JOURNAL OF CLINICAL MICROBIOLOGY, Mar. 1996, p. 530–533 Vol. 34, No. 3
0095-1137/96/$04.00+0

Rapid Colorimetric Hybridization Assay for Detecting Amplified *Helicobacter pylori* DNA in Gastric Biopsy Specimens

ANDREY P. LAGE,[1] ALAN FAUCONNIER,[1] ALAIN BURETTE,[2] YOURI GLUPCZYNSKI,[3] ALEX BOLLEN,[1] AND EDMOND GODFROID[1]*

Service de Génétique Appliquée, Université Libre de Bruxelles, B 1400 Nivelles,[1] Gastroenterology Unit, Nouvelle Clinique de la Basilique, B 1080 Brussels,[2] and Clinical Microbiology, Centre Hospitalier A. Vésale, B 6110 Montigny-le-Tilleul,[3] Belgium

Received 1 August 1995/Returned for modification 2 October 1995/Accepted 27 November 1995

A very simple, practical, sensitive, and specific colorimetric hybridization assay for detecting amplified *Helicobacter pylori* DNA is described. This assay, which combines a sensitive sandwich DNA hybridization reaction and a colorimetric protocol similar to those used in conventional enzyme immunoassays, was shown to be suitable for detecting *H. pylori*-infected gastric biopsy specimens and for monitoring the eradication of the pathogen after treatment. The specificity and sensitivity of the colorimetric hybridization assay were tested by assaying 27 *H. pylori* strains (4 reference and 23 clinical isolates), 9 strains of other *Helicobacter* spp. or *Campylobacter* spp., and 11 clinical isolates of other urease-positive bacteria. The likelihood of *H. pylori* detection in gastric biopsy specimens by the colorimetric hybridization assay was evaluated with 23 *H. pylori*-positive and 41 *H. pylori*-negative biopsy specimens on the basis of positive and negative results, respectively, of culture, rapid urease test, histological examination, and PCR. Biopsy specimens from 33 treated patients, endoscopied 4 to 8 weeks after the end of treatment, were also tested. All *H. pylori* strains showed positive results in the colorimetric hybridization assay, presenting optical densities at 450 nm (OD_{450}s) of ≥3.0. None of the other *Helicobacter* spp., *Campylobacter* spp., or the clinical isolates of other urease-positive bacteria showed OD_{450}s equal to or greater than the cutoff (mean OD_{450} cutoff, 0.208). The colorimetric hybridization assay detected all 23 *H. pylori*-positive biopsy specimens (mean OD_{450}, 2.910 ± 0.295), while none of the *H. pylori*-negative biopsy specimens was shown to be positive in the assay (mean OD_{450}, 0.108 ± 0.025). *H. pylori* was considered to be not eradicated from three of the posttreatment biopsy specimens by culture, rapid urease test, histological examination, and PCR. They were all positive by the colorimetric hybridization assay, and their OD_{450}s were ≥3.0. The colorimetric hybridization assay also detected two other *H. pylori*-positive patients. Specimens from these two patients had negative culture, rapid urease test, and histology results, and a specimen from one of them also tested negative by PCR. These results indicate that the colorimetric hybridization assay is a suitable method both for the diagnosis of *H. pylori* in biopsy specimens and for the follow-up of patients after the end of treatment.

Helicobacter pylori is a microaerophilic gram-negative bacterium which infects the gastric mucosa and which is one of the most common chronic bacterial infections worldwide (29). Infection with this bacterium is associated with various gastric pathologies in all age groups, including gastritis, peptic ulcer, and gastric cancer (3, 7). Various antimicrobial regimens have been used to treat *H. pylori* infections, and successful eradication of this bacterium was shown to prevent the relapse of duodenal and gastric ulcers (15, 27).

In this respect, it is important to rely on accurate methods for the diagnosis of *H. pylori* infection, which not only would be useful in the detection of *H. pylori*-infected patients but also would be suitable for monitoring the eradication of the pathogen after treatment. The routine invasive diagnostic assays for *H. pylori* consist of culture, rapid urease test (RUT), and histological examination of gastric biopsy sections (11, 13). Although these methods achieve good sensitivities and specificities in detecting *H. pylori* before treatment, they have decreased levels of performance in monitoring the follow-up of treatment because of the decreased number of bacteria and the presence of coccoid forms (11, 21, 25). In addition, urea breath test and serology provide two noninvasive assays useful for short- and long-term treatment follow-up, respectively, completing the panel of available systems (11, 13). In the last few years, molecular techniques that are based on nucleic acid hybridization and PCR and that target different *H. pylori* genes have been described (2, 5, 14, 20, 31, 33).

