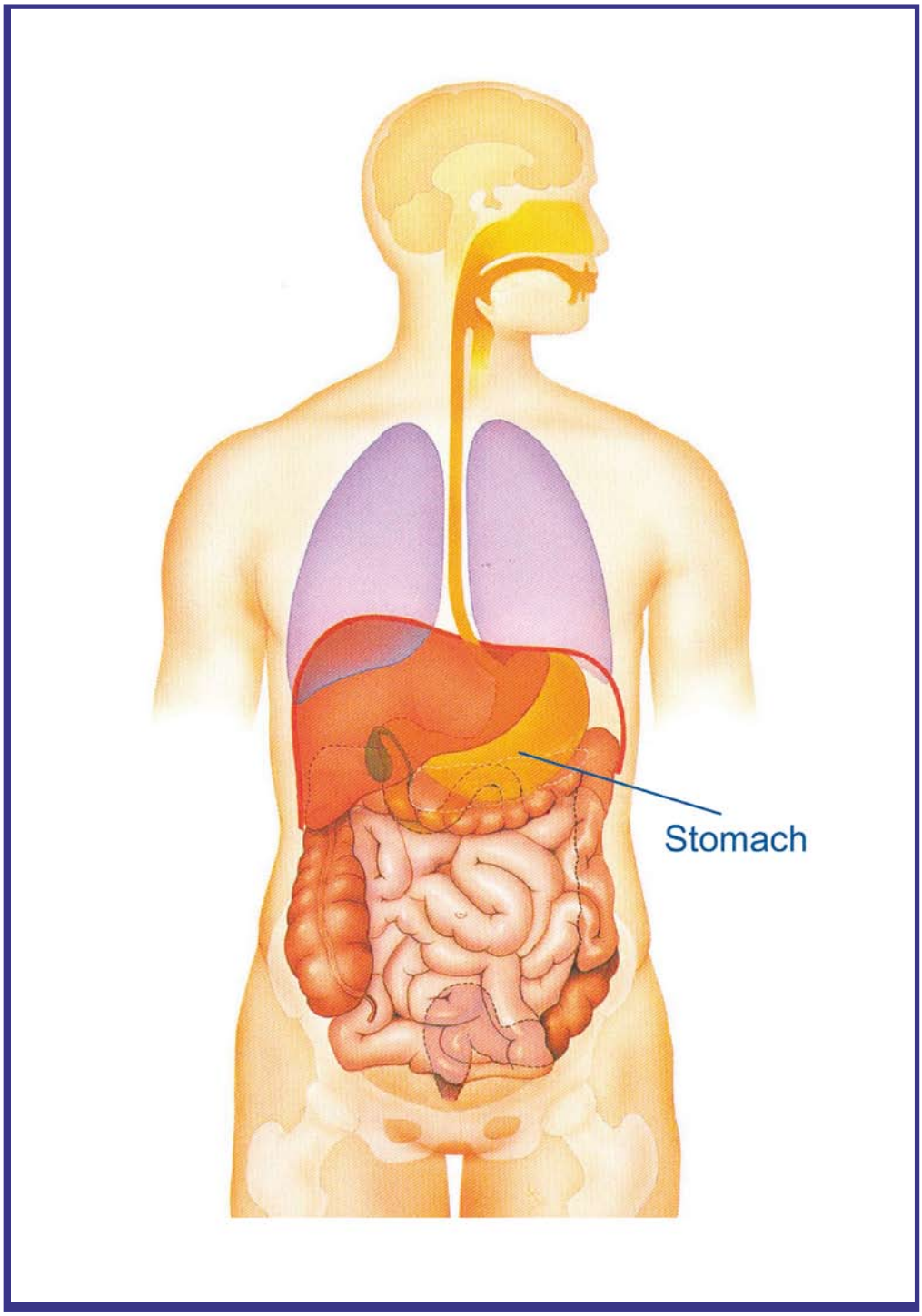
Protocols - ^{13}C Breath Tests - STOMACH



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Introduction

^{13}C Breath Test protocols

This folder contains a set of protocols describing the principles and general test procedures for today's most relevant ^{13}C 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 ^{13}C 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 ^{13}C substrates and the existing knowledge on the particular application of interest. There is no such thing as a standard protocol for all tests.

^{13}C Breath Testing: principle and requirements

A ^{13}C Breath Test consists of the administration to a patient of a ^{13}C labeled substrate that is metabolized by a specific enzyme system resulting in $^{13}CO_2$ as the end product. To monitor the enzyme response ^{13}C enrichment is measured in breath CO_2.
The total procedure of ^{13}C Breath testing includes the definition of the preparation of the patient before the test, administration of the ^{13}C labeled substrate, collection of breath samples, measurement of ^{13}C enrichment in breath CO_2 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 ^{13}C abundance. Therefore, the patient must be instructed to avoid eating ^{13}C 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 (^{13}C Lactose-Ureide breath test) the patient must be pretreated with unlabeled substrate to stimulate the involved enzyme system.

