

The Stool Antigen Test for Detection of *Helicobacter pylori* after Eradication Therapy

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Background: Current noninvasive tests to confirm the eradication of *Helicobacter pylori* must be performed 4 weeks or more after eradication therapy is completed.

Objective: To determine whether the stool antigen test, a relatively new noninvasive test for *H. pylori*, administered at various times after eradication therapy correctly identifies persons with persistent *H. pylori* infection.

Design: Prospective blinded study.

Setting: Six clinical centers in the United States and Europe.

Patients: 84 *H. pylori*-infected patients undergoing endoscopy for upper abdominal symptoms.

Measurements: At baseline and on day 35 after the completion of triple eradication therapy, all patients underwent endoscopy with histologic examination, rapid urease test and culture, urea breath test, and a stool antigen test. The stool antigen test was

also performed on days 3, 7, 15, 21, 28, and 35 after completion of therapy.

Results: Compared with the gold-standard endoscopic tests on day 35 after antimicrobial therapy, the urea breath test had a sensitivity of 94% (95% CI, 71% to 100%) and a specificity of 100% (CI, 94% to 100%). The stool antigen test had a sensitivity of 94% (CI, 71% to 100%) and a specificity of 97% (CI, 89% to 100%). On day 7 after treatment, the stool antigen test was predictive of eradication (positive predictive value, 100% [CI, 69% to 100%]; negative predictive value, 91% [CI, 82% to 97%]).

Conclusion: A positive result on the stool antigen test 7 days after completion of therapy identifies patients in whom eradication of *H. pylori* was unsuccessful.

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Noninvasive tests for *Helicobacter pylori* are important in primary care, both for initial diagnosis of *H. pylori* infection and for confirmation of eradication. Current guidelines recommend noninvasive testing and treatment of young dyspeptic patients without alarm symptoms (such as dysphagia or weight loss that suggest underlying malignant disease) in a primary care setting by using low-cost noninvasive tests (1, 2). Randomized, controlled trials have shown that a "test and eradicate" strategy toward *H. pylori* is effective in patients with dyspepsia seen in primary care settings who have not undergone investigations such as endoscopy or radiographic studies (3). Post-therapy testing is also growing in importance. Resistant strains of *H. pylori* are now widely prevalent in the United States and Europe, and eradication therapy with current regimens fails in 10% to 20% of patients (4, 5). Furthermore, some patients with ulcer disease remain symptomatic despite successful eradication of *H. pylori* and healing of the ulcer (6). In patients with persistent symptoms, testing for persistent *H. pylori* infection is important to direct further therapy. Routine testing to confirm eradication in patients with

complicated ulcer disease, such as bleeding peptic ulcer, is necessary because the risk for rebleeding is greatly increased in patients with persistent infection (7).

The choice of tests in the post-therapy setting is limited. Serologic tests are unreliable in determining eradication (8). Endoscopic tests (rapid urease test, histologic examination, or culture) are reliable, but endoscopy is expensive and inconvenient. Until recently, the only noninvasive test that reliably demonstrated whether eradication was successful was the urea breath test (9). This test has high sensitivity and specificity in the post-therapy setting but cannot be used until 4 weeks after treatment. Moreover, the breath test is still not widely available in the United States.

The fecal antigen test is a relatively new noninvasive test for detection of *H. pylori* (10). This test detects the presence of infection by measuring the fecal excretion of *H. pylori* antigens. It has been approved by the U.S. Food and Drug Administration for detection of *H. pylori* before and after therapy.

We sought to determine whether a stool antigen test administered at various times after treatment correctly

identifies persons in whom *H. pylori* infection persists despite eradication therapy.

METHODS

We prospectively studied 84 patients infected with *H. pylori* at six clinical centers (31 in Bologna, Italy; 29 in Amsterdam, the Netherlands; 9 in Rome, Italy; 8 in Lisbon, Portugal; 4 in Madrid, Spain; and 3 in Milwaukee, Wisconsin). The sample consisted of consecutive patients with dyspepsia (defined as pain or discomfort centered in the upper abdomen) who were referred by primary care physicians for upper endoscopy (11). Consenting patients were enrolled if they tested positive for *H. pylori* on endoscopic tests. Patients enrolled in this study have not been enrolled in other studies. Patients were excluded if they had taken proton-pump inhibitors, H₂-receptor antagonists, nonsteroidal anti-inflammatory agents, or antibiotics in the 4 weeks before the study. Failure to return for follow-up endoscopy was an a priori exclusion criterion. All patients gave written informed consent, and the study was approved by the human subjects review committee or equivalent at each participating institution.

