

A rapid immunochromatographic assay for *Helicobacter pylori* in stool before and after treatment

L. GATTA*, F. PERNA*, C. RICCI*, J. F. OSBORN†, A. TAMPIERI*, V. BERNABUCCI*, M. MIGLIOLI* & D. VAIRA*

*Department of Internal Medicine and Gastroenterology, S. Orsola-Malpighi Hospital, University of Bologna, Bologna, Italy;

†Department of Public Health Science, 'La Sapienza University', Rome, Italy

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SUMMARY

Background: Current guidelines recommend non-invasive testing and treatment of young dyspeptic patients without alarm symptoms.

Aim: To evaluate the accuracy of a new rapid immunochromatographic stool test to diagnose *Helicobacter pylori* infection before and after treatment compared with a gold standard.

Methods: Prospective, single-blind study, performed in a tertiary care hospital. A total of 303 consecutive dyspeptic patients underwent endoscopy with multiple biopsies. Infected patients were offered a treatment and invited to come back 4–6 weeks after the end of therapy

to repeat the endoscopy. Patients were also asked to provide a stool sample before and after therapy.

Results: About 149 patients were *H. pylori* infected. The sensitivity and specificity before treatment were 91.3 and 93.5%; after treatment 92 and 100%. The likelihood ratios were robust enough to produce significant changes from pretest to post-test probability both in pre-treatment (LR+ = 14, LR- = 0.093) and post-treatment (LR+ = 19.6, LR- = 0.095).

Conclusions: The novel immunochromatographic stool test is fast, easy to perform and provides good differentiation between positive and negative results. It might become a rapid near patients test easily performed in the doctor office.

INTRODUCTION

Current guidelines recommend non-invasive testing and treatment of young dyspeptic patients without alarming symptoms (such as dysphagia or weight loss that suggest underlying malignant disease) in a primary care setting, using low-cost non-invasive tests.^{1, 2} Randomized-controlled trials have shown that a 'test and eradicate' strategy towards *Helicobacter pylori* is effective in patients with dyspepsia in primary care who have not undergone investigations such as endoscopy or radiographic studies.^{3, 4} 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 10–20% of patients.^{5, 6} The faecal antigen test is a relatively new non-invasive tool, based on a microtitre enzyme immunoassay (EIA), which detects the presence of infection by measuring the faecal excretion of *H. pylori* antigens and its performance in diagnosing or evaluating the success of eradication therapy has been shown reliable⁷ as recently confirmed in several meta-analysis.^{8, 9} Recently, a rapid immunochromatographic assay based on monoclonal antibodies to detect *H. pylori* antigens in stool specimens has been introduced (ImmunoCard STAT! HpSA; Meridian Bioscience Inc, Cincinnati, OH, USA). It is easy to perform, provides results in only 5 min and is inexpensive. These

Correspondence to: Dr D. Vaira, Department of Internal Medicine and Gastroenterology, S. Orsola Hospital, Nuove Patologie, Via Massarenti 9, 40138, Bologna, Italy.
E-mail: vairadin@med.unibo.it

characteristics make it a potential near patient test to be used easily in the doctor's daily practice.

The aim of this prospective study was therefore to evaluate the accuracy of this new test to diagnose *H. pylori* infection before and after treatment compared with an endoscopic biopsies gold standard.

MATERIALS AND METHODS

Patients and endoscopy

We prospectively studied 303 patients (134 men and 169 women, mean age 53 years, s.d. 15 years) between May 2002 and December 2003. The sample consisted of consecutive patients with dyspepsia (defined as pain or discomfort in the upper abdomen¹⁰) who were referred by primary care doctors for upper endoscopy to our University centre. To be included in this study, patients had to be not previously investigated or treated for *H. pylori* infection, and to be free from antibiotics, bismuth preparations, or antisecretory drugs [H₂ antagonists or proton pump inhibitors (PPI)] during the 4 weeks prior to endoscopy. All endoscopies were performed by the same investigator (DV), using an Olympus GIF 100 video-endoscope (Cincinnati, OH, USA). At endoscopy, six biopsy samples were obtained. Two biopsies were taken from the antrum and two from the corpus for histology. Two more samples were taken from the antrum to perform culture and rapid urease test (RUT). According to the guidelines of the European *Helicobacter pylori* Study Group, *H. pylori*-positive patients were offered a 1-week PPI-based triple therapy (PPI standard dose b.d., clarithromycin 500 mg b.d., amoxicillin 1 g b.d.).² Endoscopy was repeated 4–6 weeks after stopping treatment using the same schedule as for pre-treatment. During these weeks patients were not allowed to take antibiotics, bismuth preparations, or antisecretory drugs (H₂ antagonists or PPIs). Patients were also asked to provide a stool sample before and 4 weeks after the end of the treatment, and these samples were stored at –20 °C before the test. Patients were classified as being infected with *H. pylori* at baseline if the RUT and histology were positive and/or if culture of gastric biopsy specimens was positive alone. All other patients were classified as negative. Nevertheless, patients were classified as eradicated at follow-up only if all three tests were negative. These criteria have been recommended by an expert panel for use in clinical trials of *H. pylori*.¹¹

