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Evaluation of Performances of Three DNA Enzyme Immunoassays for Detection of *Helicobacter pylori* PCR Products from Biopsy Specimens

LURDES MONTEIRO,[1]* JOSÉ CABRITA,[2] AND FRANCIS MÉGRAUD[1]

Laboratoire de Bactériologie, Université de Bordeaux 2, Bordeaux, France,[1] *and Faculdade de Farmácia, Lisbon, Portugal*[2]

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PCR is recognized as a promising method for the detection of *Helicobacter pylori* in gastric biopsy specimens. However, detection of PCR products by gel electrophoresis is difficult to implement in routine clinical laboratories. The aim of this study was to compare three new DNA enzyme immunoassays with the standard method in their ability to detect PCR products. The three assays were based on the amplification of a fragment of the *ureC* gene of *H. pylori* and a colorimetric hybridization assay. The first assay (GEN-ETI-K DNA enzyme immunoassay; Sorin, Sallugia, Italy) was based on the hybridization of amplified DNA with a probe bound in microtiter wells and detected with labelled anti-DNA antibody. The second assay (Pylori-prob; Biocode, Sclessin, Belgium) comprised a solid-phase sandwich hybridization system with a specific biotinylated probe being used for detection. Finally, the third assay (PCR enzyme-linked immunosorbent assay; Boehringer, Mannheim, Germany) was based on the hybridization of amplified DNA labelled with digoxigenin as a probe (used as a coating in microtiter wells) and detected with antidigoxigenin-peroxidase as conjugate. The sensitivity of the colorimetric assay was evaluated by using amplification products from PCR assays performed on several 10-fold dilutions of DNA from *H. pylori* CIP 101260, and the specificity was assessed with different urease-positive bacteria. Biopsy specimens from 199 patients were tested; 106 were classified as *H. pylori* positive, and 93 were classified as *H. pylori* negative by culture and/or histological examination as the "gold standard." The receiving operating characteristic curve was used to determine the best cutoff point for each assay. The detection of PCR products by colorimetric hybridization increases the sensitivity up to 100-fold compared to that with gel electrophoresis. The results are rapid (4 h) and easy to interpret and can be automated.

PCR is recognized as a promising method for detection of *Helicobacter pylori* in gastric biopsy specimens (9, 15, 31, 32). After amplification, PCR products are usually detected by gel electrophoresis and ethidium bromide staining. Despite the fact that this method is not considered expensive, it renders PCR difficult to implement in routine laboratories, due to (i) the specific equipment required, (ii) the use of ethidium bromide with its related handling procedures, and (iii) the need to photograph the gel to document the results.

Furthermore, human error can be a problem in the usual detection method with gel electrophoresis due to subjective evaluation of the final product. Doubtful results can occur: more than one band where only one was expected, a diffuse band, or almost no band. In these cases, the results must be confirmed by other techniques, e.g., a Southern blotting procedure, which is difficult to perform, not suitable for the analysis of large numbers of samples or for automation, and therefore not practicable in all laboratories.

Recently, several investigators have presented sensitive assays for the diagnosis of various viral (16, 17, 24, 30, 35), bacterial (3, 5, 7, 8, 20, 22, 23, 28, 33, 37), fungal (14), and protozoal (2) infections on the basis of nonradioactive (colorimetric or chemiluminescent) nucleic acid hybridization. These kinds of techniques are simple, and colorimetry requires only a conventional microtiter spectrophotometric reader and can be readily done on a large scale. Screening and confirmatory tests are performed together because a hybridization step is part of the protocol.

