

Non-invasive diagnosis of *Helicobacter pylori* infection in adult dyspeptic patients by stool antigen detection: does the rapid immunochromatography test provide a reliable alternative to conventional ELISA kits?

Stephanie A. Chisholm,¹ Claire L. Watson,¹ E. Louise Teare,² Seth Saverymuttu³ and Robert J. Owen¹

Correspondence

Stephanie A. Chisholm
Stephanie.Chisholm@hpa.org.uk

¹Specialist and Reference Microbiology Division, 61 Colindale Avenue, Colindale, London NW9 5HT, UK

²Chelmsford Microbiology Laboratory, Chelmsford, UK

³Broomfield Hospital, Chelmsford, UK

Stool antigen-testing allows non-invasive detection of *Helicobacter pylori* that is indicative of active infection. Three commercial kits are currently marketed in the UK for stool antigen-testing. The aim of this study was to conduct a comparative evaluation of the performances of each of these tests, compared with culture and histological examination of gastric biopsies, for pre-treatment diagnosis of infection in an adult dyspeptic population in south-east England. Examination of 112 stool samples by the Premier Platinum HpSA ELISA (Meridian Diagnostics) and by the Amplified IDEIA HpStAR ELISA (DakoCytomation) kits demonstrated that the latter was more sensitive (81.3 versus 93.8 %, respectively) and specific (91.7 versus 100.0 %, respectively). Additionally, the IDEIA HpStAR was easier to interpret, with OD readings of positive and negative results being far from the recommended cut-off, whereas equivocal results that were generated by the HpSA kit were difficult to interpret. Additional testing of 87 of the 112 stools by the ImmunoCard STAT! HpSA kit (Meridian Diagnostics) demonstrated that this test was easier to perform than ELISA and was more sensitive than the HpSA kit but, compared with the IDEIA HpStAR kit, the ImmunoCard test was less sensitive (87.8 versus 95.9 %, respectively) and specific (89.4 versus 100.0 %, respectively). Furthermore, the ImmunoCard test generated weakly positive results, correlating with lower OD readings for both ELISA kits, that were difficult to interpret. The Amplified IDEIA HpStAR kit is therefore the most sensitive and specific of the three tests that are available for pre-treatment, non-invasive detection of *H. pylori* in stool samples in an English adult dyspeptic population.

Received 8 October 2003

Accepted 15 March 2004

INTRODUCTION

Helicobacter pylori is the principal cause of peptic ulcer disease (NIH Consensus Conference, 1994) and is associated with lymphoproliferative disorders and development of gastric carcinoma (*Helicobacter* and Cancer Collaborative Group, 2001). Prevalence of infection is high worldwide – in England and Wales alone, over 7.5 million adults in the general population are estimated to have active *H. pylori* infection (Vyse *et al.*, 2002). Several methods, both invasive and non-invasive, are available for detection of *H. pylori* infection. Invasive methods involve endoscopy and examination of gastric biopsies, e.g. by culture, rapid urease test or histology, and are not appropriate for large-scale population studies. Non-invasive methods include the urea breath test,

serology and stool antigen test. The latter approach is non-invasive, does not require highly specialized equipment and, unlike serology, is more likely to provide evidence of active, rather than past, infection. Furthermore, it may be more appropriate for use in paediatric patients, where techniques such as serology are insensitive (Casswall *et al.*, 1999) and invasive methods are undesirable. Additionally, it may be used for treatment follow-up purposes.

Two commercial ELISAs, the Premier Platinum HpSA kit (Meridian Diagnostics) and the Amplified IDEIA HpStAR kit (formerly the FemtoLab Cnx *H. pylori* kit) (DakoCytomation), are currently marketed in the UK for stool antigen-testing. Since the original evaluative study of the Premier Platinum HpSA kit (Vaira *et al.*, 2000), numerous studies