More recently, several investigators have presented sensitive assays for the diagnosis of various viral, bacterial, fungal, and protozoal infections on the basis of nonradioactive (colorimetric or chemiluminescent) nucleic acid hybridization (1, 8, 9, 18, 19). Herein, we describe a fast, sensitive, specific, very simple, and prone-to-automation colorimetric hybridization assay for detecting amplified *H. pylori* DNA and show that it is suitable for detecting *H. pylori*-infected patients and for monitoring the eradication of the pathogen after treatment.

MATERIALS AND METHODS

Bacterial strains. The following reference bacteria were used in the study: *H. pylori* NCTC 11637^T, *H. pylori* ATCC 49503, *H. pylori* ATCC 53726, *H. pylori* Tx30A, *Helicobacter muridarum* LMG 13646, *Helicobacter mustelae* NCTC 12031, *Helicobacter fennelliae* NCTC 11612^T, *Helicobacter cinaedi* ATCC 35683^T, *Campylobacter jejuni* subsp. *jejuni* NCTC 11351^T, *Campylobacter jejuni* subsp. *doylei* NCTC 11951, *Campylobacter coli* NCTC 11366^T, *Campylobacter lari* NCTC

* Corresponding author. Mailing address: Service de Génétique Appliquée, Université Libre de Bruxelles, 24, Rue de l'Industrie, B 1400 - Nivelles - Belgium. Phone: 32 67 88 94 63. Fax: 32 67 88 94 77. Electronic mail address: godfroid@sga.ulb.ac.be.

11352^T, and a urease-positive thermophilic campylobacter, *C. lari* J116. In addition, 23 clinical *H. pylori* strains, as well as clinical isolates of other urease-positive bacteria (6 *Proteus mirabilis* and 5 *Klebsiella pneumoniae* isolates) from a previous study (17) were used. Culture and storage conditions were as described previously (17).

Clinical samples. The present study encompassed two groups of patients. Patients in group A were randomly selected among nontreated patients from a previous study (17). Biopsy specimens from 23 *H. pylori*-infected patients showing positive results by PCR, culture, RUT, and histological examination and 41 *H. pylori*-negative patients with negative results by all four tests were analyzed.

Patients in group B consisted of 33 peptic ulcer patients who had received antimicrobial therapy for *H. pylori* eradication (omeprazole, 20 mg twice daily, and amoxicillin, 1 g twice daily for 14 days, or the same regimen plus clarithromycin, 500 mg three times daily for 12 days). Four to 8 weeks after the end of treatment, patients underwent endoscopy, and biopsy specimens were taken to evaluate the efficacy of the treatment.

Six antrum and three corpus biopsy specimens were collected from each patient for RUT (1 antrum biopsy specimen), histology (3 antrum and 2 corpus biopsy specimens), culture (1 antrum and 1 corpus biopsy specimens), and PCR (1 antrum biopsy specimen).

Routine diagnostic tests. Culture was done on gastric biopsy specimens ground at 10,000 rpm for 15 s with an electric tissue homogenizer (Ultraturrax, Iena, Germany) before inoculation onto selective Columbia blood agar and incubation under microaerophilic conditions at 37°C for up to 7 days (10). Organisms were identified as *H. pylori* on the basis of morphology by Gram staining and by oxidase and catalase tests and RUT (30).

Paraffin-embedded tissue sections were stained with hematoxylin and eosin to grade the severity of gastritis and with Giemsa stain to detect microorganisms with a morphology typical of that of *H. pylori*. The classification and grading of gastritis were made in accordance with the Sydney system (24).

For the RUT, one antrum biopsy specimen was introduced with a sterile needle into a semisolid 2% urea agar, and the agar was incubated at room temperature. Results were recorded for up to 4 h after inoculation (10).