The aim of this study was to compare three new DNA enzyme immunoassays (DEIAs) with the standard method in their ability to detect PCR products. The three assays had in common the amplification of a fragment of the *ureC* gene of *H. pylori* with the primers described by Labigne et al. (18) and a colorimetric hybridization assay, but the amplification protocols were different. The principle of each of these DEIAs was also different. The first (GEN-ETI-K DEIA; Sorin, Sallugia, Italy) was based on the hybridization of amplified DNA with a probe bound in microtiter wells and detection with labelled anti-DNA antibody. It can be used for any kind of microorganism, requiring only the synthesis of a specific probe for the target DNA sequence. The second assay (Pylori-prob; Biocode, Sclessin, Belgium) comprised a solid-phase sandwich hybridization system with a specific biotinylated probe used for detection. This method was elaborated specifically for *H. pylori* detection. The third one (PCR enzyme-linked immunosorbent assay [ELISA]; Boehringer, Mannheim, Germany) was based on the hybridization of amplified DNA labelled with digoxigenin (DIG) with a probe used as a coating in microtiter wells and detection with anti-DIG–peroxidase as conjugate. As in the GEN-ETI-K DEIA, it can be used for any kind of microorganism once the specific probe has been synthesized for the target DNA sequence.

* Corresponding author. Mailing address: Laboratoire de Bactériologie, Hôpital Pellegrin, Place Amélie Raba Léon, 33076 Bordeaux Cedex, France. Phone: (33) 5 56 79 59 77. Fax: (33) 5 56 79 60 18. E-mail: bactenf@labhel.u-bordeaux2.fr.

MATERIALS AND METHODS

Bacterial strains. One reference *H. pylori* strain, CIP 101260; three clinical isolates of urease-positive bacteria (*Proteus mirabilis*, *Klebsiella pneumoniae*, and *Haemophilus influenzae*); one urease-positive thermophilic *Campylobacter* strain,

TABLE 1. Conditions for DNA amplification for each assay

Assay	Amt of buffer	$MgCl_2$ concn (mM)	dNTP[a] concn (mM)					Primer concn (μM)	Amt of *Taq* polymerase (U)	Test sample size (μl)	Final vol (μl)	No. of cycles (time [min]/temp [°C])
			dATP	dCTP	dGTP	dTTP	DIG-dUTP					
GEN-ETI-K DEIA	1×	1.5	0.2	0.2	0.2	0.2		1	1	5	50	1 (5/94, 1/50, 1/72) 39 (1/94, 1/50, 1/72) 1 (1/94, 1/50, 5/72)
Pylori-prob	1×	1.5	0.2	0.2	0.2	0.2		0.4	2.5	5	50	1 (3/94) 35 (1/94, 1/55, 0.5/72) 1 (1/94, 1/50, 10/72)
PCR ELISA	1×	1.0	0.2	0.2	0.2	0.19	0.01	0.25	2.5	5	50	1 (5/94, 1/50, 1/72) 1 (1/94, 1/50, 1/72) 39 (1/94, 1/50, 1/72) 1 (1/94, 1/50, 5/72)

[a] dNTP, deoxynucleoside triphosphate.

Campylobacter lari bv. UPTC (26); and one strain of *Helicobacter felis* were used to test the sensitivity and specificity of these methods.

Clinical samples. Biopsy specimens from 98 patients not having received therapy were obtained at the time of endoscopy. They were classified as *H. pylori* positive (72 specimens) and *H. pylori* negative (26 specimens) based on culture and/or histological examination as the "gold standard." For those which were positive for *H. pylori* upon histological examination only, the presence of histologically evident gastritis was considered to be a confirmation of *H. pylori* infection.

In addition, biopsy specimens from 101 patients having received eradication therapy were also tested 4 to 6 weeks after the end of treatment. For 94 patients, the therapy consisted of lansoprazole (30 mg twice a day [b.i.d.]), amoxicillin (1 g b.i.d.), and metronidazole (500 mg b.i.d.) for 10 days. For the remaining seven patients, the therapy consisted of nitazoxanide (500 mg b.i.d.) and omeprazole (20 mg b.i.d.) for 7 to 14 days.

A blinded colorimetric detection assay of four biopsy specimens was performed six times at different intervals in order to test reproducibility.

Culture. Culture was performed on gastric biopsy specimens ground for 2 to 3 s with an electric tissue homogenizer before inoculation onto selective in-house medium and *H. pylori* agar (bioMérieux, Marcy l'Etoile, France) and nonselective medium (Wilkins Chalgren agar [Oxoid, Unipath, Basingstoke, Hampshire, England] enriched with 10% human blood) and incubation under microaerobic conditions at 37°C for 8 days (26). The in-house medium is composed of Wilkins Chalgren agar enriched with 10% human blood and four antibiotics (vancomycin, 10 μg/ml; cefsulodin, 10 μg/ml; trimethoprim, 5 μg/ml; and actidione, 100 μg/ml). Bacteria were identified as *H. pylori* on the basis of morphology on Gram stain and of oxidase, catalase, and urease activity.