have shown it to be highly sensitive and specific when applied to adult (Odaka *et al.*, 2002; Vaira *et al.*, 2000, 2002) and paediatric (Oderda *et al.*, 2000; Konstantopoulos *et al.*, 2001) populations for testing pre-treatment (Makrithathis *et al.*, 2000; Oderda *et al.*, 2000; Gisbert & Pajares, 2001; Konstantopoulos *et al.*, 2001) and post-therapy (Makrithathis *et al.*, 2000; Konstantopoulos *et al.*, 2001; Odaka *et al.*, 2002; Vaira *et al.*, 2002). The observations of these individual studies were confirmed by a recent meta-analysis of 68 studies, which reported an overall sensitivity and specificity of 92.4 and 91.9%, respectively, for diagnosing infection in untreated patients (Gisbert & Pajares, 2001) and of 88.3 and 92%, respectively, for patient follow-up at ≥ 4 weeks to assess eradication outcome (Gisbert & Pajares, 2001). Results from individual studies suggested that the sensitivity and specificity of this test may vary according to geographical locations (Gisbert & Pajares, 2001), demonstrating the need for local evaluation of kit performance prior to routine implementation. Whilst one study has examined the performance of the HpSA kit in a paediatric patient group in south-west Scotland (Shepherd *et al.*, 2000), no other evaluative studies have been conducted in the UK. Although few evaluative studies of the IDEIA HpStAR kit have been reported, evidence to date suggests that this kit is marginally more sensitive than the HpSA kit for primary diagnosis of paediatric infection (Makrithathis *et al.*, 2000; Koletzko *et al.*, 2003) and for post-treatment follow-up of such patients (Makrithathis *et al.*, 2000). Similar results were reported in a study that investigated 148 adult dyspeptic patients after eradication therapy (Leodolter *et al.*, 2002). Additionally, the ImmunoCard STAT! HpSA test (Meridian Diagnostics) is now available in the UK, but no evaluative studies of its performance have yet been reported.

This study evaluated both ELISA kits (Premier Platinum HpSA and Amplified IDEIA HpStAR) and the ImmunoCard STAT! HpSA test, a lateral-flow immunochromatography test, to compare relative performances when applied to stools from dyspeptic adult patients in south-east England.

METHODS

Clinical samples. In total, 112 adult dyspeptic patients, aged from 23 to 89 years (mean age, 60 years) attending an open-access endoscopy clinic in south-east England (Broomfield Hospital, Chelmsford, UK), who had not taken specific eradication therapy for *H. pylori*, were included in this study. Macroscopic examination of gastric mucosae at the time of endoscopy demonstrated that patients in this group represented a wide range of disease spectra, including patients with normal mucosa, evidence of gastritis and/or duodenitis, gastric and/or duodenal ulceration, dysplasia, oesophagitis, oesophageal ulceration and Barrett's oesophagitis. Samples were selected on the basis of results from routine examinations of antral gastric biopsies that were collected at endoscopy, so that kit sensitivities could be assessed by including a high proportion of stools from *H. pylori*-positive patients. Sixty-four patients were positive for *H. pylori* by culture and/or histology, whereas the remaining 48 patients had no biopsy-based evidence of *H. pylori* infection.

Local ethical approval was obtained to collect stool samples from patients at the time of endoscopy; these were provided with the patients'

informed consent. Stools were stored immediately at -20°C until they were analysed. All 112 stools were tested for *H. pylori* antigen by the Premier Platinum HpSA and by the Amplified IDEIA HpStAR ELISA kits, as described below. Additional testing by the ImmunoCard STAT! HpSA kit was performed on 87/112 stools, 49 of which were from *H. pylori*-positive patients.

Detection of *H. pylori* stool antigen by the Premier Platinum HpSA kit. *H. pylori* antigen was detected from stools by the Premier Platinum HpSA ELISA (Launch Diagnostics) by following the protocol of the manufacturer (Meridian Diagnostics). Briefly, a 5–6 mm-sized stool sample (or 100 μl , if liquid) was emulsified in 200 μl sample diluent and mixed by vortexing. Diluted stool (50 μl) was added to an anti-*H. pylori* antibody-coated well, along with one drop of peroxidase-conjugated antibody. Plates were incubated at room temperature for 1 h and unbound material was removed by five manual washes with the buffer provided. Bound antigen-antibody complexes were detected by a colour change, following incubation in the dark with enzyme substrate at room temperature for 10 min. Reactions were stopped by the addition of stop solution and spectrophotometric absorbances were measured at 450 and 630 nm, within 30 min, using a Labsystems Multiskan RC version 6.0 (Labsystems Oy). According to the manufacturer's guidelines, samples were defined as either negative, equivocal or positive on the basis of OD₄₅₀/OD₆₃₀ readings of <0.100 , 0.100–0.120 and ≥ 0.120 , respectively.