Administration of ^{13}C 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 ^{13}C enrichment in breath CO_2, it is also necessary to obtain at least two breath samples before the ingestion of the ^{13}C substrate to determine the natural background of ^{13}C abundance. The methodology of collecting breath samples is dependent on the technology to determine the ^{13}C 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 ^{13}C enrichment

To determine the ^{13}C abundance in breath CO_2 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 anlalytical technique. It is the level of ^{13}C 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 ^{13}C 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.

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Applications

In the present update the following tests have been described:

Table of contents of applications

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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Test Protocols Stomach

1. Helicobacter pylori Infection
^{13}C-Urea Breath Test

Principle
^{13}C-Urea contains one carbon atom labeled with the non-radioactive isotope ^{13}C. After oral administration, ^{13}C-Urea passes through the stomach. The presence of Helicobacter pylori leads to the hydrolysis of ^{13}C-Urea to ammonia and $^{13}CO_2$ due to the action of urease enzyme. $^{13}CO_2$ is absorbed, transported by the blood and excreted by the lungs. The appearance of ^{13}C in breath CO_2 reflects the presence of Helicobacter pylori.

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

Applications
^{13}C-Urea Breath Test is used to detect Helicobacter pylori in patients with general gastric complaints. An increase of ^{13}C abundance outside the control range is indicative for a positive test result. The effect of eradication therapy can be monitored within one month after the completion of therapy.

Protocol
Adults: The ^{13}C-Urea Breath Test is performed after an overnight fast. A dose of 75 mg or 100 mg is administered orally after dissolution in about 100 ml water. The intake of the solution is preceeded by citric acid solution or orange juice. Breath samples are collected before (2x) and 30 minutes (2x) after ingestion of the ^{13}C-Urea. ^{13}C enrichment in breath CO_2 is determined by Isotope Ratio Mass Spectrometry (IRMS) or non-dispersive Infrared Spectrometry. The absolute increase of ^{13}C abundance after 30 minutes is compared to the baseline value and then used as the diagnostic parameter.

Children: A dose of 50 mg is sufficient 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 4 or 5 ‰ is described for the increase after 30 minutes over the baseline value before administration of ^{13}C-Urea.

Precautions
The results must be interpreted with caution when the patient has undergone gastrectomy, has been treated with antibiotics, proton pump inhibitors and antisecretory drugs, or in other cases in which acid secretion is affected such as atrophic gastritis.

Summary

	Dose	Samples	
Adults	75 mg ^{13}C-Urea or 100 mg ^{13}C-Urea	2	Before administration
		2	30 minutes (0.5 h) after administration
Children	50 mg ^{13}C-Urea	2	Before administration
		2	30 minutes (0.5 h) after administration

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2. Gastric Emptying of Solids
^{13}C-Octanoic Acid Breath Test (Leuven Model)

Principle
(1-^{13}C)-Octanoic Acid contains a carboxyl-carbon labeled with the non-radioactive isotope ^{13}C. After oral administration (1-^{13}C)-Octanoic Acid passes through the stomach and is completely absorbed by the small intestine. (1-^{13}C)-Octanoic Acid is oxidized to a large extent. The kinetics of appearance of ^{13}C in breath CO_2 reflects the rate of gastric emptying of the solid phase of a meal.

Applicability of ^{13}C-Octanoic Acid Breath Test
^{13}C-Octanoic Acid Breath Test has so far been applied to adults, children and newborn infants.

Applications
^{13}C-Octanoic Acid Breath test is used to detect impaired gastric emptying of solids by comparison with a control range. The effect of drug treatment can be monitored.

Protocol
Adults: The ^{13}C-Octanoic Acid Breath Test is performed after an overnight fast. A dose of 100 mg (1-^{13}C)-Octanoic Acid is administered orally in a solid test meal. The test meal is standardized and consists of one scrambled egg with two slices of white bread and 5 g of margarine, together with 150 ml water (swallowed immediately after ingestion of the meal). The total caloric content is 250 kcal. The egg yolk is doped with 100 mg (1-^{13}C)-Octanoic Acid and fried separately from the egg white. The meal is consumed within 10 minutes. Breath samples are collected before (2x), every 5 minutes during the first 30 minutes (0.5 h) and every 15 minutes for the next 210 minutes (3.5 h) after the ingestion of the (1-^{13}C)-Octanoic Acid. ^{13}C enrichment in breath CO_2 is determined by Isotope Ratio Mass Spectrometry (IRMS). The equation of the breath test results is obtained by 2 non-linear regression curves fitting the % dose ^{13}C recovered in breath per minute and the cumulative % dose recovered in breath. From this equation the half emptying time and the lag phase time are calculated as well as the gastric emptying coefficient (GEC).

