

¹³C urea breath test for *Helicobacter pylori*: Determination of the optimal cut-off point in a Canadian community population

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AIM: To determine the test characteristics and the optimal cut-off point for the ¹³C urea breath test (¹³C UBT) in a Canadian community laboratory setting.

METHODS: Of 2232 patients (mean age \pm SD: 51 \pm 21 years, 56% female) who completed a ¹³C UBT, 1209 were tested to evaluate the primary diagnosis of *Helicobacter pylori* infection and 1023 were tested for confirmation of eradication following treatment. Cluster analysis was performed on the ¹³C UBT data to determine the optimal cut-off point and the risk of false-positive and false-negative results. Additionally, 176 patients underwent endoscopic biopsy to allow validation of the sensitivity and specificity of the ¹³C UBT against histology and microbiology using the calculated cut-off point.

RESULTS: The calculated cut-off points were 3.09 $\delta\%$ for the whole study population (n=2232), 3.09 $\delta\%$ for the diagnosis group (n=1209) and 2.88 $\delta\%$ for the post-treatment group (n=1023). When replacing the calculated cut-off points by a practical cut-off point of 3.0 $\delta\%$, the risk of false-positive and false-negative results was lower than 2.3%. The ¹³C UBT showed 100% sensitivity and 98.5% specificity compared with histology and microbiology (n=176) for the diagnosis of active *H pylori* infection.

CONCLUSIONS: The ¹³C UBT is an accurate, noninvasive test for the diagnosis of *H pylori* infection and for confirmation of cure after eradication therapy. The present study confirms the validity of a cut-off point of 3.0 $\delta\%$ for the ¹³C UBT when used in a large Canadian community population according to a standard protocol.

Key Words: ¹³C urea breath test; Cut-off point; *Helicobacter pylori*

Helicobacter pylori infection is one of the most common human infections worldwide (1). This organism has been shown to infect over 50% of the world's population, with a prevalence of 20% to 40% in the Canadian population and up to 80% in developing countries (2-5).

H pylori is the primary cause of gastritis and peptic ulcer disease, and has been associated with gastric lymphoma and adenocarcinoma (6,7). Since the discovery of its pivotal role in many human gastroduodenal pathologies, several diagnostic

Le test respiratoire à l'urée marquée au ¹³C pour dépister l'*Helicobacter pylori* : Les limites d'inclusion optimales au sein d'une population canadienne

OBJECTIF : Déterminer les caractéristiques et les limites d'inclusion optimales du test respiratoire à l'urée marquée au ¹³C (TRUM ¹³C) dans un laboratoire communautaire canadien.

MÉTHODOLOGIE : Des 2 232 patients (âge moyen de 51 \pm 21 ans, 56 % de sexe féminin) qui ont effectué un TRUM ¹³C, 1 209 avaient subi le test pour évaluer un diagnostic primaire d'infection par *Helicobacter pylori* et 1 023 pour en confirmer l'éradication après traitement. On a exécuté une analyse typologique des données du TRUM ¹³C pour déterminer la limite d'inclusion optimale et le risque de résultats faux positifs et faux négatifs. De plus, 176 patients ont subi une biopsie endoscopique afin de valider la sensibilité et la spécificité du TRUM ¹³C par rapport à l'histologie et à la microbiologie, au moyen de la limite d'inclusion calculée.

RÉSULTATS : Les limites d'inclusion calculées étaient de 3,09 $\delta\%$ pour toute la population à l'étude (n=2 232), de 3,09 $\delta\%$ pour le groupe diagnostiqué (n= 1 209) et de 2,88 $\delta\%$ pour le groupe déjà traité (n= 1023). Lorsqu'on remplaçait les limites d'inclusion calculées par une limite d'inclusion pratique de 3,0 $\delta\%$, le risque de résultats faux positifs et faux négatifs était inférieur à 2,3 %. Le TRUM ¹³C s'associe à une sensibilité de 100 % et à une spécificité de 98,5 % par rapport à l'histologie et à la microbiologie (n=176) pour le diagnostic d'infection par *H pylori* active.