Detection of *H. pylori* stool antigen by the Amplified IDEIA HpStAR kit. *H. pylori*-specific antigen was detected by the Amplified IDEIA HpStAR ELISA kit, following the procedure provided by the manufacturer (DakoCytomation). Approximately 0.1 g stool was emulsified in 500 μl sample diluent, vortexed and then centrifuged (5000 r.p.m. for 5 min). Supernatant (50 μl) was added to antibody-coated wells, along with 50 μl peroxidase-conjugated anti-*H. pylori* antibody; this was incubated at ambient temperature for 1 h. Wells were washed by using a Well Wash 4 automated plate-washer (Denley, Life Sciences International UK) and remaining bound complexes were detected by incubation at ambient temperature for 10 min in the dark with 100 μl substrate. Following the addition of 100 μl stop solution, results were recorded as described for the HpSA kit. Samples were defined as either negative or positive on the basis of OD₄₅₀/OD₆₃₀ readings of <0.150 and ≥ 0.150 , respectively, as indicated by the manufacturer.

Detection of *H. pylori* stool antigen by the ImmunoCard STAT! HpSA kit. Stool samples were tested for *H. pylori* antigen by the ImmunoCard STAT! HpSA kit (Launch Diagnostics) by following the protocol of the manufacturer (Meridian Diagnostics). Briefly, a 5–6 mm-sized stool sample was added to vials that contained 1 ml sample diluent and emulsified by vortexing for 15 s. The tip of the vial was snapped off and four drops were added to the sample port of the test cassette. The test was read after exactly 5 min incubation at ambient temperature. Tests were interpreted as negative if there was a blue line in the control (C) window only, and as positive if there was any evidence of an additional pink line in the test (T) window. Results were defined as positive if the pink band was clearly visible and weakly positive if it was very faint.

RESULTS AND DISCUSSION

Comparison of ELISA kits

Examination of stools from 112 dyspeptic patients demonstrated that whilst both ELISA kits allowed rapid diagnosis of infection (within 1.5–2 h), the Amplified IDEIA HpStAR kit was more sensitive and specific (Table 1) and was easier to

Table 1. Comparison of HpSA and HpStAR stool antigen test performances when applied to 112 stools from patients of known *H. pylori* status

Stool antigen kit	Result	Patient status* (n)		Sensitivity (%)†	Specificity (%)†
		+ (64)	- (48)		
HpSA	+	47	2	81.3	91.7
	Equivocal	5‡	2	(73.4)	(95.8)
	-	12	44		
HpStAR	+	60	0	93.8	100.0
	-	4	48		

**H. pylori* status, confirmed by culture and/or histology of gastric biopsies.

†Sensitivities and specificities in parentheses were calculated by assuming that equivocal results were *H. pylori*-negative.

‡Of these five equivocal results, four of the patients were positive by culture and histology and one patient was positive by histology only (by gastric biopsy analyses).

use, with automation of washing steps. Overall, the Premier Platinum HpSA kit detected specific stool antigen in 47 of 64 *H. pylori*-positive patients and in two of 48 *H. pylori*-negative patients (Table 1, Fig. 1). Equivocal results were generated in

five of 64 and two of 48 samples from patients that were *H. pylori*-positive and -negative, respectively (Table 1; Fig. 1). The HpSA kit was 81.3% sensitive and 91.6% specific if equivocal results were interpreted as stool antigen-positive; however, if these were considered to be negative, sensitivity was lower (73.4%), but specificity was higher (95.8%). In contrast, the Amplified IDEIA HpStAR kit detected specific stool antigen in 60 of 64 *H. pylori*-positive patients and in none of 48 *H. pylori*-negative patients (93.8% sensitive and 100.0% specific). No equivocal results were generated with this kit (Table 1; Fig. 1). The lower sensitivity of the HpSA kit determined in this study compared with previous reports (Gisbert & Pajares, 2001) may be due to differences in the study population or conditions of specimen storage. Nevertheless, direct comparison of the results for each kit clearly demonstrated higher sensitivity and specificity of the IDEIA HpStAR ELISA.

The quality of results generated by the two kits, indicated by OD₄₅₀/OD₆₃₀ readings, also varied (Fig. 1). The mean OD₄₅₀/OD₆₃₀ values recorded for HpSA-positive and -negative specimens were 0.447 ± 0.137 units above and 0.059 ± 0.008 units below the kit's cut-off value for equivocal results of 0.10 OD units, respectively (Fig. 1). The mean OD₄₅₀/OD₆₃₀ values of the IDEIA HpStAR kit for positive and negative results were 1.930 ± 0.344 units above and 0.113 ± 0.006 units below the kit's recommended cut-off level (0.15 OD units) (Fig. 1). Comparison by an unpaired *t*-test demonstrated that the OD₄₅₀/OD₆₃₀ values of the IDEIA HpStAR kit were significantly further above or below the defined cut-off than those of the HpSA kit (*P* < 0.0001) and were therefore easier to interpret.

