

# Bile Acid Breath Test

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Warning: The radiopharmaceutical  $^{14}\text{C}$ -glycocholic acid used in this protocol is not registered.

## 1. Introduction

The bile acid breath test can be used to assess bacterial overgrowth or bile acid malabsorption in the small intestine. This test is based on the principle that many bacteria are able to split the amino acid part from conjugated bile salts. The radioactive  $^{14}\text{C}$  atom is incorporated in the amino acid part of the glycocholic acid.

Radioactive bile acid ( $^{14}\text{C}$  glycocholic acid) is orally administered. The amino acid glycine is released after splitting from the conjugated bile acid and converted to  $^{14}\text{C}$   $\text{CO}_2$  which is subsequently exhaled. Normally, 95% of bile acids are absorbed in the small intestine. The remaining 5% enters the colon where it is metabolised by natural colon bacteria. In the absence of significant small intestine bacterial overgrowth and bile acid malabsorption, very small amounts of  $^{14}\text{C}$  glycine is released. The amount of  $^{14}\text{C}$   $\text{CO}_2$  exhaled is measured every hour for 5 h during this test. The extent of bacterial overgrowth has a direct correlation with the amount of  $^{14}\text{C}$   $\text{CO}_2$  measured.

## 2. Methodology

This guideline is based on available scientific literature on the subject, the previous guideline (Aanbevelingen Nucleaire Geneeskunde 2007), international guidelines from EANM and/or SNMMI if available and applicable to the Dutch situation.

## 3. Indications

- a. Evaluation of bacterial overgrowth in the small intestine.
- b. Assessment of bile acid malabsorption in the distal ileum.

## 4. Relation to other diagnostic procedures

- a. The  $^{14}\text{C}$  bile acid breath test measures (decreased) absorption of bile acid and bacterial overgrowth at the same time. The SeHCAT test measures bile acid absorption only and is therefore more specific for that indication (see Bile Acid Malabsorption Test Pag 314).  
The non-radioactive  $\text{H}_2$ -breath test has, in most hospitals, replaced the  $^{14}\text{C}$  bile acid breath for the assessment of bacterial overgrowth. The  $\text{H}_2$ -breath test is usually performed in the gastroenterology department.
- b. Morphological damage to the ileum can be diagnosed by CT, MRI or ultrasound, but this may not provide any functional information.

## 5. Medical information necessary for planning

The patient must be able to repeatedly breath through a straw into the investigation vial. This may take several minutes, interruptions will not significantly influence the result.

## 6. Radiopharmaceutical

Tracer:	$^{14}\text{C}$ glycocholic acid sodium-salt, supplemented with 3 mg non radio-active glycocholic acid
Nuclide:	Carbon-14
Activity:	200 kBq
Administration:	Orally, liquid test meal

## 7. Radiation safety

### a. Pregnancy:

The external radiation dose received by the foetus after administration of the radiofarmacon to the mother is less than 0,001 mGy mGy/MBq). Foetal risk is thus low and the investigation can be performed during pregnancy.

### b. Lactation

According to ICRP 106 there is no need to interrupt breastfeeding.

### c. Effective dose (mSv/MBq)

1,6 mSv/MBq, 9,9 E-1 mSv/MBq, 8,0 E-1 mSv/MBq, 5,7 E-1 mSv/MBq, 5,8 E-1 mGy/MBq for respectively a 1-yr-, 5-yr-, 10-yr-, 15-yr old and an adult patient with normal biological functioning.

## 8. Patient preparation/essentials for the procedure

### Patient preparation

1. During the test, the patient is not allowed to drink coffee, eat, smoke, sleep or be very active. Water and tea may be consumed (if preferred with sugar).
2. Patients must have fasted from 10:00 p.m. the day before.
3. Check if the patient is on a diet. The patient must tolerate milk.
4. For diabetic patients the test meal consists of 200 ml sugar free lemonade and a sandwich.
5. If other radiopharmaceuticals have been administered recently, interference of activity in the exhaled air must be ruled out.
6. Document:
  - Hyaminehydroxide titer and the calculated division factor
  - Patient length
  - Patient weight
  - Whether the patient is on any special diet
  - Medication use (in some cases therapy is already initiated and the aim of the investigation is to monitor therapy effect
  - The amount of test meal consumed
7. For diagnosis of bacterial overgrowth, cholestyramine, aluminiumhydroxide and medication that increase intestinal motility should be discontinued 2-3 days prior to the investigation. If the test is used for monitoring therapy effect, medication should be taken as prescribed.

### Essentials for the procedure

1. Test meal (200 g as an emulsion in water). Composition (weight based):
  - 5,6% fat (mixture of corn oil (95 g) and lecithin (5 g))
  - 14% glucose
  - 3,1% milkprotein (Galactomin 19<sup>®</sup> of Nutricia).