CONCLUSIONS : Le TRUM ¹³C est un test précis et non effrayant pour diagnostiquer l'infection par *H pylori* et en confirmer la guérison après une thérapie d'éradication. La présente étude confirme la validité d'utiliser une limite d'inclusion de 3,0 $\delta\%$ pour le TRUM ¹³C au sein d'une vaste population du Canada, selon un protocole standard.

tests, both invasive and noninvasive, have been developed. Recently, it has been recommended that subjects younger than 45 years of age with abdominal discomfort should undergo a noninvasive and rapid diagnosis of *H pylori* infection, followed by pharmacological treatment if the test is positive (8). The ¹³C urea breath test (¹³C UBT), with a specificity of 98% and a sensitivity of 97%, allows the diagnosis of an active *H pylori* infection without the need of costly and invasive endoscopic testing (9). Thus, the ¹³C UBT has become the noninvasive

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test of choice in many jurisdictions for diagnosis and confirmation of eradication following treatment, as recommended by a number of clinical guidelines (8,10,11). The ¹³C UBT also has the advantage of assessing the global presence of *H pylori* throughout the stomach, whereas endoscopy-based tests are limited to focal assessments (at the site of biopsy) with the consequent risk of false-negative tests due to sampling errors (12).

The ¹³C UBT detects the presence of gastric *H pylori* urease, which hydrolyzes orally administered ¹³C-labelled urea (a stable isotope) and produces ammonia and ¹³C-labelled carbon dioxide (¹³CO₂). The ¹³CO₂ diffuses into the blood and is excreted by the lungs; therefore it can be detected in the breath using various methods. To distinguish between positive and negative results, a diagnostic cut-off value is defined at a specified time point after ingestion of a substrate. This cut-off point is generally determined by comparison with the 'gold standard' diagnostic technique (usually, histology and culture) in the affected population (13). However, there is still controversy about the value of the best cut-off point. The ¹³C UBT value may, for example, be affected by sociodemographic factors, concomitant medication and bacterial and host factors (14-18), leading to the possibility that the optimal cut-off point could be quite variable. Thus, before a test such as this is widely adopted, a comprehensive reassessment of the cut-off point is needed in the appropriate populations. Despite the need to validate the clinical performance of the ¹³C UBT in a Canadian population, until now, there have been no large-scale data available for this purpose. Validation of the ¹³C UBT's performance in a community setting is essential before it is adopted as part of a primary care-based 'test and treat' strategy for dyspepsia management (19).

The objective of the present study was to determine the optimal cut-off value for the ¹³C UBT by cluster analysis in a large dataset from Canadian patients who had undergone testing in community laboratories, and to support it by independent validation against histology and culture in a Canadian setting using the same study protocol.

PATIENTS AND METHODS

The protocol of the present study was approved by the McMaster University Research Ethics Board. Results of ¹³C UBT performed on 2232 patients (mean age ± SD: 51±21 years, 56% female) were analyzed. Among them, 1209 patients (mean age: 49±17 years, 54% female) were tested to evaluate the primary diagnosis of *H pylori* infection (diagnosis group), and 1023 patients (mean age: 53±25 years, 57% female) were tested for confirmation of eradication following treatment (post-treatment group). Samples were collected through community laboratories (MDS Diagnostic Services, Toronto, Ontario) and analyzed at the McMaster University Medical Centre, Hamilton, Ontario. An additional 176 patients from the McMaster University Medical Centre had both ¹³C UBT and endoscopic biopsies to determine their *H pylori* status.

Using a standardized questionnaire, patients were asked about their use of antibiotics and acid-suppressive treatment during the four weeks before testing. Exclusion criteria included the use of proton pump inhibitors, bismuth compounds or antibiotics within 14 days before the ¹³C UBT. The ¹³C UBT was performed after an overnight fast by obtaining two breath samples, one before (T₀) and the second 30 min after (T₃₀) oral administration of 75 mg ¹³C-labelled urea (Helikit, Isotechnika Diagnostics, Canada) in 100 mL of citric acid solution. The samples were obtained by

having patients gently exhale through a plastic straw, with its distal tip placed at the bottom of a 10 mL glass tube. The tube was sealed with a stopper immediately after patient exhalation. All samples were analyzed by a gas isotope ratio mass spectrometer (BreathMAT, Finnigan MAT GmbH, Germany). The difference between values at 30 min and at baseline (T₃₀-T₀) is expressed as delta (δ) over baseline (DOB, ‰).