The observed variations in performance may relate to the different capture antibodies used in each assay. The monoclonal capture antibodies in the HpStAR kit would allow highly specific binding of *H. pylori* antigen. In contrast, the polyclonal antibodies of the HpSA kit, whilst potentially more sensitive, could theoretically bind non-specifically to a

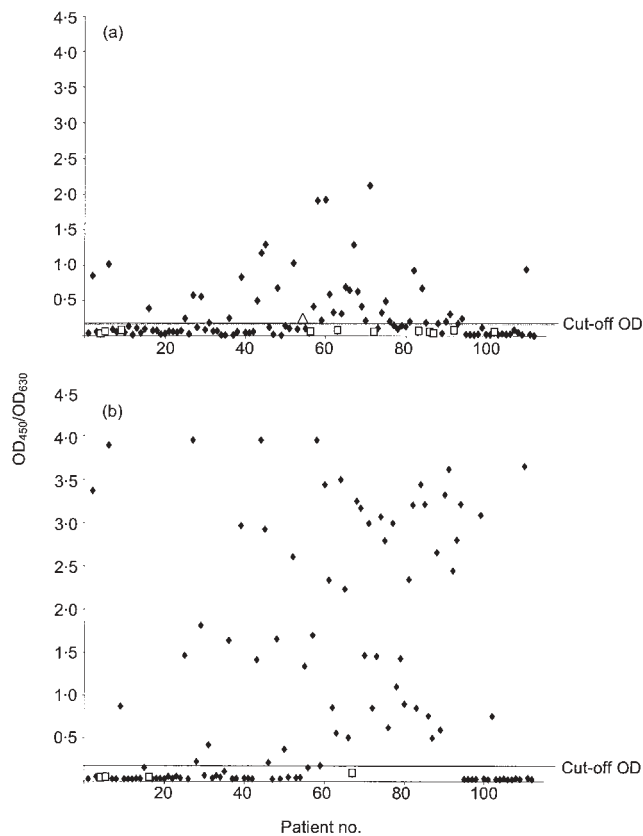


Fig. 1. Scattergraphs representing the range of OD₄₅₀/OD₆₃₀ values recorded for each stool specimen tested by (a) the Premier Platinum HpSA and (b) the Amplified IDEIA HpStAR kit. □, False-negative; △, false-positive.

wider range of faecal antigens, leading to higher background OD levels. Use of polyclonal antibodies could also lead to variation in assay performances between kit batches. Additionally, preliminary centrifugation of diluted stool in the HpStAR kit removes insoluble faecal material, prior to incubation of samples with capture and conjugate antibodies. This may improve accessibility and diffusion of soluble *H. pylori* antigen and, therefore, facilitate formation of specific antigen-antibody complexes, leading to higher OD levels for positive results. Removal of solid particles also allowed washing steps to be automated and hence standardized between runs. In contrast, the HpSA kit protocol does not include a centrifugation step and both insoluble and soluble faecal material is incubated with the capture and conjugate antibodies, which may lower the rate of specific complex formation and, ultimately, OD readings, while increasing non-specific binding and background OD levels. Automated well-washing was not possible, because of the potential for equipment blockage, and efficiency of manual washing may have varied between runs. Although care was taken to ensure adequate washing of wells, the amount and nature of insoluble material varied between specimens. Levels of residual material may also have contributed to the high non-specific, background OD levels that were observed in some samples.

Our results suggest that of the two commercially available, ELISA-based kits for *H. pylori* stool antigen detection, the IDEIA HpStAR kit is the most accurate for pre-treatment *H. pylori* diagnosis in adult dyspeptic patients in south-east England. Few studies have compared the relative performances of these two kits (Makrithathis *et al.*, 2000; Leodolter *et al.*, 2002; Koletzko *et al.*, 2003). The findings of this study of an adult dyspeptic population were in agreement with those of an earlier study that demonstrated marginally higher sensitivity (98.0%) of the IDEIA HpStAR (=FemtoLab) kit for primary diagnosis of *H. pylori* infection in 49 paediatric patients when compared with the HpSA kit (93.8%) (Makrithathis *et al.*, 2000). Additionally, that study demonstrated that both kits allowed patient follow-up post-therapy. A subsequent study of 148 adult patients also reported marginally higher sensitivity of the HpStAR kit for evaluating success of eradication therapy, although the kit cut-off OD was lowered to 0.90 to achieve this (Leodolter *et al.*, 2002).