Histology. Biopsy specimens were processed for histological examination according to the standard procedure. Hematein-eosin staining as well as a special staining for *H. pylori* (Giemsa) was performed (10). The criterion for positivity was the presence of bacteria with a morphology compatible with that of *H. pylori.*

DNA extraction from bacterial cultures. The DNA of *H. pylori* was extracted by a standard phenol-chloroform procedure (27). Briefly, the bacteria, grown under optimal conditions on Wilkins Chalgren agar plates with 10% human blood, were harvested after 2 days of incubation in 1 ml of a buffer containing 10 mM Tris-HCl and 1 mM EDTA, pH 8.0. They were then treated successively with lysozyme, sodium dodecyl sulfate, and proteinase K. Proteins were extracted twice with phenol and once with chloroform, and DNA was precipitated with ethanol in the presence of sodium acetate. The concentration of DNA was measured by absorption of light at 260 nm with the following conversion factor: 1 optical density (OD) unit of a double strand of DNA = 50 μg/ml.

DNA extraction from biopsy specimens. DNA extraction was performed as described previously (27). Briefly, each biopsy sample was ground in 1 ml of brucella broth for 2 to 3 s. The ground sample was then transferred to a microcentrifuge tube and centrifuged for 5 min at 10,000 × *g*. The supernatant was then discarded, and 300 μl of extraction buffer (20 mM Tris-HCl [pH 8.0], 0.5% Tween 20) was added to the pellet. This pellet was then resuspended, 15 μl of a proteinase K solution (10 mg/ml) was added, and the sample was incubated at 55°C for 1 h. Finally, the proteinase K was inactivated by heating the sample for 10 min at 98°C.

Synthetic primers and probes. Oligonucleotide primers and probes were synthesized by Eurogentec (Seraing, Belgium). Primer 1684 (5′AAGCTTTTAGG GGTGTTAGGGGTTT3′) and primer 1685 (5′AAGCTTACTTTCTAACACT AACGC3′), derived from the *H. pylori ureC* gene sequence (18) (accession numbers, EMBL X57132 and GenBank M60398), which amplify a 249-bp DNA fragment, were used. For GEN-ETI-K DEIA and PCR ELISA, the biotinylated probe (5′CGATGGCTTGGTGTGCG3′) was internal to the amplified *ureC* fragment. For Pylori-prob, the capture probe (5′GCGCGATTGGGGATAAG TTTGTGAGCG3′) and the detection probe (5′CCGGCGATGGCTTGGTGT GCGC3′) were also internal to the amplified *ureC* fragment.

DNA amplification. Conditions for DNA amplification are detailed in Table 1.

Colorimetric hybridization assays. (i) GEN-ETI-K DEIA. The GEN-ETI-K kit involves hybridization of amplified DNA with a single-stranded DNA biotinylated probe added to streptavidin-coated microtiter plate wells. The hybrid of the probe and the captured DNA was detected in an original way with an anti-DNA monoclonal antibody. This antibody reacts only with double-stranded DNA and not with single-stranded DNA. When the denatured DNA samples are added to the wells, the probe specifically binds the complementary hemistrand, if present, to form a hybrid. On the other hand, if a sample is negative, it will not contain the sequence sought, and the hybrid (double-stranded molecular species) will not form. After incubation and subsequent clearing of the sample, the addition of anti-double-stranded DNA antibody identifies the wells in which hybridization has taken place. The addition of an enzyme tracer detects this DNA-antibody complex. Briefly, as indicated by the manufacturer, the biotinylated probe was covalently linked to polystyrene microplates by adding 100 μl of a solution of biotinylated probe at an optimal concentration for each well. The plate was then incubated for 18 to 22 h at 2 to 8°C. Then, the plates were washed as indicated by the manufacturer. The assay was performed on denatured DNA samples which were incubated for 15 min at 95 to 100°C and then cooled by being dipped in an ice bath.

