

Protocols - ^{13}C Breath Tests - INTESTINE

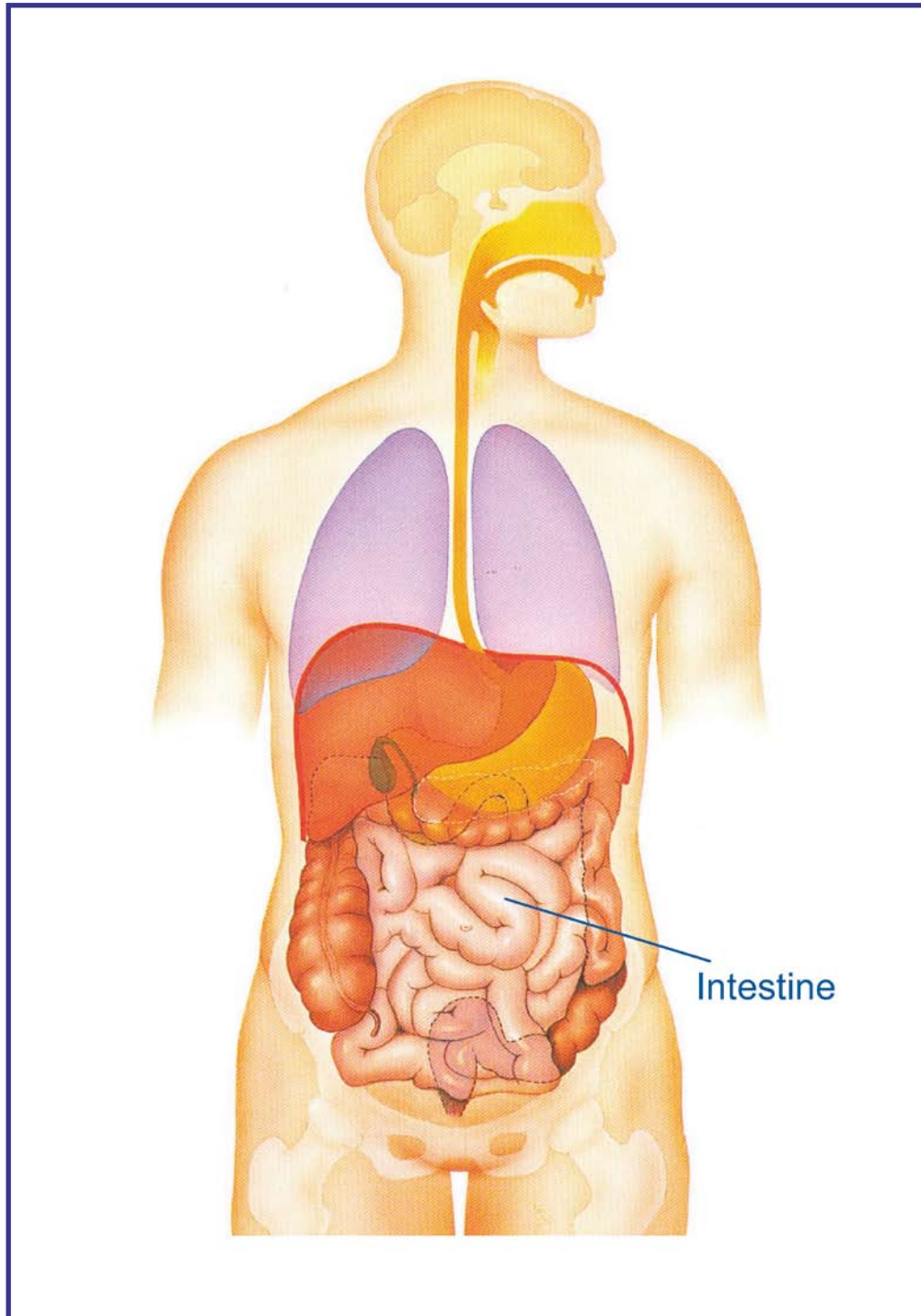


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Introduction

■ **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. 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

Great care has been taken over the composition of the text, figures and tables. The possibility of errors however, cannot be excluded completely. Therefore Campro Scientific GmbH and the authors cannot accept any legal or other liability with respect to incorrect details and their consequences. The authors would be grateful to receive suggestions for improvements and information about errors.