None of the patients examined in our study had received specific eradication therapy, but validation of each kit for adult patient follow-up is needed so that appropriate cut-offs can be established. Likewise, further studies are essential to fully evaluate the performances of these kits in paediatric patients from the UK.

Comparison of ImmunoCard STAT! HpSA test and ELISA kits

For the 87 stool specimens analysed by all three tests, specific antigen was detected by the HpSA and IDEIA HpStAR ELISA kits in, respectively, 39/49 and 47/49 *H. pylori*-positive patients and in 2/38 and 0/38 *H. pylori*-negative patients. Positive results were obtained for the ImmunoCard test in 43/49 positive patients and in 4/38 negative patients. Respective sensitivities for the HpSA, the IDEIA HpStAR and the ImmunoCard tests were thus 79.6, 95.9 and 87.8%, while test specificities were 94.7, 100.0 and 89.4%, respectively. However, if five equivocal results generated by the HpSA ELISA were considered negative, sensitivity of that test was 71.4%, while specificity was 97.4%.

Positive results by the ImmunoCard STAT! HpSA test could be subdivided into weak, moderate or strong, based on the intensity of the pink band. Stool specimens that were strongly or moderately positive generated mean OD₄₅₀/OD₆₃₀ readings of 0.527 ± 0.178 for the HpSA kit and 2.72 ± 0.333 for the HpStAR kit. In contrast, mean OD₄₅₀/OD₆₃₀ readings for stools that were weakly positive or falsely negative were significantly lower for both the HpSA kit (0.146 ± 0.057, *P* = 0.0009) and the HpStAR kit (0.774 ± 0.335, *P* < 0.0001). Further illustration of this correlation is provided by the observation that stools that were falsely negative or equivocal by ELISA were also weakly positive or falsely negative by ImmunoCard HpSA test (Table 2). Weakly positive ImmunoCard results could be difficult to interpret, particularly as faint pink bands also could be observed in negative stools beyond the recommended 5 min incubation. High sensitivity and specificities have been reported for similar ImmunoCard tests that are available for the detection of enteric pathogens *Escherichia coli* O157 (Mackenzie *et al.*, 2000) and *Giardia lamblia* and *Cryptosporidium parvum* (Garcia *et al.*, 2003). Our results suggest that the Immuno-

Table 2. Results generated by the ImmunoCard STAT! HpSA test when applied to stools from 87 dyspeptic patients, compared with results of the Premier Platinum HpSA ELISA and the IDEIA HpStAR ELISA

TP, True positive; TN, true negative; E, equivocal result; FN, false negative; FP, false positive, defined on the basis of culture and histology testing of matched gastric biopsies.

ImmunoCard STAT! HpSA	HpSA ELISA					IDEIA HpStAR ELISA			
	TP	TN	E	FN	FP	TP	TN	FN	FP
Positive (<i>n</i> = 29)	25	0	1	3	0	29	0	0	0
Weak positive (<i>n</i> = 18)	8	4	3	3	0	13	4	1	0
Negative (<i>n</i> = 40)	2	32	1	4	1	5	34	1	0

Card HpSA test was more sensitive, but less specific, than the HpSA ELISA and allows accurate detection of *H. pylori* from stools with higher levels of antigen. However, weakly positive results were defined subjectively and, as for the equivocal results of HpSA ELISA, would be difficult to interpret in a clinical setting.

Both ELISA-based kits required inclusion of a positive and a negative control in each run. It was therefore most economical to batch-test multiple stool specimens in a single experiment. In contrast, the immunochromatographical ImmunoCard STAT! HpSA test is more suitable for testing single or small numbers of stools. This test uses mAbs to capture specific antigen and results can be obtained in 5–10 minutes, compared to 2–3 h with ELISA-based methods. The speed and simplicity of this test are clearly advantageous compared to ELISA, particularly in a laboratory that examines low numbers of stool specimens.

In conclusion, our study has shown that whilst the ImmunoCard STAT! HpSA test is more convenient to use than either ELISA kit, the higher sensitivity and specificity of the IDEIA HpSTAR ELISA kit support its use for routine pre-treatment diagnosis of *H. pylori* infection in an adult population in south-east England.

