ORIGINAL ARTICLE

Use of LARA-urea breath test in the diagnosis of *Helicobacter pylori* infection in children and adolescents: A preliminary study

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AS Day, S Veldhuyzen van Zanten, AR Otley, L Best, AM Griffiths, PM Sherman. Use of LARA-urea breath test in the diagnosis of *Helicobacter pylori* infection in children and adolescents: A preliminary study. Can J Gastroenterol 2003;17(12):701-706.

BACKGROUND: An accurate diagnosis of *Helicobacter pylori* infection in children currently relies upon histological assessment or culture of gastric biopsies obtained at endoscopy. Noninvasive testing would permit simpler assessment of children with dyspeptic symptoms. The primary aim of the present study was to prospectively evaluate a novel urea breath testing method in children undergoing diagnostic assessment of dyspeptic symptoms and secondarily to consider the roles of other noninvasive tests in these children.
METHODS: Laser associated ratio analysis (LARA)-^{13}C urea breath testing was performed on children presenting with upper gastrointestinal symptoms for diagnostic endoscopy. Serum and stool were collected for performance of serology and stool antigen testing, respectively. Histology and culture of endoscopic biopsies of the gastric antrum were used to establish *H pylori* infection status.
RESULTS: Eight (36%) of 22 children were *H pylori*-positive by histology or culture of gastric biopsies. Urea breath testing showed a sensitivity of 75%, but specificity of 100%. The deletion of a test meal from the urea breath test protocol in eight patients did not alter the utility of the test. Serology provided sensitivity of 87.5%, but a specificity of only 75%. Stool antigen testing in eight available samples provided sensitivity of 50% and specificity of 100%.
CONCLUSIONS: The LARA-urea breath testing method provided less sensitivity in this group of children than suggested from previous studies. However, urea breath testing in children is easy to complete and provides rapid noninvasive results. Breath testing protocols require standardization; for instance, the addition of a test meal may not be necessary in older children. Although noninvasive tests for the presence of *H pylori* in children may provide accurate results and can be considered for use in the initial assessment of dyspeptic children, further work is required to establish the most accurate testing methods.

Key Words: *Children*; Helicobacter pylori; *Serology*; *Stool antigen testing*; ^{13}C urea breath testing

Test respiratoire à l'urée pour le diagnostic de l'infection à *Helicobacter pylori* chez les enfants et les adolescents : étude préliminaire

CONTEXTE : Le diagnostic précis d'infection à *Helicobacter pylori* chez les enfants repose actuellement sur l'examen histologique ou les cultures de biopsies de l'estomac faites au moment de l'endoscopie. Des tests non effractifs faciliteraient l'évaluation des enfants souffrant de dyspepsie. La présente étude avait pour but principal d'évaluer, de façon prospective, un nouveau test respiratoire à l'urée chez les enfants soumis à une exploration diagnostique pour des symptômes dyspeptiques et pour but secondaire d'évaluer le rôle d'autres tests non effractifs chez ces enfants.
MÉTHODE : Des enfants présentant des troubles digestifs supérieurs et devant subir une endoscopie de diagnostic ont été soumis à un test respiratoire à l'urée marquée au carbone13 (C$_{13}$) (LARA : laser associated ratio analysis). Nous avons également procédé à des prélèvements de sang et de selles pour des tests sérologiques et la recherche d'antigènes dans les fèces, respectivement. Des biopsies de l'antre pylorique ont été pratiquées au moment de l'endoscopie pour des examens histologiques et des cultures afin de déterminer la présence de l'infection à *H pylori*.
RÉSULTATS : Huit enfants sur vingt-deux (36 %) ont obtenu des résultats positifs à l'égard d'*H pylori* à l'examen histologique ou à la culture. Le test respiratoire à l'urée a montré une sensibilité de 75 % mais une spécificité de 100 %. La suppression d'un repas d'épreuve du protocole du test respiratoire chez huit patients n'a pas diminué l'utilité du test. Pour ce qui est du test sérologique, il a produit une sensibilité de 87,5 % mais une spécificité de 75 % seulement. Enfin, quant à la détection de l'antigène dans les fèces à partir de huit prélèvements, elle a donné une sensibilité de 50 % mais une spécificité de 100 %.
CONCLUSIONS : Le test respiratoire à l'urée s'est avéré moins sensible dans le présent groupe d'enfants que ne le laissaient supposer d'autres études. Toutefois, il est facile à réaliser chez les enfants et il donne des résultats rapides, et ce, sans effraction tissulaire. Par ailleurs, le protocole pour le test respiratoire doit être normalisé; par exemple, l'ajout d'un repas d'épreuve pourrait ne pas être nécessaire chez les enfants plus âgés. Même s'il existe d'autres examens non effractifs de détection d'*H pylori* chez les enfants, capables de donner des résultats précis et susceptibles d'utilisation au moment de l'exploration initiale, des études plus poussées s'imposent pour déterminer lesquels sont les meilleurs.