Preparation of samples for PCR amplification. Bacterial genomic DNA was extracted from all strains by the guanidium thiocyanate method (23). The DNA pellet was dissolved in water to a concentration of 25 μg/ml. DNAs from the biopsy specimens were prepared by the alkaline-thermal method (17). Briefly, biopsy specimens were suspended in 0.5% *N*-acetyl-L-cysteine (Sigma, St. Louis, Mo.), vortexed for 5 min, and incubated for an additional 5 min. After centrifugation (17,000 × *g*, 15 min) and discarding of the supernatant, biopsy specimens were resuspended in 100 μl of distilled water. A total of 100 μl of 50 mM of NaOH was added, and the mixtures were overlaid with light mineral oil (Sigma). The tubes were vortexed vigorously, briefly spun down, and heated to 100°C for 10 min in a water bath. After cooling, the mixtures were neutralized by the addition of 16 μl of 1 M Tris-HCl (pH 7.5). The samples were stored at 4°C until they were tested.

Synthetic primers and probes. Oligonucleotide primers and probes were synthesized via the solid-phase phosphoramidite method on a 394 DNA-RNA synthesizer (Applied Biosystems, Foster City, Calif.) and were purified as described by the manufacturer. The probe used for detection was polyaminated and multibiotinylated (eight biotins) as described recently (6).

Primer 93275 (5′-AAGCTTTTAGGGGTGTTAGGGGTTT-3′) and primer 93276 (5′-AAGCTTACTTTCTAACACTAACGC-3′) derived from the *H. pylori ureC* gene sequence (16) (accession numbers, EMBL X57132 and GenBank M60398, respectively) amplify a 294-bp DNA fragment and have already been tested for use in the diagnosis of *H. pylori* infection (2, 17).

The capture probe 95045 (5′-GCGCGATTGGGGATAAGTTTGTGAGCG-3′) and the detection probe 94216 (5′-CCGGCGATGGCTTGGTGTGCGC-3′) were internal to the amplified *ureC* fragment.

DNA amplification and analysis of amplified products. Amplifications were performed in a final volume of 50 μl containing 0.4 μM (each) primer, 0.2 mM (each) deoxynucleotide (dATP, dGTP, dTTP, and dCTP; Boehringer Mannheim GmbH, Mannheim, Germany), and reaction buffer (100 mM Tris-HCl, 500 mM KCl, 15 mM $MgCl_2$, and 0.01% [wt/vol] gelatin [pH 8.3]), and one drop of light mineral oil (Sigma) was overlaid before the addition of 10 μl of test sample. The mixture was denatured at 94°C for 10 min and was cooled on ice, and 2.5 U of *Taq* DNA polymerase (Boehringer) was added prior to 35 amplification cycles (2-min denaturing step at 94°C, 2-min annealing step at 55°C, and 2-min elongation step at 72°C). After the amplification cycles, a final cycle comprising a 1-min step at the specified annealing temperature and a 10-min elongation step at 72°C was performed (16, 17). DNAs from *H. pylori* ATCC 53726 and a tube containing water in place of DNA were assayed in each PCR run as positive and negative controls of the reaction, respectively. To avoid cross-contaminations, mixture preparation, sample handling, PCR amplification, and gel detection of amplified material were done in separate rooms.

Ten microliters of each PCR mixture was subjected to gel electrophoresis (2% agarose) and ethidium bromide staining for the detection of amplified DNA products (26).

Colorimetric hybridization assay. The capture probe 95045 was covalently linked to polystyrene microplates (Polymer Laboratories, Birmingham, United Kingdom) by chemical condensation (6). Oligonucleotide-coated plates were incubated at room temperature for 10 min with buffer 1 (0.1 M Tris-HCl, 1 M NaCl, 2 mM $MgCl_2$, 0.05% Triton X-100 [pH 7.5]) containing 5% nonfat dried milk prior to use.

