

Application Note

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CIA-001

Detection of Helicobacter Pylori Infection Using a ^{14}C -Urea Breath Test

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Introduction

For the greater part of this century, medical opinion has favored the dictum, stated by the Croatian physician Karl Schwarz; no acid, no ulcer. To this end, therapeutic measures have all focussed on either the reduction, removal or suppression of inappropriate secretion of excess gastric acid with variable but usually limited success. In 1984, Marshall et al.^{1,2} demonstrated that an organism that they identified as a Campylobacter, but subsequently was revealed to be a Helicobacter, was partly, if not totally, responsible for the development of gastric and duodenal ulceration. This spiral organism was named Helicobacter pylori. Therefore, therapy in the form of antibiotics (alone or in conjunction with acid suppressants) was designed to eradicate the infection, with spectacularly successful results (Sung, Chung, Ling et al. 1995; Mendall 1995).^{3,4}

Methods for Detection of H. Pylori Infection

Culture of the organism from gastric biopsies proved to be extremely difficult and therefore not suitable for routine detection. Thus, testing for H. pylori infection was originally carried out using conventional histological techniques on gastric and duodenal biopsies obtained during endoscopy. However, the sensitivity of this has been reported to be as low as 85% (Simor et al. 1990)⁵ and since H. pylori tends to show patchy colonization of gastric mucosa, multiple biopsies have to be taken. A more simple biopsy test was developed, the CLO

(Campylobacter Like Organism) test, in which the sample is placed on a microscope slide holding an immobilized gel containing urea and an indicator. The urease activity in the organism breaks down the urea causing a pH shift which is registered by a change in color. Apart from its simplicity, this test suffers from those disadvantages inherent in biopsy based tests. Additionally, acid suppression, gastric atrophy and contamination with bile can all result in false positives.

Measurement of $^{14}\text{CO}_2$ in expired breath following the consumption of a ^{14}C -labeled meal has been used for many years in the diagnosis of a variety of diseases. The development of a breath test for H. pylori using this principle was therefore a logical one and a significant improvement over available tests. ^{14}C -labeled urea is given in a drink; then breath samples are collected and counted for ^{14}C . In infected patients, the organism breaks down the urea to ammonia and $^{14}\text{CO}_2$, which manifests itself in increased counts in the expired air (Figure 1). In non-infected patients, no breakdown occurs and the labeled urea, which takes part in no major biochemical systems, is excreted in the urine. The significant advantage of the test is the fact that, apart from being simple and inexpensive to perform, infection in any part of the stomach will give a positive result since no discrete sampling takes place. Other urease producing organisms such as Proteus and Klebsiella express only comparatively small amounts and are rarely found in the stomach, and therefore represent no real threat of producing false positive results.



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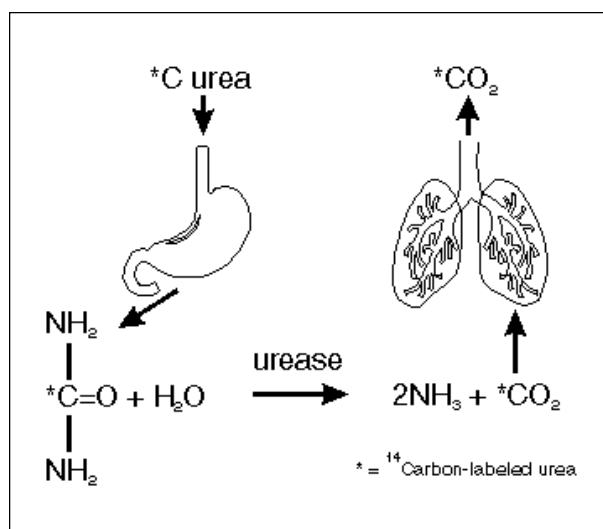


Figure 1.
The principle of the urea breath test.

Materials and Methods

After a six hour fast, a baseline breath sample is taken. The sample is collected by exhaling steadily into a plastic tube which terminates in a 20 mL glass scintillation vial containing 4 mL of 0.5M hyamine (benzethonium hydroxide) and 0.5 mL of phenolphthalein (0.04M) indicator. The tip of the tube is positioned beneath the surface of the liquid, so that expired CO_2 is absorbed by it.

The tube contains a silica gel trap to absorb water and prevent accidental ingestion of the scintillation vial contents. The point at which the hyamine is neutralized, indicated by the change in color of the phenolphthalein, represents 2 mmol of expired CO_2 and the breath collection is stopped.

Following this, the patient is given a 150 mL, 250 Kcal liquid meal containing 8 g of fat. After five minutes, 37 kBq of ^{14}C -labeled urea is given in 50 mL of the same liquid meal. The first drink is given with the aim of delaying gastric emptying so that when the labeled part of the meal is given, it remains in contact with the gastric mucosa and any *H. pylori* present, for at least five minutes. Breath samples are collected 15, 20, 30, 40, 50 and 60 minutes following the meal. Twenty mL Emulsifier Safe (Packard Instrument Company, Meriden, Connecticut USA) is added to the vials which are kept in the dark for one hour before counting on a Packard Tri-Carb[®] 2100TR for ten minutes.

Results and Discussion

Table 1 contains the mean values with 99% confidence intervals (C.I.) for both positive and negative patients. These data are graphically represented in Figure 2. Data show that the test is extremely sensitive. Positives can be identified in as little as 15 minutes after ingestion of the ^{14}C -labeled urea. Figure 3 shows typical values for patients who have undergone a single course eradication therapy.

