and diabetes may disturb the outcome of the \(^{13}\text{C}-\text{Galactose Breath Test}\).

■ Summary

<table>
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<td>Adults</td>
<td>(5 \text{ mg/kg body weight} (1-{^{13}\text{C}})-\text{Galactose} + 495 \text{ mg/kg unlabeled Galactose})</td>
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Literature

Liver

1. Microsomal Liver Function
   13C-Aminopyrine Breath Test

   Recommended literature


2. Microsomal Liver Function

**13C-Methacetin Breath Test**

### Recommended literature


3. Mitochondrial Liver Funktion

13C-Ketoisocaproic Acid Breath Test (13C-KICA)

- **Recommended literature**


4. Cytosolic Liver Funktion

$^{13}$C-Galactose Breath Test

■ Recommended literature