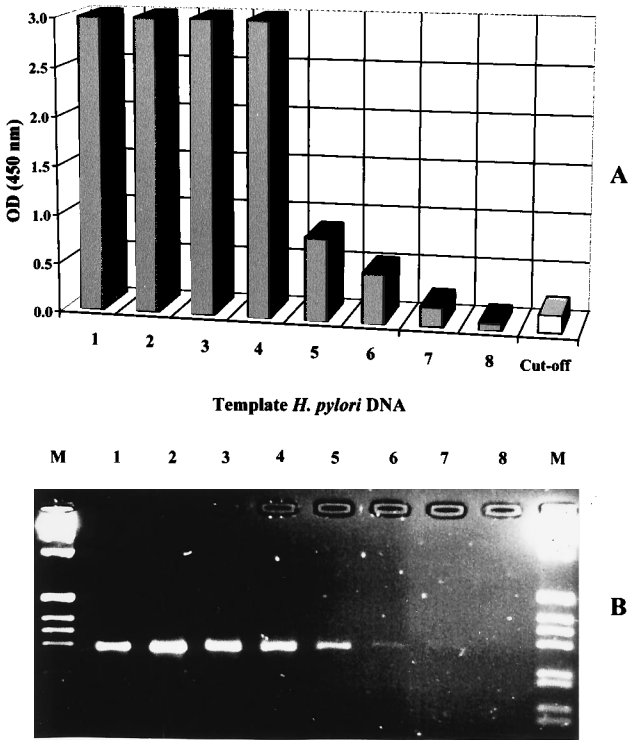

FIG. 1. Detection of amplified *H. pylori* DNA by the colorimetric hybridization assay (A) and agarose gel electrophoresis (B). Samples (lanes) 1 to 8 correspond to 10-fold dilutions, starting at 1.8 ng, of DNA from *H. pylori* ATCC 49503 introduced as template in the PCR. The cutoff value was defined as 3 standard deviations above the mean for the blank PCR mixtures. Lane M, 1-kb DNA ladder.

Twenty-five microliters of each PCR mixture was added to 100 μl of 0.25 M NaOH, and the mixture was incubated at room temperature for 5 min to denature double-stranded DNA. Fifty microliters of denatured PCR products was added to a well previously filled with 50 μl of hybridization buffer (buffer 1 plus 0.2 M acetic acid) containing 80 nM biotinylated capture probe. Each PCR mixture was tested in duplicate. Hybridization took place at 37°C under mild agitation for 90 min. After hybridization, the plates were washed five times in buffer 1 containing 5% nonfat dried milk. One hundred microliters of buffer 1 containing 3% bovine serum albumin (Sigma) and streptavidin-horseradish peroxidase (DAKO, Copenhagen, Denmark), diluted 1,000-fold, was added to each well, and the plates were incubated at room temperature for 15 min. Then, the plates were washed five times with buffer 1 prior to the addition of 200 μl of 3,3′,5,5′-tetramethylbenzidine (Sigma) to each well. After 15 min of incubation at room temperature, the reaction was stopped with 100 μl of 5% sulfuric acid per well, and the optical density (OD) was read at 450 nm (OD_{450}) in a Novapath microplate reader (Bio-Rad, Richmond, Calif.).

A blank PCR mixture (a PCR mixture containing water in place of DNA) was tested in six wells in each assay run. The cutoff value was defined as three standard deviations above the mean for the blank PCR mixtures.

RESULTS

Sensitivity and specificity of the colorimetric hybridization assay. The sensitivity of the colorimetric hybridization assay was evaluated by using amplification products from PCR assays performed with serial 10-fold dilutions of DNA from *H. pylori* ATCC 49503 (starting template concentration, 180 pg/μl). Both agarose gels stained with ethidium bromide (Fig. 1B) and the colorimetric hybridization assay (Fig. 1A) detected up to 1.8 fg of template *H. pylori* DNA, although only a very faint band was shown in agarose gels. This roughly corresponds to one *H. pylori* genome (28). The same level of sensitivity was observed when PCR amplification was performed in the pres-