It is now evident that the gastric pathogen *Helicobacter pylori* is generally acquired during childhood, even though the precise mode of transmission remains unclear (1). Following initial colonization, *H pylori* persists in the stomach throughout the life of the infected host, unless specific eradication treatment is administered. In a subset of individuals, *H pylori* gastritis is associated with the development of peptic ulceration and gastric cancers (2). Therefore, detection and eradication of *H pylori* may be particularly relevant during childhood so as to be able to modify lifelong risks of peptic ulcer disease and carcinogenesis (2).

The gold standard for detecting the presence of *H pylori* infection has been the combined use of histology and culture of gastric biopsies (3). Although these methods are accurate, upper endoscopy is relatively invasive, costly and time consuming. Consequently, attention has focused on noninvasive diagnostic tests for use either as initial screening tests or as follow-up tests to confirm bacterial eradication following the treatment of *H pylori*. Serological techniques are readily available, but their reliability is reduced in children compared with test results obtained in adults (4). Detection of *H pylori* antigen in stool specimens has been reported recently, with encouraging initial results both in adults (5) and children (6,7). In addition, exploitation of the urease activity of *H pylori* has led to the development of breath tests using labelled urea as substrate (8). Urea breath tests have proven to be efficient and accurate in the detection of *H pylori* infection both in adults and older children (9).

Laser methods of isotope ratio analysis recently have been developed for urea breath testing (10). The laser associated ratio analysis (LARA) technique has sensitivity and accuracy comparable with other detection techniques of urea breath testing (11) and has been validated in adults (12-14). The LARA analyzer was available to us as part of a network of breath testing units established across Canada for research purposes (UBTAN; principal investigator Dr Sander Veldhuyzen van Zanten).

Up to this point there has not been an attempt to establish standard urea breath testing characteristics in pediatric populations. For instance, the need to include a test meal in testing protocols has not been established in children. Adult studies note that the addition of a meal to the urea breath test protocol leads to a decrease in the rate of false-negative results by delaying gastric emptying and permitting longer time for exposure to bacterial urease (15,16). One study (17) in 61 *H pylori*-positive Taiwanese adults suggested that a meal is not required: sensitivity changed from 100% with a meal to 98% without. The effect of inclusion of a test meal on the sensitivity of the urea breath test remains undetermined in children.

The principal aim of this preliminary study was to prospectively evaluate the utility of LARA-urea breath testing in children and adolescents. Testing with and without the inclusion of a test meal was also undertaken in a subgroup in order to ascertain the importance of this. In addition, the study evaluated the accuracy of other noninvasive tests for the presence of *H pylori*.

PATIENTS AND METHODS

Patients
Children between eight and 18 years of age undergoing esophagogastroduodenoscopy for the investigation of upper gastrointestinal symptoms by any of nine consultant pediatric gastroenterologists at the Hospital for Sick Children in Toronto, Ontario, were enrolled consecutively before endoscopy. Inclusion criteria included upper gastrointestinal symptoms without a previous specific etiological diagnosis. Exclusion criteria included the administration of antibiotics in the preceding month and the use of proton pump inhibitor or histamine-2 antagonist treatment in the preceding fortnight. In addition, known history of *H pylori* infection led to exclusion. At enrolment, background demographic information detailing chronological age, sex, number of family members at home, source of drinking water, presence of household pets, and family history of *H pylori* infection or peptic ulcer disease was

obtained from each patient. The study was conducted following institutional guidelines and received approval of the hospital's Research Ethics Review Board.

Upper endoscopy and analysis of mucosal biopsies
The findings at endoscopy for each child were recorded. Biopsies from the gastric antrum were processed for both microbiological culture and histology, as described previously (18,19). Further mucosal biopsies were obtained by the endoscopist, as clinically indicated. A positive result from one or both of these analyses was used as the gold standard for the determination of *H pylori* infection status. Rapid urease testing was not performed as part of routine assessment.

