^{14}C-Urea Breath Test

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1. Introduction

The ^{14}C urea breath test is based on the principle that *Helicobacter pylori* bacteria (previously known as *Campylobacter pylori*) have the specific ability to convert urea into CO_2 and ammonia. To verify the presence of these bacteria in the stomach, ^{14}C labelled urea is administered to the patient in the form of a test meal. When these bacteria are present, ^{14}C-CO_2 will be formed. This is absorbed in the body bicarbonate pool and subsequently exhaled.
This test measures the amount of ^{14}C-CO_2 present in a fixed amount (2 mmol) of exhaled CO_2 one hour after dose administration (in duplicate). In many Dutch hospitals, the ^{14}C urea breath test has been replaced by the (non-radioactive) ^{13}C-urea breath test, which is usually performed at the gastro-enterology department.

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. Suspected gastric colonisation with *Helicobacter pylori*.
b. Confirmation of eradication. Of note, the test should not be performed sooner than one month after completion of the therapy.

4. Relation to other diagnostic procedures

a. The ^{14}C-urea breath test is a non-invasive test that specifically detects the presence of *Helicobacter pylori* in the gastrointestinal tract because conversion of urea into CO_2 and ammonia takes place only in the presence of Helicobacter pylori. As mentioned in the introduction, nowadays many gastro-enterology departments use the (non radio-active) ^{13}C-urea breath test for this indication.
b. The presence of *Helicobacter Pylori* in the gastric mucosa can also be demonstrated directly by taking gastric biopsies and histological staining or culturing. The culture can then be tested for the ability to split urea. However, this is a demanding and time-consuming procedure.
c. Blood samples can be analysed for the presence of antibodies against *Helicobacter pylori.* This technique cannot discriminate between a recent and an old infection.

5. Medical information necessary for planning

The patient must be able to exhale through a straw into a sample bottle for several minutes. A few short breaks are allowed.

6. Radiopharmaceutical

Tracer:	^{14}C-Urea, filled up to 350 mg with 'cold' urea, dissolved in 1 ml water. Store at -20 °C. Alternatively, this liquid test dose can be replaced by ^{14}C-urea capsules (37 kBq)
Nuclide:	Carbon-14
Activity:	200 kBq
Administration:	Oral, dissolved in 100 ml water

7. Radiation safety

Due to the very low amount of activity administered (200 kBq) and the very low effective dose for the foetus 0,0048 mGy (0,024 mGy/MBq), there are no precautions necessary during pregnancy or lactation.

8. Patient preparation/essentials for the procedure

Patient preparation

1. The patient should be nil by mouth for 4 h prior to the investigation and may not smoke, eat, drink, sleep or perform strenuous work during the test.
2. If the patient has recently undergone any investigations using radioactive substances it should be ascertained that there is no interference with the ^{14}C-urea breath test.
3. Proton pump inhibitors and sucralfate should not be used two weeks prior to the investigation.
4. Antibiotics and bismuth compounds should not be used in the preceding thirty days.

Essentials for the procedure

1. ^{14}C-labelled urea. The dosage consists of 200 kBq activity and 350 mg urea in a volume of 1 ml water.
2. Nutricia Nutridrink®.
3. Exhalation station: Bendable straws, mouth pieces, check valves and connection pieces to be fitted between the straw and the check valve. Scintillation vials and caps (caps with a hole for the straw), Perspex holders for the vials. A dehumidifier ($CaSO_4$) to prevent quenching by H_2O in the scintillation fluid.
4. Thymolphthalein in absolute ethanol (60 mg/l).
5. Calibrated hyamine hydroxide (approximately 1,0 N): Prior to use, titrate the hyamine hydroxide to determine the exact titre. Perform this (one-off) titre calibration per vial as follows: Pipette 2 ml of hyamine hydroxide (using the dispenser to be used in the test) and 2 ml thymolphthalein into a receptacle. Titrate this using approximately 20 ml of 0,1 M HCl.
6. Coloured stickers for the caps of the scintillation vials.
7. Scintillation fluid: e.g. Instagel® or Optiphase trisafe®.
8. Alternatively, the liquid test dose can be replaced by ^{14}C-urea capsules (37 kBq) and analysis can subsequently be performed by commercially available breathcards and a ^{14}C analyser.

Procedure

1. Record the weight of the patient.
2. Fill the scintillation vials with 2 ml of thymolphthalein and 2 ml of hyamine hydroxide and

mix. This mix should be prepared no more than 1 h before planned exhalation.

3. Before dose administration, ask the patient to exhale in duplicate until the blue area on the scintillation vials changes to colourless. Ask patients not to exhale too forcefully to prevent sputtering.
4. Decant the ^{14}C-urea dose into a plastic beaker and rinse twice with as little water as possible.
5. Pour a 60 ml portion of Nutridrink® into a plastic beaker.
6. Ask the patient to drink half of the Nutridrink® (i.e. 30 ml) and then administer the dose. Rinse the dosage cup with the remaining Nutridrink® and ask the patient to drink this as well.
7. Forty minutes after dose administration, ask the patient to exhale into one scintillation vial until the blue area changes to colourless. Breath samples should be taken every 10 min from t=0 until t=60 min to generate a curve and to minimise the chances of an artifact detracting from the reliability of the investigation. Moreover, the curve can reveal additional information.
8. After exhalation, add 5 ml of scintillation fluid to each scintillation vial, mix and use alcohol to clean the vials.

9. Measuring conditions

a. Analyse the vials in a liquid scintillation counter using the settings for ^{14}C.
b. Include references containing approximately 200, 500, 1000 dpm respectively (low, middle, high) and a ^{14}C reference standard (100.000 dpm) as controls, plus a blank vial to correct for background activity.
c. As mentioned above, analysis can also be performed using commercially available breath cards and a ^{14}C analyser (after ingestion of ^{14}C-urea capsules (37 kBq)).

10. Interpretation

a. The counts are corrected for background activity.
b. The percentage of the dose (G) that is exhaled per mmol CO_2 is calculated according to the formula below:

$$G = \frac{A}{F \times D \times T \times V} \times 100 \times BW \ \left[\% \times kg / mmol \right]$$

in which:

A = mean of both counts corrected for background activity (cpm)
F = correction factor to obtain the correct unit
D = administered dosage (kBq)
T = titre of hyamine hydroxide solution (mol/l)
V = volume of hyamine hydroxide solution (ml)
BW = body weight (kg)

Note: All conversion factors required have been combined in the correction factor in order to convert the counts in cpm first into dpm and kBq and then into the percentage.

c. The test demonstrates a higher than normal presence of Helicobacter pylori when the ^{14}C-urea breath test results exceed 0,07% × kg/mmol.
d. The test should not be performed within four weeks of antibiotic eradication treatment against *Helicobacter pylori*. Urease activity of the bacteria might temporarily be low without permanent eradication of the bacteria leading to a false negative result.

11. Report

The percentage of the dose that is exhaled per mmol CO_2 and normal values (< 0,07% x kg/mmol) is decribed.

12. Literature

- Bell GD, Weil J, Harrison, et al. C-14-urea breath analysis, a non-invasive test for Campylobacter pylori in the stomach. Lancet 1987;1367-8.
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