Positive Urea Breath Tests						
	Sample Times					
	15 min.	20 min.	30 min.	40 min.	50 min.	60 min.
Mean DPM	477.7	683.2	903.9	980.5	1100.9	1133.1
S.E.M.*	91.1	122.2	212.6	198.1	233.5	236.4
99% C.I.**	283.0	379.6	660.4	596.6	703.3	712.3
Negative Urea Breath Tests						
	Sample Times					
	15 min.	20 min.	30 min.	40 min.	50 min.	60 min.
Mean DPM	47.5	43.3	39.9	35.0	38.2	38.3
S.E.M.	11.5	7.5	3.8	4.4	4.8	4.3
99% C.I.	37.4	24.4	12.5	12.9	14.0	12.6

Table 1.
Breath test results for positive and negative patients. * S.E.M. = Standard error of the mean.
** C.I. = Confidence interval.

[†]Emulsifier Safe is sold as PolyFluor in the USA.

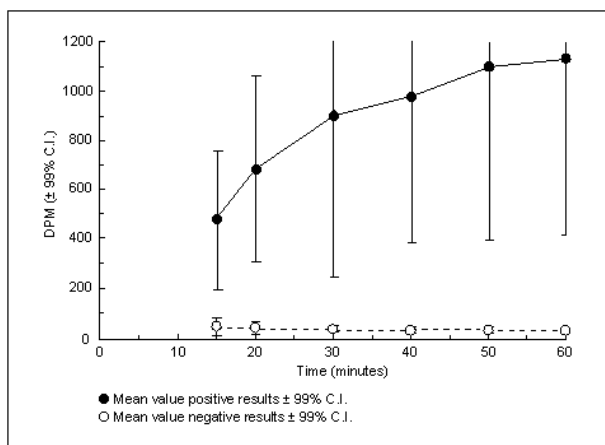


Figure 2.

Mean values for positive and negative patients (99% C.I.).

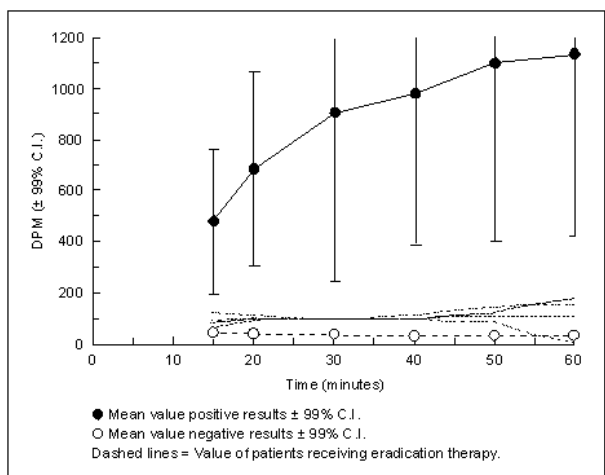


Figure 3.

Typical values for patients receiving eradication therapy.

Three scintillation fluids were tested (Table 2), which are recommended by the manufacturers for CO₂ trapping. Optiphase Safe (EG&G Wallac, Turku Finland) gave unacceptably high levels of chemiluminescence and lost dissolved CO₂, indicated by a reversion to a blue color after standing. Soluscint O (National Diagnostics, Atlanta, Georgia USA) frequently changed color to a deep yellow which resulted in a large quenching effect, rendering the samples unfit for counting. Emulsifier Safe suffered none of these problems and took less time for settling of chemiluminescence.

A performance comparison between Soluscint O (S) and Emulsifier Safe (E) was carried out by collecting samples in a double quantity of hyamine, dividing them in two and adding the respective scintillation fluids (Table 3).

Scintillation Fluid Only	tSIE	CPM	DPM*	LUM
Optiphase Safe	392	32	34	50
Soluscint O	594	26	27	4
Emulsifier Safe	404	21	22	0
Blanks				
Optiphase Safe	297	176	191	80
Soluscint O	236	19	21	7
Emulsifier Safe	191	24	27	0
370 kBq Standard				
Optiphase Safe	363	10078	10780	21
Soluscint O	237	21247	20205	0
Emulsifier Safe	193	21205	20449	0

Table 2.

Performance of several scintillation cocktails. * ¹⁴C efficiency applied.

Time After Scintillation Addition						
		DPM	LUM (%)		DPM	LUM (%)
1 hour	E	113	15	S	157	51
4 hours	E	111	8	S	138	47
12 hours	E	108	3	S	127	38
24 hours	E	104	1	S	118	23
48 hours	E	103	0	S	115	18
72 hours	E	104	1	S	108	2
96 hours	E	103	0	S	101	0

Table 3.

Performance of Emulsifier Safe (E) and Soluscint O (S) cocktails.

Summary

The ¹⁴Carbon-labeled urea breath test represents an inexpensive, reliable, sensitive and safe means for testing for *H. pylori* infection in man. By using a low dose of isotope (*i.e.*, 37 kBq) and collecting a larger amount of expired CO₂, the sensitivity of the test can be maintained while keeping the exposure to radioactive isotope to a minimum. Stubbs and Marshall (1993)⁶ calculated that the largest effective dose equivalent for the 37 kBq ¹⁴CO₂ urea breath test was 0.08 mSv/MBq equivalent to about 11 hours worth of exposure to natural background radiation.

The scope for testing patients remains unclear at present. While there is general agreement that pretreatment testing is advisable, the issue regarding posttreatment testing is less clear. Our results have shown that, in some patients, after a single course of antibiotics, urea breath test results can still exceed the values found in *H. pylori* negative patients, and therefore must be considered as evidence of residual infection. The consequences of this remain unclear and the economic consequences of either a repeat course of antibiotics or a recurrent ulcer, requiring further endoscopy and treatment, are unresolved.

References

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