REFERENCES

- Casswall, T. H., Nilsson, H.-O., Bergström, M., Aleljung, P., Wadström, T., Dahlström, A. K., Albert, M. J. & Sarker, S. A. (1999). Evaluation of serology, 13C-urea breath test, and polymerase chain reaction of stool samples to detect *Helicobacter pylori* in Bangladeshi children. *J Pediatr Gastroenterol Nutr* **28**, 31–36.
- Garcia, L. S., Shimizu, R. Y., Novak, S., Carroll, M. & Chan, F. (2003). Commercial assay for detection of *Giardia lamblia* and *Cryptosporidium parvum* antigens in human fecal specimens by rapid solid-phase qualitative immunochromatography. *J Clin Microbiol* **41**, 209–212.
- Gisbert, J. P. & Pajares, J. M. (2001). Diagnosis of *Helicobacter pylori* infection by stool antigen determination: a systematic review. *Am J Gastroenterol* **96**, 2829–2838.
- Helicobacter and Cancer Collaborative Group** (2001). Gastric cancer and *Helicobacter pylori*: a combined analysis of 12 case control studies nested within prospective cohorts. *Gut* **49**, 347–353.
- Koletzko, S., Konstantopoulos, N., Bosman, D., Feydt-Schmidt, A., van der Ende, A., Kalach, N., Raymond, J. & Rüssmann, H. (2003). Evaluation of a novel monoclonal enzyme immunoassay for detection of *Helicobacter pylori* antigen in stool from children. *Gut* **52**, 804–806.
- Konstantopoulos, N., Rüssmann, H., Tasch, C., Sauerwald, T., Demmelmair, H., Autenrieth, I. & Koletzko, S. (2001). Evaluation of the *Helicobacter pylori* stool antigen test (HpSA) for detection of *Helicobacter pylori* infection in children. *Am J Gastroenterol* **96**, 677–683.
- Leodolter, A., Peitz, U., Ebert, M. P., Agha-Amiri, K. & Malfertheiner, P. (2002). Comparison of two enzyme immunoassays for the assessment of *Helicobacter pylori* status in stool specimens after eradication therapy. *Am J Gastroenterol* **97**, 1682–1686.
- Mackenzie, A., Orrbine, E., Hyde, L. & 7 other authors (2000). Performance of the ImmunoCard STAT! E. coli O157:H7 test for detection of *Escherichia coli* O157:H7 in stools. *J Clin Microbiol* **38**, 1866–1868.
- Makrithatis, A., Barousch, W., Pasching, E., Binder, C., Kuderna, C., Apfalter, P., Rotter, M. L. & Hirschl, A. M. (2000). Two enzyme immunoassays and PCR for detection of *Helicobacter pylori* in stool specimens from pediatric patients before and after eradication therapy. *J Clin Microbiol* **38**, 3710–3714.
- NIH Consensus Conference** (1994). *Helicobacter pylori* in peptic ulcer disease. NIH Consensus Development Panel on *Helicobacter pylori* in Peptic Ulcer Disease. *JAMA* **272**, 65–69.
- Odaka, T., Yamaguchi, T., Koyama, H., Saisho, H. & Nomura, F. (2002). Evaluation of the *Helicobacter pylori* stool antigen test for monitoring eradication therapy. *Am J Gastroenterol* **97**, 594–599.
- Oderda, G., Rapa, A., Ronchi, B., Lerro, P., Pastore, M., Staiano, A., de'Angelis, G. L. & Strisciuglio, P. (2000). Detection of *Helicobacter pylori* in stool specimens by non-invasive antigen enzyme immunoassay in children: multicentre Italian study. *BMJ* **320**, 347–348.
- Shepherd, A. J., Williams, C. L., Doherty, C. P., Hossack, M., Preston, T., McColl, K. E. L. & Weaver, L. T. (2000). Comparison of an enzyme immunoassay for the detection of *Helicobacter pylori* antigens in the faeces with the urea breath test. *Arch Dis Child* **83**, 268–270.
- Vaira, D., Menegatti, M., Ricci, C., Gatta, L., Berardi, S. & Miglioli, M. (2000). Accurate diagnosis of *Helicobacter pylori*. Stool tests. *Gastroenterol Clin North Am* **29**, 917–923.
- Vaira, D., Vakil, N., Menegatti, M. & 13 other authors (2002). The stool antigen test for detection of *Helicobacter pylori* after eradication therapy. *Ann Intern Med* **136**, 280–287.
- Vyse, A. J., Gay, N. J., Hesketh, L. M., Andrews, N. J., Marshall, B., Thomas, H. I., Morgan-Capner, P. & Miller, E. (2002). The burden of *Helicobacter pylori* infection in England and Wales. *Epidemiol Infect* **128**, 411–417.