Histology and bacterial culture

Histological biopsies were stained with haematoxylin and eosin plus Giemsa stains, and gastritis was scored using the updated Sydney System.¹² The pathologist who performed histological examination (CR) was blinded to the results of all other tests. Biopsies collected for bacterial culture were streaked onto Columbia agar enriched with 5% horse blood and containing vancomycin, trimetoprim, polymixin B and nalidixic acid to inhibit the growth of microbes other than *H. pylori*. The plates were incubated in a microaerobic environment, at 37 °C, for 7 days, and inspected daily from the third day. The isolates were identified by Gram stain and by oxidase, catalase and urease tests. The microbiologist who performed bacterial cultures was blinded to the results of all other tests carried out.

ImmunoCard STAT! HpSA

ImmunoCard STAT! HpSA test is an *in vitro* qualitative procedure based on a lateral flow chromatography technique that detects bacterial antigens using monoclonal anti-*H. pylori* antibodies. Using an applicator stick, a small portion of the stool sample is transferred into a diluent vial. After vortexing or hand-shaking for 15 s, four drops are dispensed into the window of the device containing immobilized anti-*H. pylori* monoclonal antibodies and the result is read after 5 min. ImmunoCard STAT! HpSA results were interpreted according to the manufacturer's instructions. The test was considered negative if only one blue coloured band (control line) appeared across the central window of the device, and positive when, in addition to the control line, a distinguishable pink-red band (test line) also appeared across the central window of the device. As specified by the manufacturer's recommendations, any pink-red line, even weak, was considered as positive result. Tests were also considered as invalid if the control band was absent. The biologist who performed ImmunoCard STAT! HpSA (FP) was blinded to the results of all other tests carried out.

Statistics

This is a prospective comparison study designed to fulfil the Standards for Reporting of Diagnostic Accuracy (STARD) recommendations.¹³ Sensitivity, specificity, and the likelihood ratios (LRs) for a positive and

negative test were calculated against the defined *H. pylori* status as gold standard and using methods recommended by Altman.¹⁴ Being dependent on the prevalence of infection, the predictive values, positive and negative, have not been calculated, because they are not indicative of the values that might be observed in other clinical settings. Statistical analysis was performed with Intercooled STATA 8.2 (Stata Corporation, College Station, TX, USA).

Ethical committees

All patients gave written informed consent and the protocol was approved by the ethics committee of S. Orsola Hospital.

RESULTS

About 303 of 340 eligible patients agreed to participate in the study. Of which 149 patients were infected with *H. pylori* according to the gold standard (prevalence rate of 49.2%; 95% CI: 43.6–54.8). At endoscopy, there were the following findings: gastric ulcers ($n = 10$; 3.3%; 95% CI: 1.8–6), duodenal ulcers ($n = 24$; 7.9%; 95% CI: 5.4–11.5), oesophagitis ($n = 69$; 22.8%; 95% CI: 18.4–27.8), and normal ($n = 200$; 66%; 95% CI: 60.5–71.1). All infected patients accepted the eradication treatment. Following the therapy, 121 of 149

H. pylori-positive patients were available for re-examination whilst 28 refused to perform the follow-up examination. According to the gold standard, 88 of 121 patients were not infected (eradication rate 72.7%; 95% CI: 64.2–79.9). At endoscopy all the ulcers had healed and there were no pathological findings. Figure 1 shows the study design.

Performance of the Immunocard STAT! HpSA before eradication therapy

Table 1 shows the performance of the Immunocard STAT! HpSA before treatment. There were no invalid tests and when compared with the gold standard, the Immunocard STAT! HpSA had a sensitivity of 91.3% (95% CI: 85.6–94.8) and a specificity of 93.5% (95% CI: 88.5–96.4). In patients with peptic ulcer disease ($n = 34$; prevalence rate: 11.2%; 95% CI: 8.1–15.3) the new device had a sensitivity of 93.3% (95% CI: 78.7–98.2) and a specificity of 100% (95% CI: 51–100).

