JOURNAL OF CLINICAL MICROBIOLOGY, Oct. 2000, p. 3755–3758 Vol. 38, No. 10
0095-1137/00/$04.00+0

Evaluation of a PCR Primer Based on the Isocitrate Dehydrogenase Gene for Detection of *Helicobacter pylori* in Feces

FRAUKE C. ARGYROS,[1] MAYURIKA GHOSH,[2] LILI HUANG,[2] NAOKO MASUBUCHI,[2] DAVID R. CAVE,[2] AND PETER GRÜBEL[3]*

Department of Biology, Northeastern University,[1] Division of Gastroenterology, St. Elizabeth's Medical Center,[2] and Division of Gastroenterology, New England Medical Center,[3] Boston, Massachusetts

Received 11 February 2000/Returned for modification 18 April 2000/Accepted 18 July 2000

In order to improve detection and identification of *Helicobacter pylori* in highly contaminated samples, we evaluated new specific primers based on the DNA base sequence within the isocitrate dehydrogenase (*icd*) gene to amplify a 1,200-bp DNA segment. The specificity of the *icd* primer was tested against DNA derived from various bacteria, including 7 *Helicobacter* species and a panel of 1 gram-variable, 2 gram-positive, and 16 gram-negative bacteria, as well as DNA from houseflies and feces from *H. pylori*-negative patients. The primers permitted the detection of all clinical *H. pylori* isolates tested, but no reactions were observed with negative controls. Several procedures for DNA extraction from feces were evaluated using PCR with *icd* primers. The lower limits of detection of *H. pylori* DNA from two different sources containing the same number of *H. pylori* organisms, a pure culture and feces spiked with *H. pylori*, were established for each extraction method tested. The results were 8.0×10^3 CFU/ml for cultures of pure *H. pylori*, and 8.0×10^6 CFU/ml for *H. pylori* from feces, using the phenol-chloroform method; 8.0×10^2 and 7.0×10^3 CFU/ml, respectively, for a glass matrix and chaotropic solution protocol; 8.0×10^2 and 7.0×10^3 CFU/ml, respectively, for the QIAamp tissue kit; and 5.0×10^2 and 5.0×10^3 CFU/ml, respectively, for the XTRAX DNA extraction kit. We conclude that the use of the *icd* gene as a primer for PCR represents a specific and sensitive assay for detection of *H. pylori* in highly contaminated samples.

Helicobacter pylori is strongly associated with gastroduodenal disease, including chronic active gastritis, duodenal ulcers, and gastric adenocarcinoma (1). Several reliable methods for detecting *H. pylori* are used at present; most of them are performed on gastric biopsy samples. Since these methods are thus invasive and expensive, less-invasive methods such as serologic examination of blood and the urea breath test are becoming more popular (27). However, these noninvasive tests have drawbacks: positive results by blood serology do not necessarily indicate current infection by *H. pylori* (D. J. Cullen, K. J. Cullen, B. J. Collins, K. J. Christiansen, and J. Epis, Letter, Lancet **340:**1161–1162, 1992), while urea breath tests require expensive, specialized equipment and reagents (2). Considering these drawbacks of such noninvasive tests, alternative methods with feces appear attractive.