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■ Authors

1. Dr. F. Stellaard
University Hospital Groningen, the Netherlands
Dept. Pathology and Laboratory Medicine
And Centre for Liver, Intestinal and Metabolic Disease
2. Dr. Ahmad Rajabi
Campro Scientific GmbH
Berlin, Germany

© Campro Scientific GmbH

European Headquarters

P.O. Box 37 03 31
D-14133 Berlin
Germany
Tel. : +49(0)30.629.01.89.0
Fax : +49(0)30.629.01.89.89
E-mail : info@campro.eu
Web : www.campro.eu

Dutch Sales Office

P.O. Box 316
NL-3900 AH Veenendaal
The Netherlands
Tel. : +31(0)318.529.437
Fax : +31(0)318.542.181
E-mail : info@campro.eu
Web : www.campro.eu

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Test Protocols Intestine

1. Brush Border Enzymes Natural Enriched ^{13}C -Lactose Breath Test

■ Principle

^{13}C -Lactose contains one or more carbon labeled with the non-radioactive isotope ^{13}C . The ^{13}C -Lactose generally used for breath testing is labeled through a natural process. Carbon atoms in corn (a C-4 plant) have a higher abundance of ^{13}C than those in C-3 plants being the major natural food sources in humans. When corn is fed to cows the milk components become enriched in ^{13}C . The Lactose isolated from this milk contains approximately 1.097% ^{13}C , whereas the mean ^{13}C content in breath CO_2 in Europeans is approximately 1.082%. After oral administration, ^{13}C -Lactose passes the stomach and is digested in the small intestine to a certain extent of ^{13}C -Glucose and ^{13}C -Galactose, which are effectively absorbed by the intestine. ^{13}C -Galactose is rapidly converted to ^{13}C -Glucose in the liver. Glucose is oxidized to a large extent. The kinetics of appearance of ^{13}C in breath CO_2 reflects the rate of intestinal digestion of lactose.

■ Applicability of ^{13}C -Lactose Ureide Breath Test

^{13}C -Lactose Breath Test has so far been applied to adults and children.

■ Applications

^{13}C -Lactose Breath Test is used to detect impaired digestion of lactose by comparison with a control range.

■ Protocol

Adults: The ^{13}C -Lactose Breath Test is performed after an overnight fast. A dose of 50 g naturally enriched ^{13}C -Lactose is administered orally, dissolved in 250 ml water. Breath samples are collected before (2x) and every 30 minutes for 240 minutes (4 h) after ingestion of the ^{13}C -Lactose. ^{13}C enrichment in breath CO_2 is determined by Isotope Ratio Mass Spectrometry (IRMS). The cumulative percentage ^{13}C recovered in breath CO_2 after 4 hours is considered as the diagnostic parameter.

Children: a dose of 2 g/kg with a maximum of 50 gram naturally enriched ^{13}C -Lactose is used for children. The same schedule for breath collections can be applied.

■ Interpretation of test results

It is advised to obtain your own internal control values. Generally a cut-off value of 12 % for the 4 hours cumulative recovery may serve as a starting point in the case of adults and children. Values below 12% indicate maldigestion of lactose. It is advised to combine the ^{13}C -Lactose with the H_2 Breath Test, which is performed simultaneously using the same dose of 2 g/kg or 50 g ^{13}C -Lactose. In case one of the tests is positive, this is more representative of low lactase activity (sensitivity 85%).

■ Precautions

No contra-indications for the ^{13}C -Lactose Breath Test test have been described so far. Since the test is using a loading dose of lactose, intestinal complaints including diarrhea are to be expected in maldigesting patients.

■ Summary

	Dose	Samples	
Adults	50 gram naturally enriched ¹³ C-Lactose	2	Before administration
		8	Every 30 minutes for 240 minutes after administration (4 hours)
Children	2 g/kg body weight with a maximum of 50 gram naturally enriched ¹³ C-Lactose	2	Before administration
		8	Every 30 minutes for 240 minutes after administration (4 hours)

■ Recent developments

The outcome of the ¹³C-Lactose Breath Test is affected by the degree of glucose oxidation and the degree of ¹³CO₂ production due to fermentation of nondigested Lactose. Improved diagnosis is obtained by measurement of ¹³C enrichment of plasma glucose (literature reference 4) in particular when 2H-Glucose is administered simultaneously to correct for gastric emptying and glucose metabolism (literature reference 1).

2. Orocecal Transit Time ¹³C-Lactose Ureide Breath Test

■ Principle

¹³C-Lactose Ureide contains urea molecule labeled with the non-radioactive isotope ¹³C attached to an unlabeled Lactose molecule. After oral administration ¹³C-Lactose Ureide is not absorbed in the small intestine and is transferred to the colon. ¹³C-Lactose Ureide is fermented by bacterial enzymes, splitting the bond between carbohydrate and urea. The released ¹³C-Urea is then converted to ammonia and ¹³CO₂, which is absorbed and excreted in the breath. The start of appearance of ¹³C in breath CO₂ reflects the entrance of ¹³C-Lactose Ureide in the colon and therewith the orocecal transit time (OCTT).

■ Applicability of ¹³C-Lactose Ureide Breath Test

¹³C-Lactose Ureide Breath Test has so far been applied to adults and children.

■ Applications

¹³C-Lactose Ureide Breath Test is used to detect accelerated or prolonged intestinal transit by comparison with a control range. The result reflects the orocecal transit time, which may be prolonged by intestinal dysmotility or reduced by nutritional factors or drugs.