At baseline, patients underwent endoscopy with biopsy sampling for histologic examination (two samples from the antrum and two from the corpus), culture (two samples from the antrum and two from the corpus), and a rapid urease test (one sample from the antrum). All patients were infected with *H. pylori* at baseline, as demonstrated by positive results on both rapid urease testing and histologic examination or a positive culture for *H. pylori*. Within 24 hours of the endoscopy, all patients underwent a ¹³C or ¹⁴C urea breath test. The breath test was chosen according to local availability and experience, but in all cases a validated breath test analysis system was used. Cut-off values were determined according to the recommendations of the various manufacturers of these tests.

Patients collected stool using a kit consisting of a plastic spoon that is used to scoop a small amount of stool from the toilet paper or toilet bowl into an airtight container. At all sites, the stool assay was performed by using the Premier Platinum HpSA test (Meridian Diagnostics, Inc., Cincinnati, Ohio). The assay is a microwell-based enzyme immunoassay that uses polyclonal anti-*H. pylori* capture antibody adsorbed to microwells. Diluted patient samples and

Context

Standard treatment regimens do not eradicate infection in approximately 10% to 20% of people with ulcers or gastritis caused by *Helicobacter pylori*.

Symptoms do not reliably identify patients who have persistent infection despite treatment.

Although positive results on a urea breath test done 4 weeks after treatment reliably identify persistent infection, a noninvasive test that detects successful eradication earlier would be useful.

Contribution

This multicenter study shows that a positive finding on a stool antigen test done as early as 1 week after treatment identifies about 95% (range, 70% to 100%) of cases of persistent infection.

Generalization Cautions

Findings are from patients with dyspepsia who were referred for endoscopy; 20% of patients were still infected at 1 month despite eradication therapy.

—The Editors

a peroxidase-conjugated polyclonal antibody were added to the wells and incubated at room temperature for 1 hour. A wash was performed to remove unbound material. Substrate was added and incubated for 10 minutes at room temperature. Color develops in the presence of bound enzyme. Stop solution was added, and the results were inspected spectrophotometrically at 450 nm within 15 minutes of adding the stop solution. Visual determination can also be used; this has been shown to have similar results (12). A positive control and a negative control are built into the test. The cut-off values were classified as negative (<0.140), indeterminate (0.140 to 0.159), or positive (>0.160).

After completion of the baseline procedures, treatment was begun with ranitidine bismuth citrate (400 mg twice daily) or omeprazole (20 mg twice daily) in combination with amoxicillin (1 g twice daily) and clarithromycin (500 mg twice daily) for 7 to 10 days. Seven-day eradication therapy was used in Europe, where it is approved by the European Union and has been shown to be effective (5). Ten-day triple therapy with proton-pump inhibitors was used in the United States, where it is approved by the U.S. Food

Table. Use of Stool Antigen Test To Predict *Helicobacter pylori* Status 35 Days after Completion of Eradication Therapy

Day after Start of Treatment	Stool Antigen Test Result*	<i>H. pylori</i> -Positive Patients (n = 17), n†	<i>H. pylori</i> -Negative Patients (n = 64), n‡	Probability That Patient Will Still Have <i>H. pylori</i> at Day 35 (95% CI)	Probability That Patient Will Be Free of <i>H. pylori</i> at Day 35 (95% CI)
3	Positive	3	1	0.75 (0.19–0.99)	–
	Negative	12	63	–	0.84 (0.74–0.91)
	Equivocal	2	0	–	–
7	Positive	10	0	1.0 (0.69–1.0)	–
	Negative	6	64	–	0.91 (0.82–0.97)
	Equivocal	1	0	–	–
15	Positive	14	1	0.93 (0.68–1.0)	–
	Negative	3	63	–	0.95 (0.87–0.99)
	Equivocal	0	0	–	–
21	Positive	14	0	1.0 (0.77–1.0)	–
	Negative	2	65	–	0.97 (0.89–1.0)
	Equivocal	1	1	–	–
28	Positive	14	0	1.0 (0.77–0.96)	–
	Negative	2	62	–	0.97 (0.89–1.0)
	Equivocal	1	2	–	–
35	Positive	16	1	0.94 (0.71–1.0)	–
	Negative	0	63	–	1.0 (0.94–1.0)
	Equivocal	1	0	–	–

* Positive results were ≥ 0.160 units on stool antigen test; negative results were < 0.140 units on stool antigen test; equivocal results were 0.140 to 0.159 units on stool antigen test.