In a limited number of studies, infected individuals have been shown to excrete *H. pylori* in feces, since PCR, enzyme immunoassay (EIA), or even culture could detect the organisms in stool specimens (7–9, 20, 23). However, many others have not yet confirmed these findings. In fact, culture of *H. pylori* is extraordinarily problematic due to the complexity of the fecal microflora, making direct culture of *H. pylori* difficult and subject to misinterpretation. Further limiting factors of isolation are technical, such as the requirement for a selective medium to culture *H. pylori* reliably from fecal samples. Hence, PCR has been used to detect *H. pylori* DNA; however, it has been shown to have its own limitations. For example, extraction of PCR-amplifiable DNA from feces has been a complicated and lengthy process (20). A variety of inhibitors present in feces can lead to erratic results (12, 24). In addition, the correct choice of highly specific and sensitive primers and the correct conformation of the amplified product are clearly essential for optimal sensitivity and specificity (10). We have recently isolated and expressed the isocitrate dehydrogenase gene (*icd*) of *H. pylori* (6). The DNA sequence of the *icd* gene was 96% identical to that present in the total-genome sequence of *H. pylori* strain 26695 (21) and only 64% homologous to the *icd* of *Escherichia coli*, 64% homologous to *Vibrio* sp. *icd*, and 63% homologous to *Bacillus subtilis icd*. Furthermore, the 3′ sequence was unique based on gene bank analysis. The aim of our study was to evaluate several procedures for extracting *H. pylori* DNA from feces and to evaluate newly designed primers based on the DNA sequence within the *icd* gene for PCR. An effective combination of DNA extraction and amplification may improve detection of *H. pylori* in the environment and in clinical settings.

MATERIALS AND METHODS

Controls for primer specificity. Bacterial strains, flies, and fecal samples used as controls are listed in Table 1.

Clinical isolates of *H. pylori* from 10 patients with duodenal ulcers were grown on *Brucella* agar (Difco, Detroit, Mich.) with lysed sheep blood (5% [vol/vol]; Remel, Lenexa, Kans.) and were incubated under a microaerophilic atmosphere at 37°C for 72 h. *Campylobacter* spp. were also grown on this medium at 42°C for 2 days. All other bacteria were grown on blood agar plates at 37°C for 24 h; for *Enterococcus faecalis*, a 5% CO_2 atmosphere was used. Stock cultures were maintained at −80°C in 20% (vol/vol) glycerol-*Brucella* broth.

Seven different *Helicobacter* species were cultured like the clinical isolates above in order to assess the specificity of our *icd* primers. Furthermore, we tested the *icd* primers against houseflies (*Musca domestica*) and human feces. Fecal samples were obtained from patients proven *H. pylori* negative by histology of endoscopically obtained gastric biopsy specimens and were tested individually. In this study, feces from *H. pylori*-positive patients was not used for the evaluation of the sensitivity and specificity of our PCR methods, since the number of *H.*

* Corresponding author. Mailing address: St. Elizabeth's Medical Center, Division of Gastroenterology, 736 Cambridge St., Boston, MA 02135. Phone: (617) 789-2423. Fax: (617) 789-2427. E-mail: apgrubel@massmed.org.

TABLE 1. Specificity of *icd* primers in detecting *H. pylori* DNA

Control specimen	Amplification with *icd* primers[a]
Arcobacter butzleri	−
Bacteroides fragilis	−
Campylobacter coli	−
Campylobacter jejunii	−
Campylobacter lari	−
Campylobacter coli	−
Clostridium perfringens	−
Escherichia coli	−
Enterococcus faecalis	−
Gardnerella vaginalis	−
Haemophilus influenzae	−
Haemophilus parahaemolyticus	−
Helicobacter spp.	
H. bilis	−
H. canis	−
H. felis	−
H. hepaticus	−
H. mustelae	−
H. pullorum	−
H. pylori (10 isolates)	+
H. rodentium	−
Providencia rettgeri	−
Salmonella sp.	−
Serratia marcescens	−
Shigella flexneri	−
Streptococcus pneumoniae	−
Yersinia enterocolitica	−
Vibrio parahaemolyticus	−
Control flies	
Spiked with *H. pylori*	+
Nonspiked	−
Feces	
Spiked with *H. pylori*	+
Nonspiked	−

[a] +, target amplification; −, no target amplification.

pylori organisms in these samples is unknown. The purpose of our work was to evaluate our primers and DNA extraction methods; therefore, samples with defined *H. pylori* loads were used.