LARA-^{13}C urea breath testing
Children fasted for at least 4 h before each breath test. Before commencing the test, patients rinsed their mouth with a small amount of water to eliminate oral urease-forming organisms. ^{13}C urea, 75 mg in 50 mL of water, was given orally followed by the test meal, comprising 155 mL of apple juice. In a subset of eight children, a second breath test was undertaken on a different day without the administration of apple juice.

Testing was undertaken with patients in the sitting position, because changes in posture also may alter test results (20). Breath samples were collected before the ingestion of the urea and liquid meal (0 min) and then again at 20, 30 and 60 min. Breath samples were analyzed by LARA (Alimenterics Inc, USA) following calibration with positive and negative controls. The ratio of ^{13}C to ^{12}C in exhaled breath before and after the ingestion of ^{13}C-labelled urea was detected by photogalvanic excitation of the two isotopes (10,11). One reading was taken at each time point to determine the ratio of $^{13}CO_2$ to $^{12}CO_2$ in each sample. The baseline ratio was then used as a reference for the other time points. An increase in the ratio of more than 6.7 δ is reported by the manufacturer as a positive test result in adults; a value of less than or equal to 5.5 δ represents a negative test and a result of between 5.5 δ and 6.7 δ constitutes an indeterminate result (manufacturer's instruction manual, Alimenterics Inc). In evaluating the results of changing ratios in subjects, any sample featuring an increased ratio was considered positive.

Serology
Venous blood was obtained from each patient during endoscopy (while the child was anesthetized) or at the time of other clinically indicated venipuncture. Serum was stored at –70°C before determination of immunoglobulin G antibodies against *H pylori*. Flow microsphere immunofluorescent assay (FMIA), which the present authors have previously described and validated for use in children (21), and a commercial immunoassay (Meridian Diagnostics Inc, USA) were utilized to detect the presence of antibodies against *H pylori*.

Stool antigen testing
Subjects were requested to provide a single stool specimen before the determination of *H pylori* status. Stool specimens were stored at –70°C before analysis. To determine the presence of *H pylori* antigens, a commercial enzyme immunoassay kit was employed following the instructions provided by the manufacturer (HpSA, Meridian), as described previously (5). The manufacturer recommended cut-offs for adults were used, whereby a positive test is indicated by optical density readings of at least 0.160 at 450 nm or at least 0.120 at 450/655 nm, equivocal results are 0.140 to 0.160

TABLE 1
Demographics and presentations of patients

| | *Helicobacter pylori* status | | |
	+	–	P
Number of patients	8 (36)	14 (64)	Not applicable
Age (years [SD])	13.9 (2.5)	10.6 (2.1)	0.034
Male sex	5 (62)	6 (43)	0.659
Presentation with UGI bleed	2 (25)	0 (0)	0.07
Born outside Canada	2 (25)	2 (15)	0.07
Family history of *H pylori*	4 (50)	7 (50)	1.0

Eight of the 22 children were diagnosed with H pylori *infection by culture and histology. Upper gastrointestinal (UGI) bleed included presentation with either hematemesis (n=1) or melena (n=1). Data are presented as number (%) unless otherwise stated. Significance values of <0.05 utilised Fisher's exact test*

and 0.100 to 0.120 at each of the two wavelengths and a negative test is indicated by an optical density of less than 0.140 and less than 0.100, respectively (5,22).

Statistics
Sensitivity, specificity, positive predictive values, negative predictive values, false-positive rates and false-negative rates were calculated for each of the noninvasive diagnostic tests (that is, urea breath testing, *H pylori*-specific serum antibody and stool antigen analysis). The results of culture or silver staining of prepyloric gastric biopsies were employed as the gold standard. The Fisher's exact test was used to compare results of diagnostic tests compared with the gold standard. Paired Student's *t* test was used for comparison of urea breath test with and without a test meal. Results are expressed as means ± SD. P<0.05 was used for the level of significance for comparative tests. Graph Pad InStat was employed for analysis of data (GraphPad InStat version 3.00 for Windows 95, GraphPad Software, USA, www.graphpad.com).

RESULTS

Patients
Twenty-two children aged 11.8±2.7 years were enrolled into this study. As shown in Table 1, eight (36%) of the 22 children (13.9±2.5 years of age) were infected with *H pylori* as diagnosed by culture (n=6) or histological analysis (n=8). One of the 14 *H pylori*-negative children had chronic gastritis present on histology, but without evidence of *Helicobacter*-like organisms on staining of gastric tissue or culture of gastric biopsy. In each of the remaining 13 children the gastric histology was normal.