† Positive for *H. pylori* by biopsy or culture 35 days after start of treatment.

‡ Negative for *H. pylori* by biopsy or culture 35 days after start of treatment.

and Drug Administration. Patients collected stool for the stool antigen test on days 3, 7, 15, 21, 28, and 35 after completion of *H. pylori* eradication therapy. On day 35 after completion of eradication therapy, endoscopy was repeated and biopsy samples were again obtained for histologic examination, culture, and the rapid urease test, as performed at the baseline visit. The ^{13}C or ^{14}C urea breath test was repeated on day 35 by using the same method and cut-off values as at baseline. Patients were classified as being infected with *H. pylori* at baseline and having persistent infection on day 35 if culture of gastric biopsy specimens was positive for *H. pylori* or results of the rapid urease test and histologic examination were positive. All other patients were classified as negative. These criteria have been recommended by an expert panel for use in clinical trials of *H. pylori* eradication (13). At baseline, the sensitivity of the stool test and urea breath test were calculated by using the presence of infection (defined above) as the gold standard. At each time point after completion of therapy (days 3, 7, 15, 21, 28), predictive values were calculated by

using continued infection on day 35 as the gold standard (positive result on culture or on rapid urease test and histologic examination).

Trained investigators who were blinded to the results of the other diagnostic studies performed the stool assays. The first endoscopy procedure was performed before the stool and breath tests. Therapy was given on the basis of results on endoscopic testing. Endoscopists were blinded to the results of post-treatment stool studies and the breath test until all evaluations were completed.

Long-Term Follow-up

Patients in whom eradication of *H. pylori* was successful were eligible for entry into a long-term study evaluating the stool antigen test. For 6 months, stool antigen tests were done monthly and a urea breath test was obtained every 3 months.

Statistical Analysis

Statistical analysis was performed by using StatView for Windows, version 5.01 (SAS Institute, Inc., Cary,

North Carolina). Results are presented as the mean (\pm SD). Sensitivity, specificity, probabilities, and predictive values are presented with 95% exact binomial CIs. Equivocal stool tests are considered by inclusion in the denominator of sensitivity and specificity. Stool antigen concentrations at individual time points were compared by using the Mann–Whitney test with downward adjustment of the *P* values for repeated observations (14).

Role of the Funding Source

The manufacturer (Meridian Diagnostics, Inc.) provided the stool kits. The study had no other funding source. Collection, analysis, and interpretation of the data, including the decision to publish, were solely the decision of the authors; the manufacturer of the test had no role in this process.

RESULTS

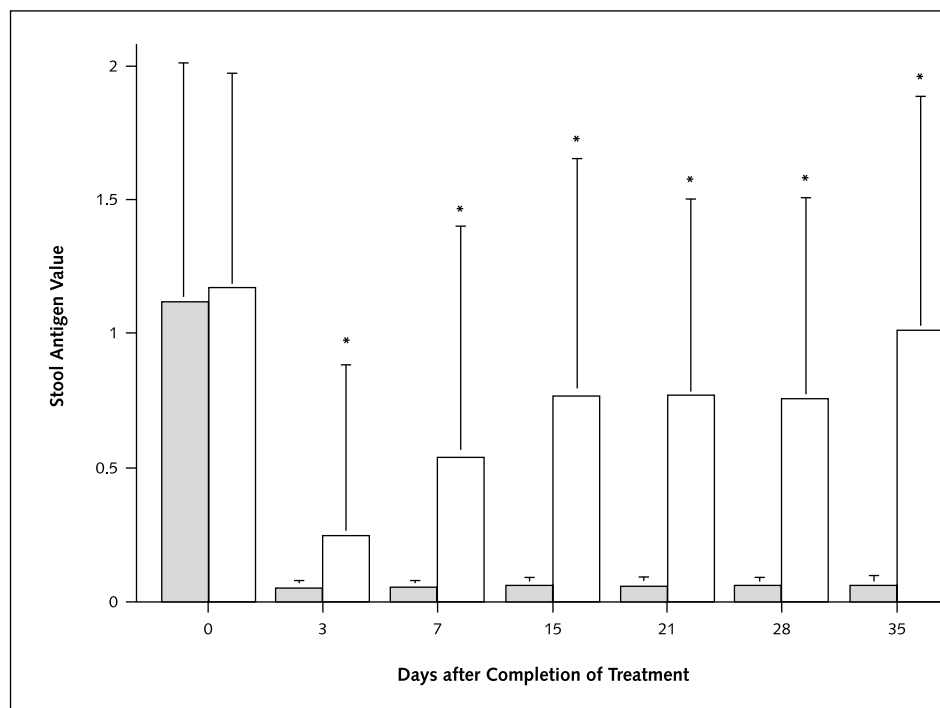
The mean age of the 84 study patients was 52 years (range, 18 to 81 years). Fifty-three patients were women,