In nine of 303 (3%; 95% CI: 1.6–5.5) patients there were weak coloured test lines: six were true positive results whilst three were false positive results. None of these patients had peptic ulcer disease. Omitting these nine patients from the analysis, the sensitivity was of 90.9% (95% CI: 85.1–96.4), and the specificity was of 95.4% (95% CI: 90.7–97.7).

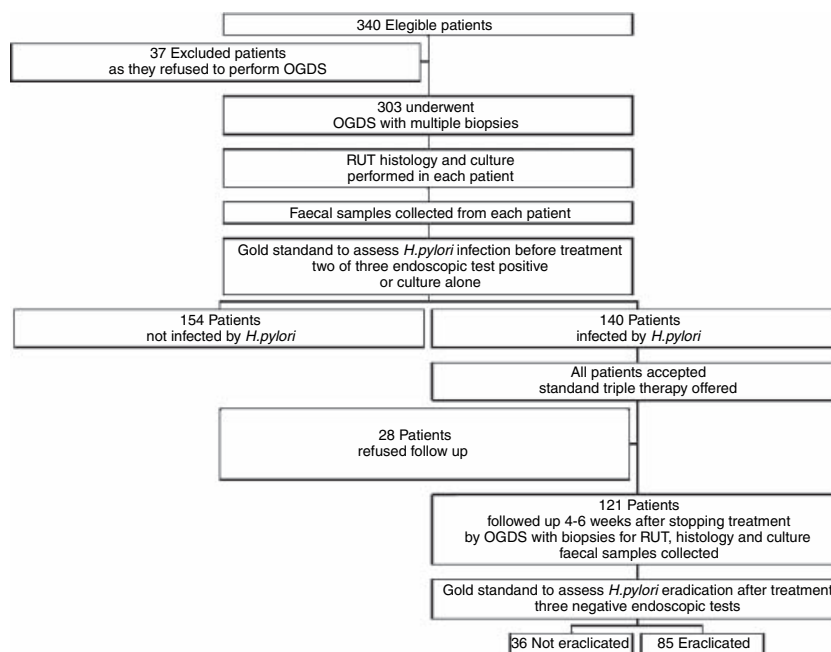


Figure 1. Studyflow chart.

	Overall population (<i>n</i> = 303)	Patients with strongly coloured test line only (<i>n</i> = 294)
Sensitivity (95% CI)	91.3% (85.6–94.8)	90.9% (85.1–96.4)
Specificity (95% CI)	93.5% (88.5–96.4)	95.4% (90.7–97.7)
LR+ (95% CI)	14 (7–25.6)	19.6 (9.49–40.5)
LR– (95% CI)	0.093 (0.05–0.15)	0.095 (0.6–0.16)

CI, confidence interval; LR+, likelihood ratio for a positive test; LR–, likelihood ratio for a negative test.

Performance of the Immunocard STAT! HpSA after eradication therapy

Table 2 shows the performance of the Immunocard STAT! HpSA after therapy. As in pre-treatment, there were no invalid tests and when compared with the gold standard, the Immunocard STAT! HpSA had a sensitivity of 92% (95% CI: 78–97) and a specificity of 100% (95% CI: 96–100). In three of 121 (2.5%; 95% CI: 0.8–7) patients there were weak coloured test lines. These patients were different from those seen before treatment and all were true positive results. Omitting these three patients from the analysis, the sensitivity was of 91% (95% CI: 76–97), and the specificity was of 100% (95% CI: 96–100).

DISCUSSION

Non-invasive testing for *H. pylori* is recommended for dyspeptic patients in primary care, in particular if they are aged < 55 years.⁴ Although serological tests are still used in different health systems, their accuracy is no longer adequate to justify their use on clinical or economic grounds.¹⁵ Furthermore, to follow-up patients with enough accuracy, they need to be performed at least 6 months after the end of the therapy.¹⁶

The choice of a test in the clinical setting depends not only on the cost of the test but also the convenience with which the test can be administered, read and interpreted. The conventional urea breath test is

	Overall population (<i>n</i> = 121)	Patients with strongly coloured test line only (<i>n</i> = 118)
Sensitivity (95% CI)	92% (78–97)	91% (76–97)
Specificity (95% CI)	100% (96–100)	100% (96–100)
LR+ (95% CI)	Infinite (21.2–∞)	Infinite (21.01–∞)
LR– (95% CI)	0.08 (infinite–∞)	0.09 (infinite–∞)

CI, confidence interval; LR+, likelihood ratio for a positive test; LR–, likelihood ratio for a negative test.