Presenting clinical symptoms included hematemesis (one patient), melena (one patient), nausea or emesis (seven patients), epigastric pain (17 patients), night-time pain (four patients) and isolated anemia of unknown origin (two patients). Although both children who presented with gastrointestinal bleeding were *H pylori*-positive, the limited numbers of patients entered into this study prevented demonstration of an association between these symptoms and *H pylori* status (P=0.07). Eighteen (82%) of the 22 subjects were born in Canada. Two children were born in the developing world (India and Iran) and two were from developed countries (Australia and United Kingdom). Both of the children born in developing countries were *H pylori*-positive.

Eleven (50%) of the patients reported a family history of *H pylori* infection and related complications in either immediate family members or in second-degree relatives. There was no
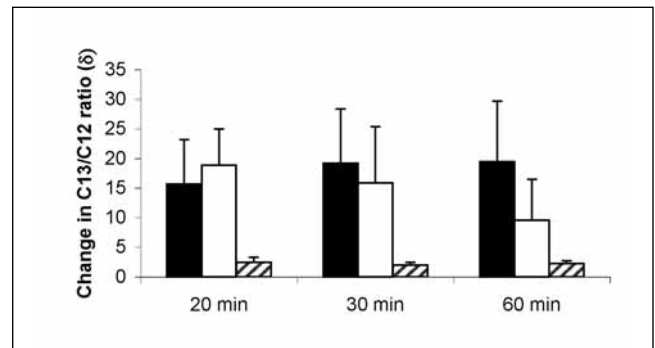

Figure 1) *Results of urea breath testing in* Helicobacter pylori-*positive and -negative children. Urea breath testing was performed in eight children infected with* H pylori *and 13 uninfected children with a test meal included in the test protocol. The change in ratio between* ^{13}C *and* ^{12}C *(δ) between baseline measurements and measurements at 20, 30 and 60 min is depicted for children with* H pylori *infection (solid bars) in comparison with uninfected children (hatched bars). In addition, three* H pylori-*positive children repeated breath testing with the only change being the exclusion of the test meal (open bars). An increase in the ratio of more than 6.7 δ is used as indicating a positive test result with a value of less than or equal to 5.5 δ representing a negative test. Data represent the mean change in ratios (mean ± SD) for each group*

association between the report of family history of *H pylori* infection and *H pylori* status of the child (50% of both groups had family members with positive history of *H pylori*, P=1.0).

Urea breath testing
Breath tests were performed in 21 of 22 patients (Figure 1). All of the subjects were able to follow instructions and completed the breath sample protocol without difficulty. Eighty-five (94.4%) of the 90 breath samples collected after the ingestion of urea were able to be processed. On two occasions, samples from one of the three time points measured were unable to be processed, because of inadequate sample collection. On one occasion all three samples collected from one patient (repeat testing without test meal) were unable to be processed.

Six of eight children with positive histology or culture results had positive breath tests. The two children with negative breath tests both had negative culture, but with organisms present on histological analysis of biopsies. For five of these six children with positive urea breath tests, positive values were obtained at each of the three time points tested. The breath test from the remaining child was positive at 30 min but provided indeterminate results at both 20 and 60 min.

Each of the thirteen *H pylori*-negative children had negative breath test results at all three time points. In this study, urea breath testing had a sensitivity of 75% (CI 35 to 97), specificity of 100% (CI 75 to 100), positive predictive value of 100% (CI 54 to 100) and negative predictive value of 87% (CI 59 to 98).

Urea breath tests without the addition of apple juice were completed in a subset of nine children (but in one case the breath samples were unable to be processed, giving eight children). In this setting, the sensitivity and specificity of breath testing were both 100%. Three *H pylori*-infected children with positive urea breath testing when performed with apple juice continued to have positive test results when the liquid test