Flies were chosen as controls because they contain a large variety of viral, bacterial, and parasite DNA, as well as food and host DNA (5). In addition, we recently reported that flies are able to carry and excrete viable *H. pylori* and may therefore represent a vector for the transmission of *H. pylori* (4). Several hundred housefly pupae (Carolina Biological Supply Company, Burlington, N.C.) were placed in well-ventilated autoclaved buckets maintained at 25°C. Emerging adult flies were removed, and pools of five flies were transferred into sterile test containers and frozen at −80°C until subsequent preparation for PCR. For DNA extraction, flies were thawed. The abdomen of each fly was separated aseptically from the chest under a dissecting microscope. The bodies of each fly pool were homogenized in 0.5 ml of sterile water by using a sterile glass grinder. All bacterial and fecal samples, as well as fly homogenates, were processed by using the XTRAX kit (see below) to purify genomic DNA for PCR.

PCR. PCR was used to identify the *icd* gene of *H. pylori* in our samples. Chromosomal DNA was prepared as described under "DNA extraction and method sensitivity" below. Oligonucleotide primers (Tufts University Protein and Nucleic Acid Analysis Unit, Boston, Mass.), were specific for the 5′ and 3′ termini of the *icd* gene respectively, with the sequences 5′-ATGGCTTACAAC CCTAAAATTTTACAAAAGCC-3′ and 5′-TCACATGTTTTCAATCATCAC GC-3′. The reaction was performed in a volume of 100 μl, comprising 50 pM each primer, 10 μl of chromosomal DNA, 2 U of *Taq* DNA polymerase (Boehringer Mannheim Biochemicals), 0.2 mM deoxynucleoside triphosphates, and 1.5 mM $MgCl_2$, in a single block thermal cycler (Ericomp, San Diego, Calif.). Cycle profiles were as follows: DNA denaturation at 94°C for 5 min, followed by 30 cycles consisting of 1-min denaturation at 94°C, 2-min annealing at 55°C, and 2-min extension at 72°C. Samples were run simultaneously with a clinical *H. pylori* isolate as a positive control. Negative-control reactions with distilled water were performed with each batch of amplification to exclude the possibility of contamination. The specificity of *icd* primers was compared with that of species-specific protein antigen primers, which have been reported to be highly specific for *H. pylori* (14). Species-specific protein antigen and *icd* primers were tested against control flies, *H. pylori*, and the non-*Helicobacter* control bacteria listed in Table 1. In addition, *icd* primers were studied with seven *Helicobacter* species. PCR products were analyzed by electrophoresis of a 10-μl aliquot using a 1.3% (wt/vol) agarose gel. Bands were visualized by excitation under UV light after staining with 1 μg of ethidium bromide/ml.

Target DNA sequence determination. DNA sequencing was performed on the amplified products in order to eliminate false-positive results. DNA was extracted from 90 μl of the reaction mixture by using a PCR purification kit (QIAGEN, Hilden, Germany). Double-stranded DNA PCR was carried out by the dideoxy chain termination method (15) with *Taq* DNA polymerase (GIBCO BRL) by using the same oligonucleotide primers specific for the *icd* gene as in the PCR.

DNA extraction and method sensitivity. In the present study, four methods to purify DNA from one pure clinical *H. pylori* isolate in suspension as well as from spiked fecal samples were compared, as follows.

(i) The classical phenol-chloroform method (11). Protein was denatured using STE buffer with 1% sodium dodecyl sulfate and 25 μg proteinase of K, and DNA was concentrated in the aqueous layer using phenol-chloroform. Ethanol was then used to remove residual phenol-chloroform and salts and to precipitate the DNA.

(ii) Glass matrix and chaotropic solution (18). Guanidine-thiocyanate was used to lyse cells and denature proteins. DNA was bound to glass beads (GlasPac; National Scientific Supply Company, San Rafael, Calif.), and the suspension was washed in wash buffer (50% ethanol, 10 mM Tris-HCl [pH 7.5], 100 mM NaCl).