Twenty microliters of denatured PCR products was added to each well, containing 100 μl of hybridization buffer. Hybridization took place at 50°C under mild agitation for 1 h. After hybridization, the plates were washed and 100 μl of the anti-double-stranded DNA was then added. The plates were incubated at room temperature for 1 h and then washed prior to the addition of 100 μl of enzyme tracer. Plates were then incubated at room temperature for 1 h and washed. Finally, 100 μl of chromogen substrate was added. After 30 min of incubation at room temperature, the reaction was stopped with 200 μl of blocking reagent, and the OD was read at 450 nm (OD_{450}) in a Titertek Multiskan Plus reader (Life Sciences International, Helsinki, Finland). A blank (hybridization buffer-chromogen substrate-blocking reagent), two DEIA negative controls, one PCR negative control (a PCR mixture containing water instead of DNA), a first positive control (a synthetic oligonucleotide complementary to the probe), and a PCR positive control (*H. pylori* DNA amplified under the same conditions as the samples) were tested at the same time.

(ii) Pylori-prob detection kit. The Pylori-prob kit is a solid-phase sandwich hybridization system. It associates the use of a specific capture probe covalently bound to microtiter wells and a specific biotinylated detection probe. Amplified DNA, sandwiched between these molecules, is then detected via streptavidin-conjugated horseradish peroxidase and a colorimetric substrate.

A microtiter plate whose wells have been coated with *H. pylori* capture probe is provided in the kit. As indicated by the manufacturer, 200 μl of denaturation solution was added to each amplification tube. After 10 min of incubation at room temperature, 50 μl of denatured amplified product was added to the appropriate wells, previously filled with 50 μl of hybridization buffer. Hybridization took place at 37°C under mild agitation for 90 min. After hybridization, the plates were washed five times in buffer. One hundred microliters of a dilute conjugate, streptavidin-horseradish peroxidase, was added to each well, and the plates were incubated at room temperature for 30 min. Plates were then washed prior to the addition of 200 μl of chromogen substrate to each well. After 15 min of incubation at room temperature, the reaction was stopped with 100 μl of stop reagent, and the OD_{450} was read in a Titertek Multiskan Plus reader. Four Pylori-prob negative controls, one PCR negative control (a PCR mixture containing water instead of DNA), a Pylori-prob positive control, and a PCR positive

TABLE 2. ODs for the different urease-positive bacteria, tested by the three colorimetric hybridization assays

Strain	OD_{450} for GEN-ETI-K DEIA	OD_{450} for Pylori-prob	OD_{405} for PCR ELISA
Helicobacter felis	0.071	0.232	0.008
Campylobacter lari bv. UPTC	0.064	0.122	0.144
Proteus mirabilis	0.086	0.118	0.141
Haemophilus influenzae	0.092	0.173	0.026
Klebsiella pneumoniae	0.063	0.126	0.080
Helicobacter pylori	>1.000	>1.500	>1.400

control (*H. pylori* DNA amplified under the same conditions as the samples) were also tested.

(iii) PCR ELISA kit. The PCR ELISA involves labelling of PCR products with DIG during the amplification process. The labelled PCR products were analyzed by solution hybridization to a specific capture probe that was complementary to the inner part of the amplification product. This specific capture probe was labelled with biotin to allow immobilization of the hybrid (DNA-probe) to a streptavidin-coated microtiter plate surface. Nonspecific amplification products do hybridize to this capture probe and are thus removed during the following washing steps. The bound hybrid is detected by an anti-DIG peroxidase conjugate and by use of the colorimetric substrate ABTS [2,2′-azinobis(3-ethylbenzothiazoline-6-sulfonic acid), diammonium salt]. Briefly, as indicated by the manufacturer, 40 μl of denaturation DNA solution was added to 20 μl of amplification product. After 10 min of incubation at room temperature, hybridization solution containing biotinylated probe at an optimal concentration was added to a volume of 500 μl. Two hundred microliters of this mix was added to the appropriate well. Hybridization took place at 50°C under mild agitation for 1 h. After hybridization, the plates were washed and 200 μl of the anti-DIG–peroxidase solution was then added. The plates were incubated at 37°C for 30 min and then washed prior to the addition of 200 μl of ABTS substrate solution to each well. After 30 min of incubation at 37°C, the OD_{405} was read in a Titertek Multiskan Plus reader.