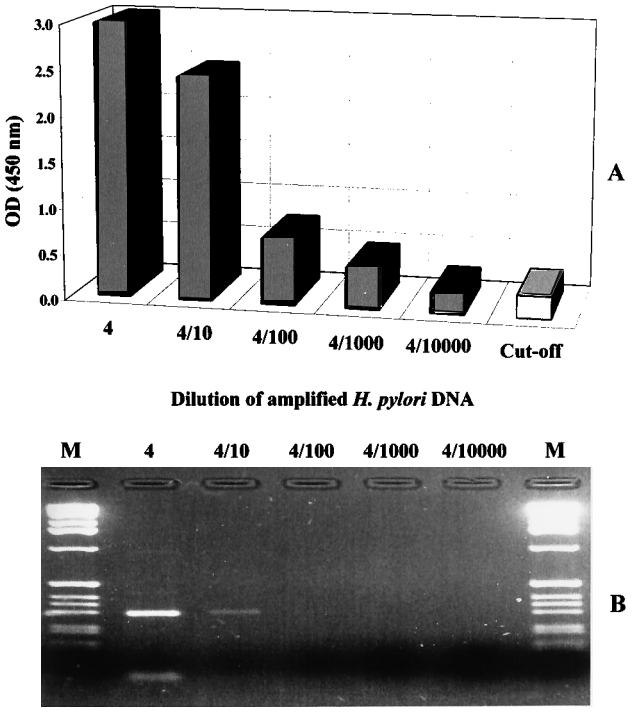

FIG. 2. Detection of 10-fold-diluted amplified DNA from *H. pylori* ATCC 49503 by the colorimetric hybridization assay (A) and agarose gel electrophoresis (B). Dilutions from a reaction in which 1.8 pg of *H. pylori* DNA was used as the template (Fig. 1, tube 4) were made up to 10,000-fold. The cutoff value was defined as three standard deviations above the mean for the blank PCR mixtures. Lane M, 1-kb DNA ladder.

ence of *H. pylori*-negative gastric biopsy specimens from different patients (data not shown). Furthermore, the sensitivity of the assay was also estimated with serial 10-fold dilutions of amplified DNA from a reaction in which 1.8 pg of *H. pylori* DNA was used as the template (Fig. 1, tube 4). The detection limit on agarose gels was 1/10 dilution (Fig. 2B), whereas the colorimetric hybridization assay detection limit reached 1/1,000 dilution (Fig. 2A), thus being 100-fold more sensitive than gel detection of the PCR products.

The specificity of the colorimetric assay was assessed by using 27 *H. pylori* strains (4 reference strains and 23 clinical isolates), 9 reference strains from various other *Helicobacter* spp. and *Campylobacter* spp., as well as clinical isolates of various urease-positive bacteria (6 *P. mirabilis* and 5 *K. pneumoniae* isolates). All *H. pylori* strains had an OD_{450} of ≥3.0. In contrast, the mean OD_{450}s were only 0.107 ± 0.013 (range, 0.083 to 0.125) and 0.132 ± 0.036 (range, 0.091 to 0.150) for the reference strains of other *Helicobacter* spp. and *Campylobacter* spp. and for the clinical isolates of other urease-positive bacteria, respectively. The average OD_{450} cutoff for these assays, as defined in the Materials and Methods section, was 0.208.

Colorimetric detection of *H. pylori*-amplified DNA in biopsy specimens from patients with defined *H. pylori* status. The colorimetric hybridization assay was able to identify all *H. pylori*-infected biopsy specimens. None of the 41 *H. pylori*-negative biopsy specimens showed OD_{450}s above the cutoff. The mean OD_{450}s were 2.910 ± 0.295 (range, 1.705 to ≥3.0) and 0.108 ± 0.025 (range, 0.083 to 0.140) for the *H. pylori*-positive and the *H. pylori*-negative biopsy specimens, respectively, with an average cutoff of 0.222.

TABLE 1. *H. pylori* detection in 33 patients after treatment

Result by:					No. of patients
Culture	RUT	Histological examination[a]	PCR (gel)	Hybridization assay	
+	+	+	+	+	3
−	−	−	+	+	1
−	−	−	−	+	1
−	−	−	−	−	28

[a] Presence of bacteria with typical *H. pylori* morphology.

Colorimetric detection of *H. pylori*-amplified DNA in biopsy specimens from treated patients. Of the 33 treated patients, *H. pylori* was considered to be not eradicated from 3 patients by the standard methods and by PCR (Table 1). In the colorimetric hybridization assay, the OD_{450}s of the specimens from these patients were ≥3.0. In addition, the specimen from only one patient tested positive by PCR on gels and the colorimetric hybridization assay (OD_{450}, 0.931), while the specimen from another patient was negative by the routine diagnostic tests as well as PCR detection on gels, but it tested positive by the colorimetric assay (OD_{450}, 0.237 versus 0.201 for the cutoff). The average OD_{450} cutoff was 0.210, and the mean OD_{450} for the *H. pylori*-negative biopsy specimens was 0.101 ± 0.023 (range, 0.070 to 0.149).