TABLE 2
Urea breath testing in pediatric patients

Children (n)	Infected (n)	Urea dose	Test meal	Sensitivity (%)	Specificity (%)	Reference
95	26	2 mg/kg	ice cream	96	93	24
63	20	50–75 mg	glucose polymer	100	97	26
100	38	75 mg	citric acid	100	100	25
178	94	2 mg/kg	ice cream/formula	96	92	27
136	91	2 mg/kg	ice cream	98	96	28
50	19	100 mg	Calogen*/Ensure†	89	90	37
79	12	50–75 mg	citric acid	100	100	29
53	16	100 mg	fatty meal	94	97	30
39	28	50 mg	citric acid	96.5	91.5	31
287	92	120 mg	grape juice	N/A	N/A	32
115	54	50–100 mg	fatty/carbohydrate meal	98	98	33
159	61	75 mg	apple juice	100	93	34
53	27	100 mg	drinking water	100	100	35
59	22	75 mg	citric acid	91	92	36

*Calogen: SHS International, United Kingdom; †Ensure: Abbot Laboratories, Montreal, Quebec. Published reports of the use of urea breath testing in children are listed chronologically. N/A Not available

meal was omitted from the protocol (Figure 1). However, in one child, when the test was performed without juice the results were positive at just one of the three time points (20 min).

There was no difference between the results of urea breath tests undertaken in *H pylori*-infected children with duodenal ulceration (n=3) and children with gastritis alone (n=5) at 20 min (P=0.46), 30 min (0.48) or 60 min (P=0.32). In addition, there was no correlation between presenting clinical symptoms and values recorded in breath tests (data not included).

Serology
Serum was available in 21 of 22 patients. When the results of a commercial immunoassay were compared with the results of histology and culture of antral biopsies, the sensitivity was high at 100% (CI 64 to 100) but the specificity was only 54% (CI 25 to 81). The FMIA provided equivalent specificity (54%, CI 25 to 81) but lower sensitivity (87.5%, CI 47 to 100). If antibody status was considered positive only when both immunoassay and flow cytometry testing gave positive results, then sensitivity of 87.5% (47 to 100), specificity of 69.2% (39 to 91), positive predictive values of 63.6% (31 to 89) and negative predictive values of 90% (56 to 100) were obtained in comparison with histology and culture.

Stool antigen
Stool specimens were provided by only eight (36%) of the 22 children; two of these children were infected with *H pylori*. Using the cut-off values as recommended by the manufacturer, one *H pylori*-infected child had a positive test and the other had an indeterminate result. Each of the *H pylori*-negative children had negative test results (sensitivity of 50% and specificity of 100%).

DISCUSSION
The current prospective study of a cohort of children and adolescents indicates that noninvasive testing with the LARA-urea breath test in children can provide excellent specificity but sensitivity below that expected from comparable studies. Both serological tests provided false-positive results, with greater sensitivity than specificity values. Stool antigen testing demonstrated low sensitivity and good specificity, albeit with

only very small numbers of stool samples. Overall, however, the small sample size of the current study needs to be taken into account when interpreting the current findings.

In adults, the accuracy of breath tests for the detection of *H pylori* is reliable and consistent across a variety of settings, with sensitivity values of up to 90% and specificity between 78% and 100% (23). Studies in children are fewer, but appear equally promising. For instance, in one study of 95 children (24), urea breath testing for *H pylori* detection showed sensitivity of 96% and specificity of 93%. Kalach et al (25) reported a sensitivity of 100% and specificity between 90% and 100% (dependant on the timing of breath specimens). Rowland et al (26) demonstrated sensitivity of 100% and specificity of 92% to 98% in 63 Irish children. Other studies evaluating urea breath testing in children (Table 2) have provided sensitivity values between 89% and 100% with specificity values of 90% to 100% (27-37). The specificity results of the current much smaller study are comparable with results from these published studies of the urea breath test in children; however, the sensitivity is lower. This may be a feature of the small sample size. Further study in a larger cohort of children would be necessary before this LARA-urea breath test could be adopted more generally.

A secondary aim of the current study was to ascertain the role of a test meal in the urea breath testing protocol. In a small group of eight children, the current results suggested that the test utility was not adversely altered when a test meal was excluded. This concurs with results from one adult study from Taiwan (17). The effect of the inclusion of a test meal on the sensitivity of the urea breath test has remained controversial in children. Moreover, the composition of test meals has not become standardized as illustrated in Table 2, although each of the reported studies undertaken in children have been completed with a test meal included in the test protocol. Rowland et al (26) did study the utility of the urea breath test in non-fasted children and reported that a test meal decreased sensitivity in their study group. These authors suggested that food may alter interactions with urease due to the lower bacterial load present in children. Fruit juice as a test meal for urea breath testing has been used in two recent studies (32,33). In the present study, the addition of apple juice in the test protocol provided a palatable fluid, which permitted satisfactory test

performances. Paired tests in a subgroup of children demonstrated that the deletion of the test meal did not alter the sensitivity or specificity of the test. Only in one child was the quantitative value of recovered ^{13}C altered such that the test was not positive at two of the three time points when the juice was not given. However, this change did not alter the interpretation of test results.