A blank (ABTS substrate solution), a negative detection control (water), a positive detection control (control PCR product provided in the kit), a positive labelling control (the control for the PCR ELISA kit), a PCR ELISA blank-negative PCR control (the negative control for the DIG labelling step), and a PCR positive control (*H. pylori* DNA amplified in the same conditions as the sample) were tested at the same time.

RESULTS

Sensitivity and specificity of the colorimetric hybridization assays. The sensitivity of the colorimetric hybridization assay was evaluated by using amplification products produced from several 10-fold dilutions of DNA from *H. pylori* CIP 101260 (starting template concentration, 1 μg/ml) in comparison to detection by gel electrophoresis. The detection limit of starting DNA on agarose gels was 10 ng/ml for GEN-ETI-K DEIA, 100 ng/ml for Pylori-prob, and 10 ng/ml for PCR ELISA, whereas the colorimetric hybridization assay detection limit of starting DNA reached 0.1, 1, and 1 ng/ml, respectively. The first two cases were thus 100-fold more sensitive than gel detection of the PCR products, and the third case was 10-fold more sensitive. The specificity of the colorimetric assay was assessed by using different urease-positive bacteria (*P. mirabilis*, *K. pneumoniae*, *H. influenzae*, *C. lari* bv. UPTC, and *H. felis*). The results show that the ODs of all these species were significantly lower than the results obtained with *H. pylori* (Table 2). The ODs obtained when the blinded colorimetric detection of four biopsy specimens was repeated under the same conditions were consistent.

Colorimetric detection of *H. pylori*-amplified DNA in biopsy specimens. For GEN-ETI-K DEIA, the distribution of OD_{450} obtained is shown in Fig. 1A. The mean OD_{450} was 0.896 ± 0.626 for biopsy specimens from untreated patients and 0.402 ± 0.440 for biopsy specimens from treated patients. For the *H. pylori*-positive biopsy specimens from untreated patients, the mean OD_{450} was 1.081 ± 0.569, while it was 0.857 ± 0.505 for the *H. pylori*-positive biopsy specimens from treated patients. This difference was not statistically significant (P = 0.05). For Pylori-prob, the distribution of OD_{450} obtained is shown in Fig. 1B. The mean OD_{450} was 1.322 ± 1.031 for biopsy specimens from untreated patients and 0.668 ± 0.830 for biopsy specimens from treated patients. For the *H. pylori*-positive biopsy specimens from untreated patients, the mean OD_{450} was 1.674 ± 0.947, while it was 1.528 ± 0.877 for the *H. pylori*-positive biopsy specimens from treated patients. This difference was not statistically significant (P = 0.4). For PCR ELISA, the distribution of OD_{405} obtained is shown in Fig. 1C. The mean OD_{405} was 0.733 ± 0.514 for biopsy specimens from