(iii) QIAamp tissue kit (Qiagen). Proteins were denatured using proteinase K. DNA was bound to the silica membrane of spin columns, leading to the removal of other contaminants by washing with buffers provided in the QIAamp kit.

(iv) XTRAX DNA extraction kit (Gull Laboratories, Salt Lake City, Utah) (26). Samples were microwaved in the presence of an extraction buffer containing detergents and salts, followed by centrifugation and DNA precipitation with isopropyl alcohol.

DNA was extracted from an *H. pylori* dilution series (10^0 to 10^{-6}, beginning with an optical density at 600 nm [OD_{600}] of 1.0), using 100 μl of a pure *H. pylori* suspension in one series and 50 μl of the creamy layer of a stool sample spiked with 100 μl of bacterial suspension in the other. The creamy layer contains fecal bacteria in a more concentrated form and was prepared as described by Thomas (19). In brief, stool samples were centrifuged (7,000 × *g* for 10 min) to separate three identifiable layers: a liquid supernatant on top, a creamy layer in the middle, and a solid layer on the bottom.

Quantification of *H. pylori* present in bacterial suspensions and in aliquots used to spike stool samples was performed by plate counts on Skirrow's agar (Difco, San Jose, Calif.).

PCR using the *icd* primers was performed to determine the sensitivities of the extraction methods (see the protocols above).

RESULTS

Our *icd* primers proved to be very specific for *H. pylori* only and did not result in false-positive results with any of the other bacterial species, including seven *Helicobacter* species (Table 1). In addition, these primers specifically amplified *H. pylori* DNA from feces and fly abdomens, which represent a pool of heterogeneous DNA. This underlines the usefulness of these primers in the detection of *H. pylori* in highly contaminated samples, such as flies and stool. In contrast, the species-specific antigen primers (14) produced false-positive signals in our initial screening tests and reacted with negative-control flies, *Campylobacter jejuni*, and *E. faecalis* (data not shown).

The DNA sequence of the amplified target DNA of our controls confirmed the PCR products with more than 96% homology to the *icd* gene. The control clinical isolates of *H. pylori* showed 95% to 97% homology to the *icd* gene of reference strain 26695 (21). The fact that the sequences are not identical minimizes the chance that contamination occurred during sample preparation.

In comparison with two other DNA extraction techniques, the QIAamp kit and the XTRAX kit proved to be the preferred methods due to their sensitivity and the fact that complete kits reduce the length and number of preparatory steps (see Table 2). Extraction using guanidine-thiocyanate, however, was as sensitive as the QIAamp and XTRAX techniques for pure *H. pylori* as well as for bacteria from feces but was less

TABLE 2. Comparison of DNA extraction methods for detection of *H. pylori* in pure solution and in human feces

Method	Sensitivity (CFU/ml) for:		Duration (min)	Cost/sample ($)	Convenience
	Pure *H. pylori*	*H. pylori* and feces			
Phenol-chloroform	8.0×10^3	8.0×10^6	600	0.50	Low
Guanidine	8.0×10^2	7.0×10^3	180	1.90	Moderate
QIAamp	8.0×10^2	7.0×10^3	330	1.80	High
XTRAX	5.0×10^2	5.0×10^3	60	1.60	High

convenient, since it required more preparation. Phenol-chloroform extraction recovered the smallest amount of DNA from pure and fecal *H. pylori*, and exposes one to harmful fumes (Table 2).

DISCUSSION

This study shows that the *icd* gene PCR assay is a sensitive and specific method for the detection of *H. pylori* in feces. Comparison of four DNA extraction protocols that included columns, centrifugation, glass matrices, or patented extraction matrices showed that the QIAamp method and XTRAX kit were the best procedures due to their sensitivity and convenience of execution.