Children: A pancake is made of 5 g sugar, 12.5 g flour, 10 g full cream milk and one egg. Half of the egg yolk is baked separately after solubilising 50 mg (1-^{13}C)-Octanoic Acid by mixing. The obtained paste is baked around the labeled half of the egg yolk. Total energy content is 150 kcal. Meal consumption, breath sampling and calculations are the same as described above for adults.

Newborn infants: A volume of water, necessary for the baby's usual intake, is heated. 50 mg (1-^{13}C)-Octanoic Acid and 1 g PolyEthylene Glycol 3350 needed for solubilization are mixed and cooked. The adequate amount of milk powder is added, and the bottle kept in the refrigerator overnight. The infants receive the test meal at the time of their usual first morning feeding, which allows for 3 hours fasting. The test meal is re-cooked and cooled prior to administration. Breath samples are collected using a nasal prong carefully inserted into the nasopharynx. 10 ml exhaled air is slowly aspirated in a syringe during the expiration phase. Breath samples are collected before (2x), every 5 minutes during the first 30 minutes (0.5 h) and every 15 minutes during the next 210 minutes (3.5 h) after drinking the meal.

Interpretation of test results
It is advised to obtain your own internal control values. The control values will depend on the population (age group) and the test meal used.

Precautions
No contra-indications for the ^{13}C-Octanoic Acid Breath Test have been described so far.

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Summary

	Dose	Samples	
Adults	100 mg (1-^{13}C)-Octanoic Acid	2	Before administration
		6	Every 5 minutes for the first 30 minutes after administration (0.5.h)
		14	Every 15 minutes for the next 210 minutes after administration (3.5 h)
Children / Newborn	50 mg (1-^{13}C)-Octanoic Acid	2	Before administration
		6	Every 5 minutes for the first 30 minutes after administration (0.5.h)
		14	Every 15 minutes for the next 210 minutes after administration (3.5 h)

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3. Gastric Emptying of Liquids
^{13}C-Acetate Breath Test

Principle
(1-^{13}C)-Sodium Acetate contains a carboxyl-carbon labeled with the non-radioactive isotope ^{13}C. After oral administration (1-^{13}C)-Sodium Acetate passes through the stomach and is completely absorbed by the small intestine. (1-^{13}C)-Sodium Acetate is oxidized to a large extent. The kinetics of appearance of ^{13}C in breath CO_2 reflects the rate of gastric emptying of the liquid phase of a meal.

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

Applications
^{13}C-Acetate Breath Test is used to detect impaired gastric emptying of liquids by comparison with a control range. The effect of drug treatment can be monitored.

Protocol
The ^{13}C-Acetate Breath Test is performed after an overnight fast. A dose of 150 mg (1-^{13}C)-Sodium Acetate is administered orally in a liquid test meal. So far the test meal has not yet been standardized and should be adapted to the individual question (neonatal feeding, exercise experiments, food matrix studies). Breath samples are collected before (2x) and every 5 minutes for the next 120 minutes (2 h) and every 10 minutes for the following 120 minutes (2 h) after the ingestion of the (1-^{13}C)-Sodium Acetate. ^{13}C enrichment in breath CO_2 is determined by Isotope Ratio Mass Spectrometry (IRMS). The equation of the breath test results is obtained by non-linear regression curve, fitting of the % dose ^{13}C recovered in breath per minute. From this equation the half emptying time and the lag phase are calculated. Due to a high correlation between the half emptying time and the time of the peak maximum of ^{13}C appearance in breath, the time to peak maximum appears a simple diagnostic parameter for the interpretation of the rate of gastric emptying.

Interpretation of test results
It is advised to obtain your own internal control values. The control values will depend on the population (age group) and the test meal that has been used. For accurate kinetic data the half emptying rate should be calculated. For screening purposes, the time to peak maximum is a simple but accurate marker.

Precautions
No contra-indications for the ^{13}C-Acetate Breath Test test have been described so far.

Summary

	Dose	Samples	
	150 mg (1-^{13}C)- Sodium Acetate	2	Before administration
		24	Every 5 minutes, the first 120 minutes after administration (2 h)
		12	Every 10 minutes, the second 120 minutes after administration (2 h)

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Literature

Stomach

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^{13}C-Urea Breath Test

Recommended literature

Recent literature

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2. Gastric Emptying of Solids
^{13}C-Octanoic Acid Breath Test

Recommended literature

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3. Gastric Emptying of Liquids
^{13}C-Acetate Breath Test

Recommended literature

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