Data were examined visually by plotting the logarithmic transformation of measurements taken from ¹³C UBT. The distribution of the natural logarithms (log_n) of the DOB values for each breath sample test interval identified two normal subpopulations that were considered to represent *H pylori*-positive and *H pylori*-negative patients. The normal distributions of the positive and negative populations allowed cluster analysis to be performed on these data to determine the minimal interclass variance, and thus, the log_n (T₃₀-T₀) value, which best separated the presumed *H pylori*-negative and *H pylori*-positive populations. Thereafter, the parameters (mean DOB [‰], and SD) for the *H pylori*-negative and *H pylori*-positive populations were established. The cut-off value was calculated as the point equidistant between the mean DOB values of the *H pylori*-negative and *H pylori*-positive populations. This was the basis for determining the probability of an *H pylori*-negative patient producing a ¹³C UBT result above the cut-off point (ie, a false-positive result), and the probability of an *H pylori*-positive patient producing a ¹³C UBT result below the cut-off point (ie, a false-negative result). This analysis was performed on the whole patient group and on the subgroups (primary diagnosis and confirmation of cure after eradication therapy).

To compare the ¹³C UBT with histology and microbiology, upper gastrointestinal endoscopy was performed on 176 patients before the ¹³C UBT was administered. During an examination, three biopsy specimens each from the gastric antrum and corpus were obtained for histology and bacterial culture. Silver staining (Warthin-Starry) was used to identify *H pylori* in the specimens. Culture samples were inoculated in *Brucella* blood agar, Mueller-Hinton sheep blood agar and egg yolk emulsion; the plates were incubated at 35°C for four days. *H pylori* growth was confirmed by Gram-staining, a rapid urea test, an oxidase test and a wet preparation for motility. Negative cultures were reincubated and examined seven to 10 days later. *H pylori* status was determined when both methods (histology and culture) produced concordant results. Sensitivity, specificity and positive and negative predictive values for the ¹³C UBT were calculated using the optimal cut-off point.

RESULTS

Cut-off point determined by cluster analysis

Data were examined visually by plotting the logarithmic transformation of the DOB values obtained from 2232 ¹³C UBT results. It was evident that the normal distributions of the DOB values could describe two distinct classes of results: *H pylori*-negative and *H pylori*-positive populations (Figure 1). From this, the means and SDs of the log_n (T₃₀-T₀) for the presumed subpopulations were calculated. The point equidistant between the mean values of the subpopulations was determined to be an appropriate cut-off point. The calculated cut-off point for the whole study population was 3.09 ‰. The distances (normal deviate) between the cut-off point and the means of the negative and positive *H pylori* distributions were calculated. The normal deviates were found to be greater than 2 SD (normal deviate = 2.28 SD), indicating that by comparison with the table of proportions of the normal curve,

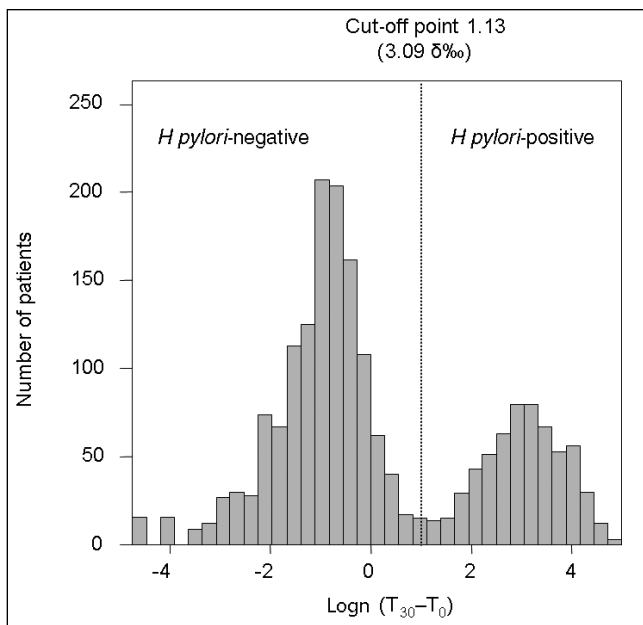


Figure 1) Cluster analysis in the whole study population. The histogram of the logarithmically transformed ¹³C urea breath test values shows two distinct populations: *Helicobacter pylori*-negative and *H pylori*-positive. The cut-off value was calculated as the point equidistant from the means of the *H pylori*-negative and *H pylori*-positive populations. Logn Natural logarithm; T₀ Time (0 min) before oral administration; T₃₀ Time (30 min) after oral administration