Isolation of *H. pylori* from feces as a diagnostic tool or for research purposes for detecting colonization is attractive because it is noninvasive. However, culture of *H. pylori* from patients' fecal samples has been problematic because currently available selective culture media for isolation of *H. pylori* are frequently overgrown by other, faster-growing gram-negative bacteria. Similarly, detection of *H. pylori* in feces by using PCR and standard extraction methods has proved difficult, producing erratic results due to a variety of fecal inhibitors, such as acidic polysaccharides, metabolic products, and large amounts of irrelevant DNA (12). Furthermore, previous PCR analysis for *H. pylori* in feces has shown low sensitivity (24) due to by multiple factors such as variations in the sensitivity and amplification performance of the polymerase and in the annealing affinity of the chosen primers to their target sequence (17). For this reason, DNA extraction procedures that remove inhibitors of PCR and concentrate the pathogen or total DNA were developed. In concert with these effective techniques, our highly specific *icd* primer represents a specific and sensitive method for detection of *H. pylori*.

Makristathis et al. (8) described species-specific protein antigen primers as specific for *H. pylori*, using only one other *Helicobacter* species to assess specificity. Our screening experiments, which included all the bacterial species listed in Table 1, as well as flies, showed that the use of these primers resulted in false-positive amplifications with control flies, *C. jejuni*, and *E. faecalis*. It is therefore possible that Makristathis' assay may have amplified non-*H. pylori* DNA, resulting in false positives. We demonstrated that the *icd* primers were more specific for *H. pylori* than the species-specific protein antigen primers when tested against a variety of DNA samples. Others have used primers based on the 16S rRNA, which have also displayed good specificity when tested against many microorganisms, including several *Helicobacter* species (16, 25), although no studies have been performed to assess the detection limit of these primers for *H. pylori* in feces.

The minimum number of organisms in feces required for a positive PCR result was 3.3×10^4 CFU/ml of stool in our most sensitive assay, which is comparable to other reported sensitivities (24). Despite concentrating *H. pylori* organisms by immunomagnetic-bead separation techniques, Osaki et al. showed comparable detection limits of 2×10^4 CFU for fecal samples obtained from gnotobiotic mice (13). Similar numbers, 1.8×10^4 CFU, are required for the recently developed stool antigen test (HpSA-EIA) (3). The HpSA-EIA is another attractive, noninvasive fecal test that seems to predict *H. pylori* status reliably (23), but it has been less suitable for evaluating the outcome of eradication therapy (22).

Another variable that may limit routine clinical use of fecal assays is the fact that *H. pylori* proteins or DNA, to be tested, have to pass unharmed through the entire gastrointestinal tract despite exposure to numerous digestive enzymes and bacteria. Furthermore, it is unknown how many *H. pylori* organisms an infected individual excretes, whether there is patchy distribution in a bowel movement, and how long *H. pylori* fragments remain in the intestinal tract after eradication therapy. Two recent studies showed that there was still significant detection of *H. pylori* debris by PCR as well as EIA at a 1-month follow-up for patients from whom *H. pylori* had been eradicated (8, 22). Hence, more data on the intestinal elimination kinetics of *H. pylori* are necessary in order to use molecular or immunoassay tests for evaluation of eradication treatment.

In conclusion, the *icd* gene assay has been shown to allow differentiation of *H. pylori* from seven other *Helicobacter* species. This newly designed primer pair may be useful for PCR used in epidemiological studies (P. Grübel, L. Huang, N. Masubuchi, F. J. Stutzenberger, and D. R. Cave, Letter, Lancet **352:**788–789, 1998). In a next step, we will test the *icd* primers on feces from *H. pylori*-positive patients to evaluate their potential for clinical application.

ACKNOWLEDGMENTS

The support given by C. Mepani, N. Stern, and F. J. Stutzenberger in providing the bacterial control specimen and by Z. Shen and J. G. Fox in providing the *Helicobacter* species is gratefully acknowledged.