and 31 were men. Endoscopic findings were as follows: normal (7 patients), esophagitis (2 patients), erythema in the antrum (45 patients), erosions in the antrum (11 patients), erosive duodenitis (9 patients), duodenal ulcers (8 patients), gastric ulcer (2 patients). Despite repeated reminders, 3 patients did not return for follow-up endoscopy and were excluded from the analysis. Satisfactory breath and biopsy samples were obtained from all patients. Results of the stool test are shown in the Table.

At baseline (before treatment), only sensitivity could be calculated because the sample consisted only of *H. pylori*-infected patients. The sensitivity of the urea breath test at baseline was 99% (95% CI, 96% to 100%) (true-positive in 80 patients and false-negative in 1 patient). The sensitivity of the stool antigen test at baseline was 99% (CI, 96% to 100%) (true-positive in 77 patients, false-negative in 1 patient, and indeterminate in 3 patients).

Eighty-one patients were prescribed 7-day therapy, and 3 patients were prescribed 10-day therapy. Of the

Figure. Mean stool antigen values over time in patients with (gray bars) and without (white bars) eradication of *Helicobacter pylori*.



Extensions of the bars represent the SD. The stool antigen value was determined spectrophotometrically. The time point of 0 represents baseline (before eradication therapy). Subsequent time points are measured from completion of eradication therapy. **P* < 0.05 compared with patients in whom *H. pylori* was eradicated.

81 patients who completed the study, 78 received 7-day therapy and 3 received 10-day therapy. The overall eradication rate was 79% (66 patients). Compared with the gold-standard endoscopic tests on day 35, the urea breath test had a sensitivity of 94% (CI, 71% to 100%; true-negative in 64 patients, true-positive in 16 patients, and false-negative in 1 patient) and a specificity of 100% (CI, 94% to 100%). The stool antigen test had a sensitivity of 94% (CI, 71% to 100%; true-negative in 63 patients, true-positive in 16 patients, false-positive in 1 patient, and indeterminate in 1 patient) and a specificity of 97% (CI, 89% to 100%). The result on rapid urease test was positive in all patients with positive findings on histologic examination.

The mean stool antigen concentration at baseline did not significantly differ (1.11 ± 0.89) between patients who eventually had successful eradication and patients without successful eradication (1.16 ± 0.81). In patients with successful eradication, the stool antigen values decreased into the negative range for the stool assay (below the cut-off value of 0.140) 3 days after completion of therapy and remained in this range for the remainder of the study. Three days after antimicrobial treatment was stopped, stool antigen concentrations were significantly higher in patients with persistent infection (Figure) and continued to be significantly higher at days 7, 15, 21, 28, and 35. The Table shows the value of a positive or negative test result in predicting eradication (determined by the gold standard test on day 35).

Nineteen of 66 patients with successful eradication of *H. pylori* in the initial phase of the study agreed to participate in a long-term study and were followed for 6 months. Stool specimens were collected monthly, and the urea breath test was administered at 3 and 6 months. Results of the urea breath test and the stool antigen test remained negative in every patient at each time point throughout this follow-up.