Table 1. Diagnostic accuracy of Immunocard STAT! HpSA before treatment

sensitive and specific but it requires either radioactive (¹⁴C) or expensive substrate (¹³C), trained staff and expensive instruments such as a mass spectrometer.¹⁷ Conventional EIAs to detect *H. pylori* antigens in stools is cost-effective,¹⁸ but it is a laboratory procedure, justified when multiple specimens are tested in batch.

The novel immunochromatographic Immunocard STAT! HpSA test allows inexpensive 5 min testing of single stool sample and can be easily performed in the doctor office, also overcoming the delays of batch testing.

The clinical worth of a diagnostic test is largely determined by the accuracy with which it identifies its target disorder. LRs are a useful means for clinicians to assess accuracy because they indicate by how much a given diagnostic test results will raise or lower the pretest probability of the target disorder. In this study, we found that LRs of Immunocard STAT! HpSA, shown in Tables 1 and 2, seem large enough to produce significant changes from pretest to post-test probability of the target disorder both in pre- and post-treatment setting.¹⁹

Wu *et al.* also reported a high accuracy of this new device before and after treatment in 257 Taiwanese residents.²⁰ The population of this study is largely formed by patients with peptic ulcer diseases (81.8%; 95% CI: 76.6–86.1), and therefore it characterizes the performance of the device in a subset of patients. Immunocard STAT! HpSA is an antigen detection test and its accuracy may be limited by the antigenic

Table 2. Diagnostic accuracy of Immunocard STAT! HpSA after treatment

diversities of *H. pylori* in different geographical areas as well as in patients with different pathologies. However, these results seem indicate that the device might not significantly suffer from antigen variations.

In this study, roughly 3% of the cards analysed showed weak test lines. Leodolter *et al.* (who studied 50 patients before and 50 after treatment) reported also in their abstract the appearance of very low intensity bands that were difficult to interpret, although they did not provide the number of these findings and how these were able to alter the accuracy in pre- and post-treatment.²¹ Chisholm *et al.* (who studied 40 patients before treatment) reported in their abstract that Immunocard STAT! HpSA were classified as strong, moderate and weak. Even in this case, the author did not provide any information concerning the number of tests classified as weak or moderate positive.²²

In our study, patients with weak coloured test lines were different in pre- and post-treatment, indicating that there should be nothing about the host itself (or the colonizing strains) but something about the stool sample. According to manufacturer's recommendations, any pink-red line, even weak, should be considered as positive result. Our main analysis included these weak positive results, and the accuracy assessed should be therefore considered as the worst-case scenario. However, when weak positive results were not included, the accuracy did not change importantly (Tables 1 and 2).

The 3% with weak positive results, which we could conservatively label as 'indeterminate results', might be regarded as a reasonable for an inexpensive tool that it is fast and easy to perform. Nevertheless, it might be worth testing these patients again asking them to collect a new stool sample.

A rigorous evaluation of diagnostic tests before their introduction into clinical practice should reduce, not only the number of unwanted clinical consequences related to inaccuracy, but also limit health care costs by preventing unnecessary testing. For this reason, the study was designed to fulfil the STARD suggestions'.⁹ Furthermore, as no single simple technique can be considered to be ideal for the detection of *H. pylori* infection, for evaluating a new tool for diagnosing and following up the infection, we choose as the gold standard a combination of invasive diagnostic tests in order to improve the accuracy.

In conclusion, the novel immunochromatographic stool test is fast, easy to perform and provides good

differentiation between positive and negative results in pre- and post-treatment setting. This test might become a good rapid near patients test to be simply performed in the doctor's office.