REFERENCES

1. **Anonymous.** 1994. NIH Consensus Conference. *Helicobacter pylori* in peptic ulcer disease. NIH Consensus Development Panel on *Helicobacter pylori* in Peptic Ulcer Disease. JAMA **272:**65–69.
2. **Bazzoli, F., M. Zagari, S. Fossi, P. Pozzato, L. Ricciardiello, C. Mwangemi, A. Roda, and E. Roda.** 1997. Urea breath tests for the detection of *Helicobacter pylori* infection. Helicobacter **2**(Suppl. 1)**:**S34–S37.
3. **Grübel, P., R. W. Frenck, D. H. Willis, P. J. Goddard, R. R. Abu-Elyazeed, A. Naficy, and D. R. Cave.** 1999. HpSA enzyme immunoassay for detection of *H. pylori* from stool samples collected from a field setting in Egypt. Gastroenterology **116:**G0772. (Abstract.)
4. **Grübel, P., J. S. Hoffman, F. K. Chong, N. A. Burstein, C. Mepani, and D. R. Cave.** 1997. Vector potential of houseflies (*Musca domestica*) for *Helicobacter pylori*. J. Clin. Microbiol. **35:**1300–1303.
5. **Grübel, P., and D. R. Cave.** 1998. Sanitation and houseflies (*Musca domestica*): factors for the transmission of *Helicobacter pylori*. Bull. Inst. Pasteur **96:**83–91.
6. **Huang, L. L., D. R. Cave, and A. Wright.** 1996. Sequencing and expression of the gene encoding a novel protein with gastric acid inhibitory properties from *Helicobacter pylori*. Gut **39:**A66. (Abstract.)
7. **Kelly, S. M., M. C. Pitcher, S. M. Farmery, and G. R. Gibson.** 1994. Isolation of *Helicobacter pylori* from feces of patients with dyspepsia in the United Kingdom. Gastroenterology **107:**1671–1674.
8. **Makristathis, A., E. Pasching, K. Schutze, M. Wimmer, M. L. Rotter, and**