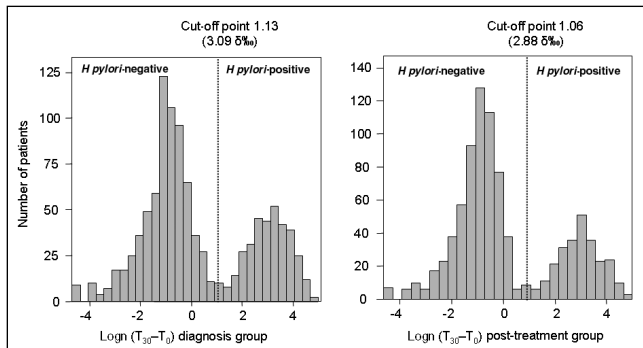


Figure 2) Cluster analysis in the diagnosis (left) and post-treatment (right) groups. The histograms of the logarithmically transformed ¹³C urea breath test values show two distinct populations: *Helicobacter pylori*-negative and *H pylori*-positive. The cut-off value was calculated as the point equidistant from the means of the *H pylori*-negative and *H pylori*-positive populations. Logn Natural logarithm; T₀ Time (0 min) before oral administration; T₃₀ Time (30 min) after oral administration

the proportions of *H pylori*-negative and *H pylori*-positive populations producing a ¹³C UBT result greater or smaller than the cut-off point were always lower than 2.3%. Thus, the risk of a false-positive or a false-negative result from the ¹³C UBT for a diagnosis of *H pylori* infection was lower than 2.3% when using the cut-off point 3.09 ‰. Using a practical cut-off point of 3.0 ‰, the corresponding normal deviates for the *H pylori*-negative and *H pylori*-positive distributions were still greater than 2 SD; therefore, according to the table of the proportions of the normal curve, the risks of error were still lower than 2.3%. Using a cut-off point of 3.0 ‰, 73.3% of the population studied was negative and 26.7% was positive for *H pylori* infection.

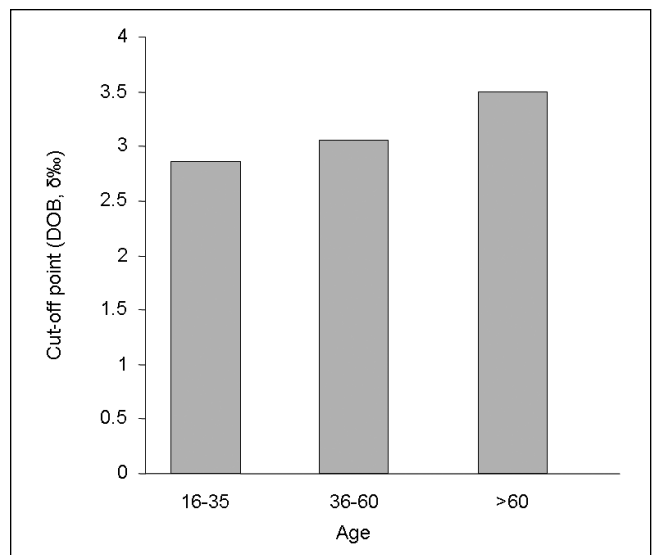


Figure 3) Cut-off points in different age (years) groups. The delta over baseline (DOB) value increased with age. When a cut-off point of 3.0 ‰ in all age groups was used, the risk of error was lower than 2.3%. The cut-off point was not determined for patients younger than 16 years of age due to insufficient data

These calculations were also performed for the diagnosis and post-treatment groups. The calculated cut-off point for the ¹³C UBT was 3.09 ‰ for the diagnosis group and 2.89 ‰ for the post-treatment group (Figure 2). Replacing the cut-off points obtained in the two groups with 3.0 ‰ (as determined for the whole population), the normal deviates for the *H pylori*-negative and *H pylori*-positive distributions in both groups were greater than 2 SD in all cases, which is again indicative of a risk lower than 2.3% for false-positive or false-negative results. Overall, 29.4% of patients were positive for *H pylori* infection in the diagnosis group and 22.4% were positive in the post-treatment group. There was no difference between women and men when a cut-off point of 3.0 ‰ was used; the risk of error was lower than 2.3% in both groups.

A cut-off point was also determined for different age groups. Although DOB values increased with age (Figure 3), using a cut-off point of 3.0 ‰ showed a risk of error lower than 2.3% in the population older than 16 years of age. The cut-off point was not determined in patients younger than 16 years of age because of insufficient data.

Validation of ¹³C UBT by histology and microbiology

In 176 patients, the ¹³C UBT results at a cut-off point of 3.0 ‰ were compared with histology and culture (considered to be the gold standard). In this analysis, the ¹³C UBT showed a sensitivity of 100% and a specificity of 98.5% for the diagnosis of *H pylori*. Based on an *H pylori* incidence of 20% to 40% in the Canadian population (16), the positive predictive value of the ¹³C UBT would be 94.5% to 97.9% and the negative predictive value would be 100% (Figure 4).