2. Bendable straws, mouth pieces, backflow valves and connectors between the straw and the backflow valve.
3. Liquid scintillation vials with caps. The caps must have a hole through which the straw will fit, perspex holder for the vials.
4. Thymolfaleine in absolute ethanol (60 mg/l).
5. Calibrated hyaminehydroxide (1,0 N): prior to use, determine the exact titer of the hyaminehydroxide: add 2 ml hyaminehydroxide to 2 ml thymolfaleine and titrate this with 20 ml HCl 0,1 M.
6. Coloured stickers to place on the vial caps (do not write on the vials itself).
7. Scintillation liquid: for example Instagel plus® or Optiphase® trisafe.

### Procedure

- a. The test meal and the dose ( $^{14}\text{C}$  glycocholic acid) should be taken from the freezer one day prior to the test and kept in a refrigerator until use.
- b. Write the patient's name and evaluation time points ( $T_0$  = baseline,  $T_1$ ,  $T_2$ ,  $T_3$ ,  $T_4$  and  $T_5$  for 1-5 h after ingestion of  $^{14}\text{C}$ ) on stickers and stick them on the vial caps. Measurements are taken in duplicate, thus 12 vials are required per patient. Use different colours for different patients.
- c. Prepare the measuring device.
- d. Place the vial holder on absorbent material e.g. a tissue for easier clean-up in case of spills.
- e. One hour prior to use, fill the liquid scintillation vials with 2 ml hyaminehydroxide and 2 ml thymolfaleine and mix.
- f. Prior to ingestion of the test meal, the patient should blow into the test vials labeled  $T_0$  until the blue area turns colourless (in duplicate). Forceful blowing will result in spillage.
- g. A new straw should be used for each time point.
- h. The test dose ( $^{14}\text{C}$  glycocholic acid) should be mixed with a small amount of water and put in a plastic beaker.
- i. The patient should drink the test dose and a part of the test meal. The plastic beaker that contained the test dose is rinsed repeatedly using the rest of the test meal. This must also be consumed by the patient. The test meal need not be fully consumed, as long as the amount consumed is noted.
- j. The patient should blow into the vials in duplicate, 1,2,3,4 and 5 h post  $^{14}\text{C}$  ingestion. Use new straws between duplicates.
- k. After  $T_3$  (i.e. the fourth measurement time point), the patient consumes bread/ a sandwich.
- l. After blowing in the vials, add 5 ml scintillation liquid, mix and clean the vials with alcohol.
- m. Count the vials in the scintillation counter (with  $^{14}\text{C}$  setting).
- n. Use known references (200, 500 and 1000 dpm (low, middle, high)) and a  $^{14}\text{C}$  calibration standard (100.000 dpm) for internal control. An empty vial is used for background correction.

### **9. Interpretation**

- a. The  $T_0$  value is used as baseline value and is subtracted from all the other measurements.

- b. The percentage of the administered dose (G) exhaled per mmol CO<sub>2</sub> is calculated using the following formula:

$$G = \frac{A}{F \times D \times T \times V} \times 100 \% [x10^{-3}/\text{mmol}]$$

In which:

- A = measured signal of the vial corrected for background (cpm)  
 F = correction factor to gain the correct unit  
 D = administered dose (kBq)  
 T = titer hyaminehydroxide solution (mol/l)  
 V = volume hyaminehydroxide solution (ml)

- c. If measurements remain under  $10^{-3} \times 1,5\%$  of the dose per mmol CO<sub>2</sub>, bacterial overgrowth is absent.
- d. If measurements are increased, the shape of the curve and the area under the curve, which is drawn through the 5 measuring points, represent the extent and location of the overgrowth:
- Peak within the first 4 measuring hours: bacterial overgrowth in the small intestine.
  - Peak after the 4th hour: dysfunction of the distal ileum.
  - After resection of the ileum the peak can occur on the 4th hour.
  - After gastrectomy or if there is gastrointestinal hypermotility, the time indications above are not applicable.

## 10. Report

The percentage of the administered dose exhaled per mmol CO<sub>2</sub> should be stated. It should be documented if bacterial overgrowth or dysfunction of the terminal ileum is present. Also, medication use during the test should be noted.

## 11. Literature

- Fromm H, Hofmann AF. Breath test for altered bile-acid metabolism. *Lancet* 1971;2:621-5.
- James OFW, Agnew JE, Bouchier IAD, et al. Assessment of the C-14-Glycocholic acid breath test. *Brit J Med* 1973;3:191-5.
- Sherr HP, Sasaki Y, Newman A, et al. Detection of bacterial deconjugation of bile salts by a convenient breath analysis technique. *N Engl J Med* 1971;285:656-61.