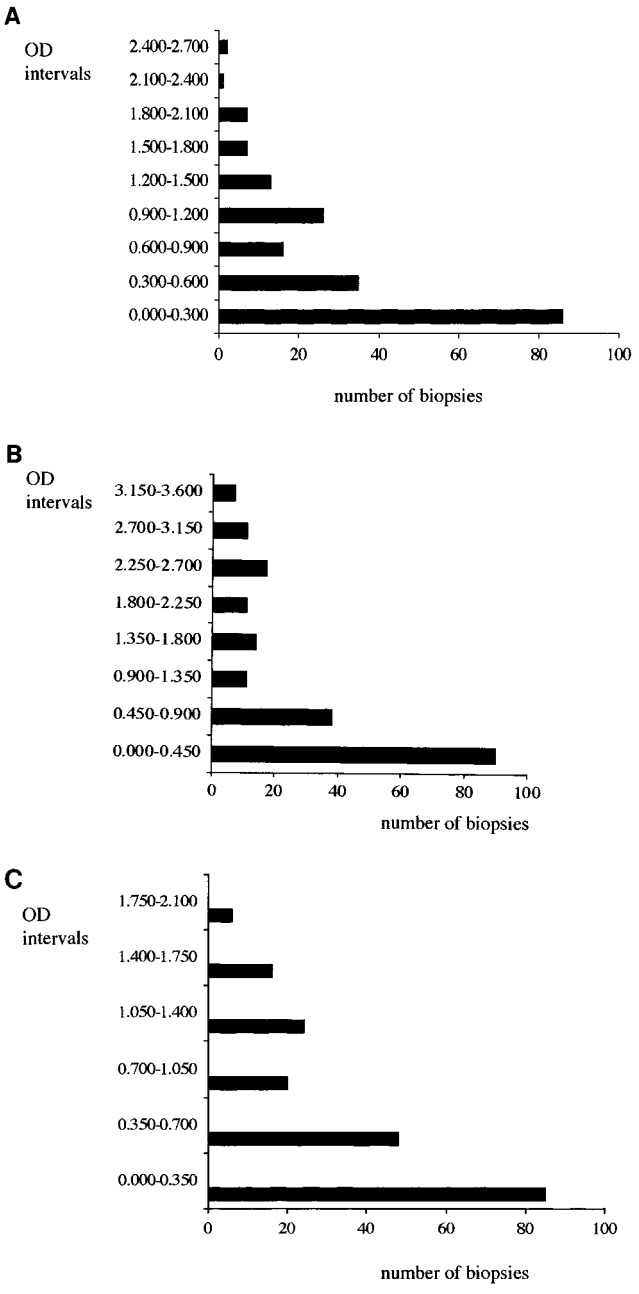

FIG. 1. OD_{450} distribution of biopsy specimens with GEN-ETI-K DEIA kit (A) and Pylori-prob kit (B) and OD_{405} distribution of biopsy specimens with PCR ELISA kit (C).

untreated patients and 0.485 ± 0.493 for biopsy specimens from treated patients. For the *H. pylori*-positive biopsy specimens from untreated patients, the mean OD_{405} was 0.988 ± 0.417, while it was 0.846 ± 0.503 for the *H. pylori*-positive biopsy specimens from treated patients. This difference was not statistically significant ($P = 0.1$).

Determination of the cutoff value of the two colorimetric hybridization assays. The determination of the cutoff value was performed by the receiving operating characteristic (ROC) curve construction method (12). Briefly, different virtual cutoff values between 0 and 4 (OD) were assigned, and for each cutoff value, the numbers of true positives, true negatives, false positives, and false negatives were calculated. True positives were those that were *H. pylori* positive by the standard method and had an OD above the virtual cutoff value, false positives were those that were *H. pylori* negative by the standard method with an OD above the virtual cutoff value, etc. Thus, each cutoff gives a sensitivity (sensitivity = number of true positives/number of *H. pylori*-positive patients) and a specificity (specificity = number of true negatives/number of *H. pylori*-negative patients) value. These data are then used to choose a cutoff value which provides both the best sensitivity and the best specificity. The ROC curves obtained for each assay are shown in Fig. 2. The chosen cutoff values for each assay considering the three possible situations (biopsy specimens obtained from untreated patients, biopsy specimens obtained from treated patients, and all the biopsy specimens combined) are shown in Table 3.

DISCUSSION

PCR has revolutionized molecular biology research and is currently broadening considerably the field of microbial diagnosis, including diagnosis of *H. pylori* infection. Nevertheless, there is still a potential for increasing its sensitivity. Several studies comparing PCR detection (amplified products being detected by gel electrophoresis) with other diagnostic methods have been performed (11, 19, 29, 34, 36). The results of these studies show that PCR can compete as an alternative diagnostic technique with culture, which is considered today as the gold standard. It is at least as sensitive as culture for primary detection of *H. pylori* and can give better results at treatment follow-up (21), when the number of bacteria in the gastric mucosa is usually small and the organisms may go undetected by other diagnostic methods.