In the present study, three time points were used for collection of breath samples after the administration of urea. Overall, the test utility was similar for each of the three time points, suggesting that three time points are not required. Accordingly, in the future, breath tests could be performed with a single 20 min or 30 min collection time. In addition to the elements of the test protocol considered in the current study, further aspects of the test also require standardization. For instance, variables of urea breath testing protocols not considered in the current study include the dose of ^{13}C administered, the composition of the test meal and the method of analysis. Standardization is required to ensure consistent and reliable test performances across various population settings. Furthermore, urea breath test protocols must be flexible to cater to patients of varied ages. Testing in children younger than eight years of age, for instance, may require adaptation of urea breath test protocols (38,39). Because the focus of the current study was to validate the test in children older than eight years of age, younger children were not included. Further specific study is needed in this younger age group (38).

Initial studies examining urea breath testing used isotope ratio mass spectrometry (IRMS) for the analysis of breath samples, which provides accurate results (23,40). The use of IRMS is associated with high costs, which has limited widespread usage of this method for urea breath test analysis. As a result, alternative methods of breath sample analysis have been described. Infra-red spectrometry has been developed for urea breath testing with the aim of lowering costs and increasing portability. Results have been compared directly with IRMS and show equivalent accuracy (40). Infrared spectrometry, however, requires a larger volume of breath for sampling, making the use of this test in children more difficult.

The LARA technique has sensitivity and accuracy comparable with IRMS when used for urea breath testing (11) and has been validated in adults (12-14). The specific advantages of the LARA analyzer are in terms of cost, ease of use and rapidity of results. The results of the current study demonstrate that this technique delivers satisfactory results when performed in children older than eight years of age. Further studies are required to confirm these results in different populations, including the primary care setting, and to establish standardized testing protocols for use in children.

Studies in adults have validated stool antigen testing as a useful noninvasive test (5,41). Similarly, pediatric studies performed in Europe note good diagnostic accuracy (6,7). However, more recent reports note lot-to-lot variability and low specificity of this polyclonal antibody test (42,43). A newer

test utilizing monoclonal antibodies may provide enhanced utility (44). Unfortunately, collection of samples from all children in the current study was not possible, which limited the ability to assess this test adequately. Two factors likely contributed to this. First, because the study was conducted at a tertiary centre, many subjects lived at great distances making collection of samples impractical. Second, there was a reluctance of many of the older children to provide a stool sample – apparently for aesthetic reasons. Thus, compliance issues may limit the use of stool antigen testing, especially in adolescent patients. In addition, stool antigen testing in pediatric patients may require different cut-off values (45). The current study was unable to assess this aspect of stool antigen testing.

Serological tests also may be population-specific and require local validation in the age group under consideration. The FMIA technique used in the current study has previously been validated in Canadian children and proven to impart reliable results (21). The results of serological testing in the current study, using the FMIA technique as well as a commercial assay, provided good sensitivity. However, neither test gave adequate specificity in these children. These findings highlight the difficulty in interpreting serological tests in children, but may again reflect the small sample size.

Serological tests for the presence of *H pylori* are not generally recommended in children, either as diagnostic or screening tests. Recent consensus guidelines formulated by the Canadian Helicobacter Study Group (46) and by the North American Society of Pediatric Gastroenterology and Nutrition (47) have both concluded that antibody tests for *H pylori* are not of value for the diagnosis of this bacterial infection in children. Furthermore, because antibody tests may remain positive for a long period of time after eradication, these tests are not of value in assessing clearance of infection.

CONCLUSIONS

The current study suggests that the LARA-^{13}C urea breath test provides excellent specificity as a simple and rapid noninvasive test for the diagnosis of *H pylori* in children and adolescents. Further analysis in a larger group of children would be required to confirm whether the low sensitivity is a feature of this small cohort or due to test characteristics. Additional endeavours are required to establish the role of stool antigen testing in the assessment of children with upper gastrointestinal symptoms, or indeed in the confirmation of eradication of *H pylori* after treatment.

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