■ Protocol

Adults: The subjects are pre-treated with 1 gram unlabeled Lactose Ureide during the evening preceding the study day to stimulate bacterial enzyme production. The ¹³C-Lactose Ureide Breath Test is performed after an overnight fast. A dose of 500 mg ¹³C-Lactose Ureide is administered orally after dissolution in about 100 ml water or cold tea after a standardized breakfast (one wheat flour roll, marmelade, butter and coffee). Breath samples are collected before (2x) and every 30 min for 600 minutes (10 h) after ingestion of the ¹³C-Lactose Ureide. A light lunch containing no ¹³C rich ingredients can be supplied about 4 hours after administration of ¹³C-Lactose Ureide. ¹³C enrichment in breath CO₂ is determined by Isotope Ratio Mass Spectrometry (IRMS). The OCTT is calculated as the time interval between ingestion of ¹³C-Lactose Ureide and the observation of a rise in ¹³C in breath CO₂ of 2 delta values or more followed by a sustained rise of enrichment.

Children: Pre-treatment is performed with 500 mg unlabeled Lactose Ureide. A dose of 250 mg ¹³C-Lactose Ureide is used for the test. The test meal can be adapted to 200 ml chocolate milk. The same time schedule for breath collections can be used reducing the end point to 600 minutes (10 h)

■ Interpretation of test results

It is advised to obtain your own internal control values. Literature data are still scarce. A normal range of 290 ± 60 min has been described for OCTT in adults as well as values between 165 and 390 min for children.

■ Precautions

Bacterial enzyme activity must be stimulated by the pre-treatment protocol. No drugs or food ingredients are allowed during the test that may affect the intestinal transit of the test meal including ¹³C-Lactose Ureide.

■ Summary

	Dose	Samples	
Adults	1 gram (pre-treatment) unlabeled Lactose Ureide, 500 mg ¹³ C-Lactose Ureide	2	Before administration
		20	Every 30 minutes for 600 minutes after administration (10 hours)
Children	500 mg (pre-treatment) unlabeled Lactose Ureide, 250 mg ¹³ C-Lactose Ureide	2	Before administration
		20	Every 30 minutes for 600 minutes after administration (10 hours)

3. Bacterial Overgrowth ¹³C-Xylose Breath Test

■ Principle

¹³C-Xylose is a labeled monosaccharide which is only partially absorbed from the small intestine. The non absorbable part is fermented by colonic bacteria producing ¹³CO₂. The presence of xylose fermenting bacteria in the small intestine leads to an early rise in ¹³CO₂ in breath. The degree of ¹³C enrichment reflects the degree of bacterial overgrowth.

■ Applicability of ¹³C-Xylose Breath Test

So far only a limited number of applications have been described both in adults and in children.

■ Applications

The ¹³C-Xylose Breath Test result reflects small intestinal bacterial mass flow. Correlations have been shown with bacterial cultures in intestinal aspirates.

■ Protocol

Adults: The ¹³C-Xylose Breath Test is performed after an overnight fast. A dose of 250 mg ¹³C-Xylose is administered orally after dissolution in 50 ml water. Breath samples are collected before (2x) and every 30 minutes for 180 minutes (3 h) after ingestion of the ¹³C-Xylose. ¹³C enrichment in breath CO₂ is determined by Isotope Ratio Mass Spectrometry (IRMS). The ¹³C enrichment after the 180 minutes collection period is used as the diagnostic parameter.

Children: A dose of 50 mg is used for children in 5 ml water or in capsules together with 30 ml water. The same time schedule for breath collections can be used. The maximum enrichment has been used as the diagnostic parameter.

■ Interpretation of test results

It is advised to obtain your own internal control values. The number of published data is too small to indicate a general cut off value.

■ Precautions

Since ¹³C-Xylose is partially malabsorbed, colonic bacteria also produce ¹³CO₂. Therefore the small intestinal fermentation should be detected at the earliest time point. However, ¹³C-Xylose is also partly absorbed and metabolically oxidised into ¹³CO₂. This probably explains the control range. Patients with abdominal complaints may generally suffer of malabsorption phenomena and motility disorders. The latter may cause intersubject variation in the rate of Xylose delivery to the fermenting bacteria. A delayed response may be caused by delayed gastric emptying and slow intestinal transit and be interpreted as colonic response. Vice versa increased intestinal motility may cause a rapid colonic response which may be confused with small intestinal response. Furthermore the bacteria should contain active carbohydrate fermenting enzymes.

■ Summary

	Dose	Samples	
Adults	250 mg ¹³ C-Xylose	2	Before administration
		6	Every 30 minutes for 180 minutes after administration (3 hours)
Children	50 mg ¹³ C-Xylose	2	Before administration
		6	Every 30 minutes for 180 minutes after administration (3 hours)

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Intestine

1. Brush Border Enzymes Natural Enriched ¹³C-Lactose Breath Test

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