DISCUSSION

We have described here a simple, practical, reliable, and sensitive colorimetric hybridization assay for the detection of amplified *H. pylori* DNA. This assay combines a sensitive sandwich DNA hybridization reaction and a colorimetric protocol, similar to those of conventional enzyme immunoassays which are widely used in routine clinical microbiology laboratories. It is based on a new phosphoramidite chemistry which enables multilabeling of the detection probe and the covalent coupling of the capture probe to polystyrene microplates (6).

The colorimetric hybridization assay proved to be more sensitive than gel detection of PCR products. It detected amplified DNA starting with 1.8 fg of template *H. pylori* DNA, i.e., approximately one *H. pylori* genome (28), and had a 100 times lower threshold limit than the gel detection limit. The increased sensitivity of the hybridization assays compared with that of gel detection of PCR products has already been observed by investigators who have used radioactive probes (5).

Although the *ureC* primers used in the PCR already offer a high degree of specificity for *H. pylori* (2, 17), the colorimetric hybridization assay provides additional specificity since it uses capture and detection probes internal to the amplified *ureC* product.

The results of the colorimetric hybridization assay for detecting *H. pylori* in gastric biopsy specimens were shown to be very sensitive, specific, and clear-cut. The OD_{450}s for the *H. pylori*-infected biopsy specimens were at least sevenfold above the average cutoff, while the mean OD_{450} for the *H. pylori*-negative biopsy specimens was lower than the cutoff in all cases.

One of the most promising applications of the colorimetric hybridization assay is in the short-term (4 to 6 weeks) follow-up after treatment, a condition in which the routine diagnostic tests may not always perform well.

In fact, in the present series of 33 treated patients who were examined 4 to 8 weeks after the end of treatment, the colorimetric hybridization assay was able to detect 2 patients from

whom *H. pylori* was not eradicated but who were negative by the routine diagnostic assays. One of them was PCR negative by gel detection, confirming the higher degree of sensitivity of the colorimetric hybridization assay compared with that of PCR detection on gels. Although the moderately low OD_{450}s of specimens from these patients (only a few units above the cutoff for one of them) could suggest false-positive results, the presence of chronic active gastritis (12) in both patients and the precautions taken to avoid sample contamination suggest that *H. pylori* has not been eradicated from these patients. The reduction in the number of bacteria and the presence of coccoid forms induced by the antimicrobial agents could account for the failure of the culture to detect *H. pylori* shortly after the end of treatment (4, 22), although culture has been shown in one study (32) to be as sensitive as PCR 3 months after treatment. Furthermore, RUT and histological examination have been described to have decreased sensitivities when used in treatment follow-up (21, 25).

In view of its performance, the colorimetric hybridization assay could constitute a method of choice for short-term treatment follow-up and is advantageous with respect to the ^{14}C-urea breath test, which uses radioisotopes, and to the expensive ^{13}C-urea breath test, for which special facilities are still required (11, 13). Furthermore, for the colorimetric hybridization assay special conditions for the conservation and transport of the biopsy specimens are not needed (10, 14) and the assay could be performed in centers where culture is not available.

In conclusion, the colorimetric hybridization assay is a very simple, sensitive, and specific assay for the detection of *H. pylori*. It can be used on gastric biopsy specimens and is suitable for short-term follow-up. Its similarity to the widely used enzyme-linked immunoassays is a characteristic that makes the colorimetric hybridization assay familiar to any clinical microbiology laboratory and enables automation of the assay. Furthermore, it can be easily adapted to a chemiluminescent detection format and can be applied to the detection of other pathogens (8).

ACKNOWLEDGMENTS

This study was supported in part by a grant from the Belgian *Helicobacter pylori* Contact Group. A.P.L. is indebted to the National Research Council (CNPq), Brazil, for a Ph.D. scholarship. A.F. is supported by the First-University Program of the Région Wallonne of Belgium.