REFERENCES

- 1 American Gastroenterological Association. Medical position statement: evaluation of dyspepsia. *Gastroenterology* 1998; 114: 579–81.
- 2 Malferteiner P, Megraud F, O'Morain C, *et al.* Current concepts in the management of *H. pylori* infection – The Maastricht 2-2000 Consensus Report. *Aliment Pharmacol Ther* 2002; 16: 167–80.
- 3 Lassen AT, Pederson F, Bytzer P, *et al.* *Helicobacter pylori* test and eradicate vs. prompt endoscopy for management of dyspeptic patients: a randomized trial. *Lancet* 2000; 356: 455–60.
- 4 McColl KE, Murray LS, Gillen D, *et al.* Randomised trial of endoscopy with testing for *Helicobacter pylori* compared with non-invasive *H. pylori* testing alone in the management of dyspepsia. *BMJ* 2002; 324: 999–1002.
- 5 Meyer JM, Silliman NP, Wang W, *et al.* Risk Factors for *Helicobacter pylori* resistance in the United States: The Surveillance of *H. pylori* Antimicrobial Resistance Partnership (SHARP) Study, 1993–1999. *Ann Intern Med* 2002; 136: 13–24.
- 6 Lind T, Mégraud F, Unge P, *et al.* The MACH2 study: role of omeprazole in eradication of *Helicobacter pylori* with 1-week triple therapies. *Gastroenterology* 1999; 116: 248–53.
- 7 Vaira D, Malferteiner P, Megraud F, *et al.* Diagnosis of *Helicobacter pylori* infection with a new non-invasive antigen based assay. *Lancet* 1999; 354: 30–3.
- 8 Lantz H, Partington S, Vakil N. Systematic review of the efficacy of the stool antigen test in the detection of *H. pylori* before and after treatment in adults and children. *Gastroenterology* 2004; 126(Suppl. 2): A-184.
- 9 Gisbert JP, Pajares JM. Stool antigen test for the diagnosis of *Helicobacter pylori* infection. A systematic review. *Gastroenterology* 2004; 126(Suppl. 2): A-1.
- 10 Talley NJ, Stanghellini V, Heading RC, *et al.* Functional gastroduodenal disorders. *Gut* 1999; 45(Suppl. 2): II37–42.
- 11 Working Party of the European *Helicobacter pylori* Study Group. Technical annex: tests used to assess *Helicobacter pylori* infection. In: Guidelines for Clinical Trials in *Helicobacter* Infection. *Gut* 1997; 41(Suppl. 2): S10–18.
- 12 Dixon MF, Genta RM, Yardley JH, *et al.* Classification and grading of gastritis: the updated Sydney system. *Am J Surg Pathol* 1996; 20: 1161–81.
- 13 Bossuyt PM, Reitsma JB, Bruns DE, *et al.* Standards for reporting of diagnostic accuracy. Towards complete and accurate reporting of studies of diagnostic accuracy: the STARD initiative. *BMJ* 2003; 326: 41–4.
- 14 Altman DG. Diagnostic Tests. Statistics with Confidences. 2nd edn. In: Altman, DG, Machin, D, Trevor, NB, Gardner, MJ, eds. London: BMJ Books, 2000.

- 15 Stevens M, Livsey S, Swann R, *et al.* Evaluation of Sixteen EIAs for the Detection of antibodies to *Helicobacter pylori*. London: Department of Health, 1997: 1–46.
- 16 Bergey B, Marchildon P, Peacock J, *et al.* What is the role of serology in assessing *Helicobacter pylori* eradication? *Aliment Pharmacol Ther* 2003; 18: 635–9.
- 17 <http://www.sigmaaldrich.com/cgi-bin/hsrun/Distributed/HathShop/firmCatalogSearchPost?Brand=ALDRICH&ProdNo=299356>. Accessed June 2004.
- 18 Vaira D, Vakil N. Blood, urine, stool, breath, money and *Helicobacter pylori*. *Gut* 2001; 48: 287–9.
- 19 Jaeschke R, Guyatt G, Sackett DL. User's guide to the medical literature. How to use an article about a diagnostic test. What are the results and will they help me in caring for my patients? *JAMA* 1994; 271: 703–7.
- 20 Wu IC, Ke HL, Lo YC, *et al.* Evaluation of a newly developed office-based stool test for detecting *Helicobacter pylori*: an extensive pilot study. *Hepato-Gastroenterology* 2003; 50: 1761–65.
- 21 Leodolter A, Wolle K, Peitz U, *et al.* Evaluation of a novel rapid *H. pylori* stool antigen test: is a reliable diagnosis now possible in the doctor's office? *Gastroenterology* 2003; 124: A-58.
- 22 Chisholm SA, Teare L, Saverymuttu S, Owen R. Non-invasive diagnosis of *Helicobacter pylori* infection by stool antigen test – a comparison of HpSA ELISA and new rapid ImmunoCard STAT! HpSA test. *Helicobacter* 2003; 3: A-17.14.