DISCUSSION

The ¹³C UBT is now widely used to document *H pylori* infection (20). The test has been recommended as the preferred method for epidemiological studies and for screening patients with dyspeptic symptoms (19). Because it is a test for active

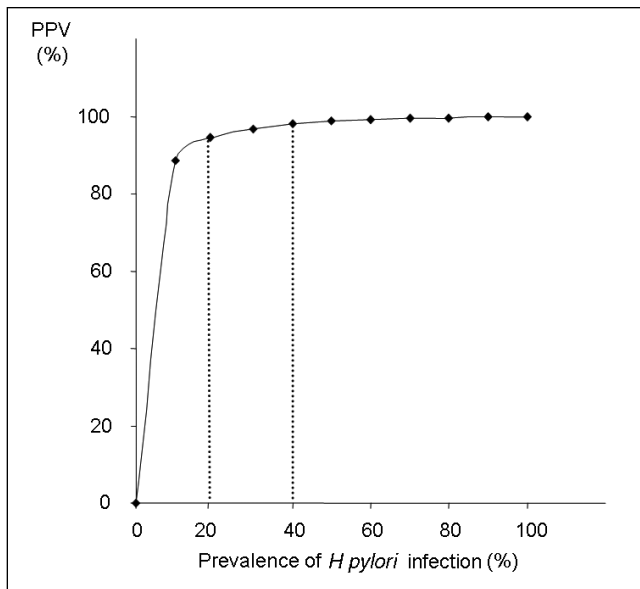


Figure 4) Positive predictive value (PPV) and negative predictive value for ¹³C urea breath test. Based on a prevalence between 20% to 40% of *Helicobacter pylori* infection in the Canadian population, the PPV would be between 94.5% and 97.9%, and the negative predictive value would be 100%

infection, it is also recognized as the best noninvasive test to assess the efficacy of anti-*H. pylori* treatments (21,22).

Considering the increasing application of the ¹³C UBT and the fact that different test meals, fasting states, nationalities, bacterial and host factors, and concomitant medication use may affect this test, we assessed a large set of Canadian patients with dyspeptic symptoms to determine the cut-off point for the ¹³C UBT in community practice (14-18,23-25).

Using cluster analysis, we found that the optimal cut-off point for the ¹³C UBT for our population was 3.09 ‰, which showed 100% sensitivity and 98.5% specificity compared with histology and culture. This is a lower cut-off point than that reported in earlier studies ($T_{30}-T_0=5.0$ ‰) (26,27), but it has been confirmed by others in comparison with histology (28) and by cluster analysis in a large set of data (29). The risk of false-negative or false-positive results was less than 2.3% when a practical cut-off point of 3.0 ‰ was used, a more than adequate efficacy for a noninvasive test. The risk of error was less than 2.3% when the same cut-off point was used in the groups that came for diagnosis and for confirmation of cure;

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TABLE 1
Calculated cut-off point and risk of error in the whole study population and in the diagnosis and post-treatment groups

	Patients, n	Optimal cut-off point, ‰	Risk of error with optimal cut-off point, %	Risk of error with cut-off point at 3.0 ‰, 2.5–3.5 ‰, %	Number of results in zone n (%)
All patients	2232	3.09	<2.3	<2.3	14 (0.62)
Diagnosis group	1209	3.09	<2.3	<2.3	10 (0.82)
Post-treatment group	1023	2.88	<2.3	<2.3	4 (0.39)

thus, depending on the clinical situation (ie, before or after treatment), it appears unnecessary to use different cut-off points. Like others (29), we recommend an indeterminate zone (2.5 ‰ to 3.5 ‰) for the ¹³C UBT, in which a second test would be recommended to assess a patient's *H. pylori* status more accurately rather than adhering to a very strict cut-off point. This intermediate zone would take into account spontaneous variations of respiratory ¹³CO₂ related to fasting, a patient's metabolism and the limits of analytical precision of ¹³CO₂ measurements. Only 0.62% (14/2232) of patient results fell in this indeterminate zone (Table 1).

CONCLUSION

The ¹³C UBT is an accurate, noninvasive test for the diagnosis of *H. pylori* infection and for the confirmation of cure after eradication therapy. The present study confirms the validity of a cut-off point of 3.0 ‰ for a Canadian community population using a standard protocol. Thus, the ¹³C UBT is a practical and accurate test on which to base a 'test and treat' strategy for the management of dyspepsia in community practice in Canada. These data provide further support for making the ¹³C UBT more widely available in community practice because it is convenient, accurate and likely more cost-effective than endoscopy or *H. pylori* serology (30).

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