A. M. Hirschl. 1998. Detection of *Helicobacter pylori* in stool specimens by PCR and antigen enzyme immunoassay. J. Clin. Microbiol. **36:**2772–2774.
9. **Mapstone, N. P.** 1993. PCR identification of *Helicobacter pylori* in faeces from gastritis patients. Lancet **341:**447.
10. **Mapstone, N. P.** 1997. The detection of *Helicobacter pylori* by the polymerase chain reaction, p. 31–36. *In* C. L. Clayton and H. L. T. Mobley (ed.), *Helicobacter pylori* protocols. Humana Press, Totowa, N.J.
11. **Monteiro, L., C. Birac, and F. Megraud.** 1996. Detection of *Helicobacter pylori* in gastric biopsies by polymerase chain reaction, p. 112–120. *In* A. Lee and F. Megraud (ed.), *Helicobacter pylori*: techniques for clinical diagnosis and basic research. W. B. Saunders Ltd., London, England.
12. **Monteiro, L., D. Bonnemaison, A. Vekris, K. G. Petry, J. Bonnet, R. Vidal, J. Cabrita, and F. Megraud.** 1997. Complex polysaccharides as PCR inhibitors in feces: *Helicobacter pylori* model. J. Clin. Microbiol. **35:**995–998.
13. **Osaki, T., H. Taguchi, H. Yamaguchi, and S. Kamiya.** 1998. Detection of *Helicobacter pylori* in fecal samples of gnotobiotic mice infected with *H. pylori* by an immunomagnetic-bead separation technique. J. Clin. Microbiol. **36:** 321–323.
14. **O'Toole, P. W., S. M. Logan, M. Kostrzynska, T. Wadstrom, and T. J. Trust.** 1991. Isolation and biochemical and molecular analyses of a species-specific protein antigen from the gastric pathogen *Helicobacter pylori*. J. Bacteriol. **173:**505–513.
15. **Sanger, F., S. Nicklen, and A. R. Coulson.** 1977. DNA sequencing with chain-terminating inhibitors. Proc. Natl. Acad. Sci. USA **74:**5463–5467.
16. **Scholte, G. H.** 1997. Polymerase chain reaction for the detection of *Helicobacter pylori* in formaldehyde-sublimate fixed, paraffin-embedded gastric biopsies. Diagn. Mol. Pathol. **6:**238–243.
17. **Sidhu, H., M. Allison, and A. B. Peck.** 1997. Identification and classification of *Oxalobacter formigenes* strains by using oligonucleotide probes and primers. J. Clin. Microbiol. **35:**350–353.
18. **Stacy-Phipps, S., J. J. Mecca, and J. B. Weiss.** 1995. Multiplex PCR assay and simple preparation method for stool specimens detect enterotoxigenic *Escherichia coli* DNA during course of infection. J. Clin. Microbiol. **33:**1054–1059.
19. **Thomas, J.** 1996. Culture of *Helicobacter pylori* from feces, p. 206–212. *In* A. Lee and F. Megraud (ed.), *Helicobacter pylori*: techniques for clinical diagnosis and basic research. W. B. Saunders Ltd., London, England.
20. **Thomas, J. E., G. R. Gibson, M. K. Darboe, A. Dale, and L. T. Weaver.** 1992. Isolation of *Helicobacter pylori* from human faeces. Lancet **340:**1194–1195.
21. **Tomb, J. F., O. White, A. R. Kerlavage, R. A. Clayton, G. G. Sutton, R. D. Fleischmann, K. A. Ketchum, H. P. Klenk, S. Gill, B. A. Dougherty, K. Nelson, J. Quackenbush, L. Zhou, E. F. Kirkness, S. Peterson, B. Loftus, D. Richardson, R. Dodson, H. G. Khalak, A. Glodek, K. McKenney, L. M. Fitzegerald, N. Lee, M. D. Adams, and J. C. Venter.** 1997. The complete genome sequence of the gastric pathogen *Helicobacter pylori*. Nature **388:** 539–547. (Erratum, **389:**412.)
22. **Trevisani, L., S. Sartori, F. Galvani, M. R. Rossi, M. Ruina, C. Chiamenti, and M. Caselli.** 1999. Evaluation of a new enzyme immunoassay for detecting *Helicobacter pylori* in feces: a prospective pilot study. Am. J. Gastroenterol. **94:**1830–1833.
23. **Vaira, D., P. Malfertheiner, F. Megraud, A. T. Axon, M. Deltenre, A. M. Hirschl, G. Gasbarrini, C. O'Morain, J. M. Garcia, M. Quina, and G. N. Tytgat.** 1999. Diagnosis of *Helicobacter pylori* infection with a new noninvasive antigen-based assay. HpSA European Study Group. Lancet **354:**30–33.
24. **van Zwet, A. A., J. C. Thijs, A. M. Kooistra-Smid, J. Schirm, and J. A. Snijder.** 1994. Use of PCR with feces for detection of *Helicobacter pylori* infections in patients. J. Clin. Microbiol. **32:**1346–1348.
25. **Weiss, J., J. Mecca, E. da Silva, and D. Gassner.** 1994. Comparison of PCR and other diagnostic techniques for detection of *Helicobacter pylori* infection in dyspeptic patients. J. Clin. Microbiol. **32:**1663–1668.
26. **Wu, L., J. Coombs, S. Malmstrom, and M. Glass.** 1997. Simultaneous multianalyte nucleic acid detection for gastrointestinal bacterial pathogens using GeneSTAR technology. Clin. Lab. Med. **17:**129–145.
27. **Zagari, R. M., F. Bazzoli, P. Pozzato, S. Fossi, L. De Luca, G. Nicolini, D. Berretti, and E. Roda.** 1999. Non-invasive methods for the diagnosis of *Helicobacter pylori* infection. Ital. J. Gastroenterol. Hepatol. **31:**408–415.