DISCUSSION

Several diagnostic tests are available for *H. pylori*. They may broadly be divided into tests that indirectly determine the presence of the microorganism (antibody tests in blood, urine, or saliva) or direct tests that detect the intact organism (histology and culture), antigens shed from the organism (stool antigen test), or metabolic functions of the organism (rapid urease test and

urea breath test). Although indirect tests are the mainstay of initial diagnosis in primary care, they are not reliable after therapy (15). The performance of a test after therapy depends on that test's characteristics. Several studies have shown that the rapid urease test, the breath test, and histologic examination may be falsely negative during the first few weeks after therapy (9). Current guidelines therefore recommend use of the urea breath test 4 weeks after completion of therapy (16).

The stool antigen test has been widely studied in the initial (pretreatment) diagnosis of *H. pylori* infection. The largest study reported to date included 501 patients undergoing endoscopy and a ^{13}C urea breath test (10). Patients were considered positive if they had a positive result on rapid urease testing and histologic examination or a positive culture for *H. pylori*. The stool test had a high sensitivity and specificity compared with the urea breath test. Studies from the United States have had similar results (17).

In the post-therapy setting, a multicenter European trial evaluated the sensitivity and specificity of the stool test and the urea breath test (compared with multiple endoscopic tests as the gold standard) 4 weeks after eradication therapy in 235 patients (18). The gold standard for comparisons in that study was histologic examination plus culture. The sensitivity of the stool test was 95.6% (CI, 89.6% to 100%), and the specificity was 94.7% (CI, 91.5% to 97.9%), values similar to those in our study. A smaller (142 patients) single-center study reported poorer results, with a sensitivity of 70% (CI, 50% to 86%) and a specificity of 82% (CI, 75% to 89%) 6 weeks after completion of eradication therapy (19). In that study, a single test (urea breath test) was used as the gold standard for eradication.

The choice of gold-standard test greatly affects the results of noninvasive testing for *H. pylori* (20). Use of a single test as the gold standard increases the error rate. The European *Helicobacter pylori* Study Group proposed that in comparative studies, the gold standard should consist of at least two tests that differ from the ones being examined (13). Similarly, the U.S. Food and Drug Administration limits the use of single tests in the evaluation of *H. pylori* infection status (21). This agency considers a positive culture to be evidence of *H. pylori* infection before and after treatment. A positive result on histologic examination after eradication is considered evidence of persistent *H. pylori* infection, but a negative

result on histologic examination alone is not adequate to confirm eradication. We used three independent tests (rapid urease test, histology, and culture) as the gold standard to provide the most accurate assessment of *H. pylori* status. Our gold standard would meet the criteria of the U.S. Food and Drug Administration and the European *Helicobacter pylori* Study Group.

We found that in patients in whom eradication is successful, stool antigen concentrations decrease rapidly after completion of eradication therapy. In patients in whom eradication has failed, stool antigen concentrations increase rapidly after completion of such therapy. It is currently recommended that the urea breath test to determine whether *H. pylori* eradication be done at least 4 weeks after completion of therapy because the test is not reliable at earlier time points. This is sometimes a problem in clinical practice because some patients remain symptomatic after antimicrobial therapy or develop recurrent symptoms shortly after treatment is completed. In these patients, a stool antigen test performed 7 days after completion of therapy may help direct further therapy, and a second course of antimicrobial therapy can be administered without further delay if the stool test result is positive. Other patients in whom retesting should be considered are those who develop recurrent symptoms, those with a bleeding ulcer at initial presentation who are likely to have recurrent bleeding if they remain infected with *H. pylori*, and those who request confirmation of eradication. The predictive values of the stool test depend on the prevalence of the infection in the study sample. With modern therapy, eradication rates are high, both in controlled trials and in community practice; the prevalence of *H. pylori* infection after therapy should therefore be similar to that in our study (22). Eradication rates in our study are consistent with those in recent multicenter studies and may be related to increased rates of antimicrobial resistance in the population (23).

The choice of test after therapy depends on its cost and availability. In Europe, the urea breath test is inexpensive and widely available, but in the United States, this test is considerably more expensive and is still not generally available. Currently, Medicare reimburses the urea breath test at \$103.95 (codes 83103 and 4). The stool antigen test does not have a specific code at this time but is reimbursed at \$50 in most states (under code 87338) (24).

Our study has limitations. Patients were referred for

endoscopy and may not be representative of patients with dyspepsia seen in primary care settings. However, the endoscopic findings in our study were similar to those reported in unselected patients undergoing endoscopy in primary care settings (25, 26). In addition, the sample size was modest, resulting in relatively wide CIs.