REFERENCES

1. **Ayyanathan, K., and S. Datta.** 1995. A nonradioactive DNA diagnostic procedure for the detection of malarial infection: general application to genome with repetitive sequences. Mol. Cell. Probes **9:**83–89.
2. **Bickley, J., R. J. Owen, A. G. Fraser, and R. E. Pounder.** 1993. Evaluation of the polymerase chain reaction for detecting the urease C gene of *Helicobacter pylori* in gastric biopsy samples and dental plaque. J. Med. Microbiol. **39:**338–344.
3. **Blaser, M. J., and J. Parsonnet.** 1994. Parasitism by the "slow" bacterium *Helicobacter pylori* leads to altered gastric homeostasis and neoplasia. J. Clin. Invest. **94:**4–8.
4. **Cellini, L. N. Allocati, E. di Dampi, and B. Dainelli.** 1994. *Helicobacter pylori*: a fickle germ. Microbiol. Immunol. **38:**25–30.
5. **Clayton, C. L., H. Kleanthous, P. J. Coates, D. D. Morgan, and S. Tabaqchali.** 1992. Sensitive detection of *Helicobacter pylori* by using polymerase chain reaction. J. Clin. Microbiol. **30:**192–200.
6. **de Vos, M.-J., A. van Elsen, and A. Bollen.** 1994. New non nucleosidic phosphoramidites for the solid phase multi-labelling of nucleotides: comb- and multifork-like structures. Nucleosides Nucleotides **13:**2245–2265.
7. **Eurogast Study Group.** 1993. An international association between *H. pylori* infection and gastric cancer. Lancet **341:**1359–1362.
8. **Fauville-Defaux, M., N. Maes, E. Severin, C. Farin, E. Serruys, M. Struelens, N. Younes, J.-P. Vincke, M.-J. de Vos, A. Bollen, and E. Godfroid.** 1995. Rapid identification of *Mycobacterium xenopi* from bacterial colonies or "Bactec" culture by the polymerase chain reaction and a luminescent sandwich hybridization assay. Res. Microbiol. **146:**349–356.
9. **Fujita, S.-I., B. A. Lasker, T. J. Lott, E. Reiss, and C. J. Morrison.** 1995. Microtitration plate enzyme immunoassay to detect PCR-amplified DNA from *Candida* species in blood. J. Clin. Microbiol. **33:**962–967.
10. **Glupczynski, Y.** 1993. Ph.D. thesis. Université Libre de Bruxelles, Brussels, Belgium.
11. **Glupczynski, Y.** 1994. The diagnosis of *Helicobacter pylori* infection: a microbiologist's perspective. Rev. Med. Microbiol. **5:**199–208.
12. **Glupczynski, Y., A. Burette, M. Labbé, C. de Prez, M. Dereuck, and M. Deltenre.** 1988. *Campylobacter pylori* associated-gastritis: a double-blind placebo controlled trial with amoxycillin. Am. J. Gastroenterol. **83:**365–372.
13. **Glupczynski, Y., and A. M. Hirschl.** 1994. The diagnosis of *Helicobacter pylori*. Curr. Opin. Gastroenterol. **10**(Suppl. 1)**:**12–16.
14. **Hammar, M., T. Tyszkiewicz, T. Wadström, and P. W. O'Toole.** 1992. Rapid detection of *Helicobacter pylori* in gastric biopsy material by polymerase chain reaction. J. Clin. Microbiol. **30:**54–58.
15. **Hentschel, E., G. Brandstatter, B. Dragosics, A. M. Hirschl, H. Nemec, K. Schutze, M. Taufer, and H. Wurzer.** 1993. Effect of ranitidine and ampicillin plus metronidazole on the eradication of *Helicobacter pylori* and the recurrence of duodenal ulcer. N. Engl. J. Med. **328:**308–312.
16. **Labigne, A., V. Cussac, and P. Courcoux.** 1991. Shuttle cloning and nucleotide sequences of *Helicobacter pylori* genes responsible for urease activity. J. Bacteriol. **173:**1920–1931.
17. **Lage, A. P., E. Godfroid, A. Fauconnier, A. Burette, J.-P. Butzler, A. Bollen, and Y. Glupczynski.** 1995. Diagnosis of *Helicobacter pylori* infection by PCR: comparison with other invasive techniques and detection of the *cagA* gene in gastric biopsy specimens. J. Clin. Microbiol. **33:**2752–2756.
18. **Mabilat, C., S. Desvarenne, G. Panteix, N. Machabert, M.-H. Bernillon, G. Guardiola, and P. Cros.** 1994. Routine identification of *Mycobacterium tuberculosis* complex isolates by automated hybridization. J. Clin. Microbiol. **32:**2702–2705.
19. **Mallet, F., C. Hebrard, D. Brant, E. Chapuis, P. Cros, P. Allibert, J. M. Besnier, F. Barin, and B. Mandrand.** 1993. Enzyme-linked oligosorbent assay for detection of polymerase chain reaction-amplified human immunodeficiency virus type 1. J. Clin. Microbiol. **31:**1444–1449.
20. **Mapstone, N. P., D. A. Lynch, F. A. Lewis, A. T. R. Axon, D. S. Tompkins, and M. F. Dixon.** 1993. Identification of *Helicobacter pylori* DNA in the mouth and stomachs of patients with gastritis using PCR. J. Clin. Pathol. **46:**540–543.
21. **Mégraud, F. (Pellegrin University Hospital, Bordeaux, France).** 1995. Personal communication.
22. **Nilius, M., A. Ströle, G. Bode, and P. Malfertheiner.** 1993. Coccoid like forms (CLF) of *Helicobacter pylori*: enzyme activity and antigenicity. Zentralbl. Bakteriol. Parasitenkd. Infektionskr. Hyg. Abt. 1 Orig. **280:**259–272.
23. **Pitcher, D. G., N. A. Saunders, and R. J. Owen.** 1989. Rapid extraction of bacterial genomic DNA with guanidium thiocyanate. Lett. Appl. Microbiol. **8:**151–156.
24. **Price, A. B.** 1991. The Sydney system: histological division. J. Gastroenterol. Hepatol. **6:**209–222.
25. **Rezende, L. M., D. M. M. Queiroz, E. N. Mendes, G. A. Rocha, L. G. Coelho, M. C. Passos, L. P. Castro, C. A. Oliveira, and G. F. Lima, Jr.** 1993. Comparison of the urease test and of the direct smear examination in the control of treatment of *Helicobacter pylori*-induced infection. Braz. J. Med. Biol. Res. **26:**699–702.
26. **Sambrook, J., E. F. Eristisch, and T. Maniatis.** 1989. Molecular cloning: a laboratory manual, 2nd ed. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y.
27. **Seppälä, K., P. Pikkarainen, P. Sipponen, E. Kivilaakso, M. H. Gormsen, and the Finnish Gastric Ulcer Study Group.** 1995. Cure of peptic gastric ulcer associated with erradication of *Helicobacter pylori*. Gut **36:**834–837.
28. **Taylor, D. E., M. Eaton, and N. Chang.** 1992. Construction of a *Helicobacter pylori* genome map and demonstration of diversity at the genome level. J. Bacteriol. **174:**6800–6806.
29. **Thomas, J. E., and S. Pretolani.** 1994. Epidemiology of the infection. Curr. Opin. Gastroenterol. **10**(Suppl. 1)**:**6–11.
30. **Tompkins, D. S.** 1992. Isolation and characteristics of *Helicobacter pylori*, p. 19–28. *In* B. J. Rathbone and R. V. Heatley (ed.), *Helicobacter pylori* and gastroduodenal diseases. Blackwell Scientific Publications, Oxford.
31. **Valentine, J. L., R. R. Arthur, H. L. T. Mobley, and J. Dick.** 1991. Detection of *Helicobacter pylori* by using the polymerase chain reaction. J. Clin. Microbiol. **29:**689–695.
32. **van Zwet, A. A., J. C. Thijs, A. M. D. Kooistra-Smid, J. Schirm, and J. A. M. Snijder.** 1993. Sensitivity of culture compared with that of polymerase chain reaction for detection of *Helicobacter pylori* from antral biopsy samples. J. Clin. Microbiol. **31:**1918–1920.
33. **Westblom, T. U., S. Phadnis, P. Yang, and S. J. Czinn.** 1993. Diagnosis of *Helicobacter pylori* infection by means of a polymerase chain reaction assay for gastric juice aspirates. Clin. Infect. Dis. **16:**367–371.