We conclude that a positive result on stool antigen testing 7 days after completion of therapy identifies patients in whom eradication of *H. pylori* was unsuccessful.

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COMMENTARY

This study is interesting because it illustrates the contrast between two ways in which physicians use tests. Sometimes, we use a test to predict a future event. More often, we use a test to predict the concurrent true state of the patient. Vaira and colleagues emphasize the

former of these uses. They show us that performing positive stool antigen tests as recently as 1 week after antibiotic therapy helps predict persistent *Helicobacter pylori* infection 35 days later (probability, 69% to 100%). The authors correctly presented this result as a probability rather than as a measure of test performance, such as

Table. Likelihood Ratio, Sensitivity, and Specificity of the Stool Antigen Test

Stool Antigen Value at Day 35	Positive for <i>Helicobacter pylori</i> (n = 17), n	Negative for <i>Helicobacter pylori</i> (n = 64), n	Probability of Test Result If Disease Is Present	Probability of Test Result If Disease Is Absent	Likelihood Ratio (95% CI)*	Sensitivity	Specificity
≥0.160	16	1	0.94	0.016	60.2	–	–
0.140–0.159	1	0	–†	–†	–†	–	–
<0.140	0	63	0	0.98	0	–	–
≥0.160 as positive result	16	1	–	–	60.2 (29–74)	0.94	0.016
<160 as negative result	1	63	–	–	0.061 (0–0.23)	0.06	0.984

* The likelihood ratio is the probability of a test result if disease is present divided by the probability of the test result if disease is absent. According to Bayes theorem, the post-test odds of disease equals the pretest odds of disease times the likelihood ratio. Thus, the likelihood ratio is the amount by which a test result changes the odds of disease.
 † Data for this range of test results were too sparse to calculate a meaningful measure of test performance.

sensitivity and specificity. They could calculate the latter only at day 35, when the reference standard test and the stool antigen test were performed together.

This study also illustrates the importance of reporting the test performance of all results, if possible. When a test such as the stool antigen test generates numbers on a continuous scale, the authors should ideally provide test performance data for defined values along the scale. In reality, most studies of test performance are too small to reliably estimate performance characteristics for many values. This study is a case in point. The authors classified the range of stool antigen results as positive (>0.160), equivocal (0.140 to 0.159), or negative (<0.140) (see the Table accompanying this commentary). According to the likelihood ratios, a stool antigen level greater than 0.160 increases the odds that *H. pylori* is present by a factor of 60, and a value less than 0.140 reduces the odds of *H. pylori* infection to zero. There were too few patients with equivocal stool antigen values to reliably estimate how much this result changes the odds that *H. pylori* is present. The authors could have defined a single cut-point (either 0.140 or 0.160) and combined the intermediate values with one of the other two groups. Many other studies (for example, studies of ventilation–perfusion lung scanning) have included enough patients with intermediate test results to make a valid estimate of the likelihood ratio and, thus, the information content of intermediate test results.

To report sensitivity and specificity when a test has many possible results, researchers must choose one value

(the cut-point) that defines positive and negative results with respect to the study end point—in this case, failure to eradicate *H. pylori*. According to probability theory, the cut-point depends on the prevalence of disease and on the ratio of benefit gained by treating patients to the harm incurred. If the prevalence of disease is high or the benefits of treatment are high relative to the harms, one should choose a cut-point at which the test has a low likelihood ratio (that is, close to zero), so that a negative result implies a very low probability that disease is present. Either of the two possible cut-points (≥ 160 or ≥ 140) satisfy this criterion (Table).

What does the likelihood ratio tell us? It is the link between test performance and clinical practice. It tells us how much the odds change after a test result. Suppose the pretest odds of antibiotic therapy failure were 1 to 3 and the stool antigen test result was positive. The post-test odds would be the product of the pretest odds (1:3) and the likelihood ratio (60.2), or about 20:1—very strong evidence that the patient was still infected with *H. pylori*. If the test result had been negative, the post-test odds would be the product of 1:3 and 0.06, that is, 0.06:3, or 1:50, which is strong evidence that *H. pylori* infection is absent. These probability estimates imply that the stool antigen test should be useful in deciding whether to re-treat the patient. However, the wide CI of the likelihood ratio (0 to 0.23) when the test result is negative (<0.160) tells us to be cautious in interpreting a negative result.

—The Editors