The use of a colorimetric hybridization assay instead of gel electrophoresis to detect amplification products has been shown to increase the sensitivity of PCR for several infectious agents, i.e., *Mycobacterium tuberculosis* (37) and *Candida* species (14). For *H. pylori*, Lage et al. had a threshold 100 times lower with gastric biopsy as a specimen source (20). Furthermore, when gel electrophoresis was compared to colorimetric hybridization, Basso et al. showed that some positive samples were detected only by the latter (4). Such results are not unexpected, as an assay based on hybridization is normally far more sensitive than an assay based on agarose gel electrophoresis. In the present study, the sensitivity of two kits was 100-fold greater than that of gel electrophoresis and the sensitivity of the third kit was 10-fold greater. A possible cause for the relative lack of sensitivity of PCR ELISA kits is the high proportion of GC nucleotides in the *ureC* gene of *H. pylori*, which renders the incorporation of DIG-dUTP difficult. Incorporation could be increased by changing the ratio of dTTP to DIG-dUTP in the PCR ELISA (DIG labelling), and consequently the sensitivity of the PCR ELISA (DIG detection) would be improved.

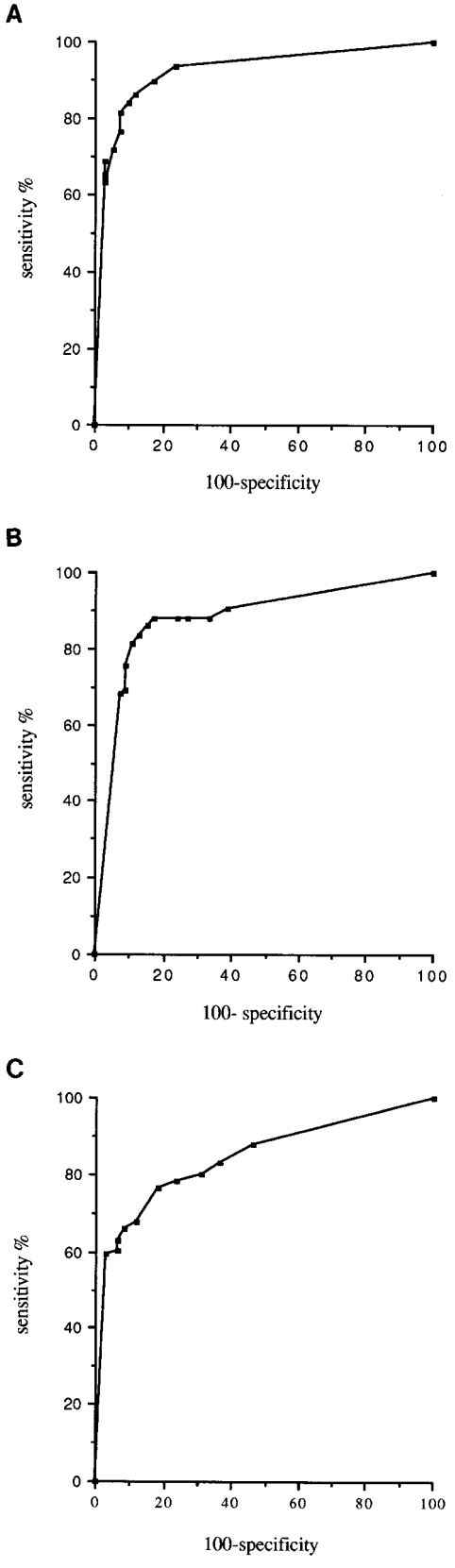

FIG. 2. The ROC curves obtained for GEN-ETI-K DEIA (A), Pylori-prob (B), and PCR ELISA (C), with sensitivity values (true-positive rates) plotted on the *y* axis and the complementary specificity values (true-negative rates) plotted on the *x* axis.

TABLE 3. Sensitivity and specificity of the three DEIAs[a]

Assay	Value for biopsy specimen type (*n*)					
	Pretreatment (98)		Posttreatment (101)		All combined (199)	
	Sensitivity (%)	Specificity (%)	Sensitivity (%)	Specificity (%)	Sensitivity (%)	Specificity (%)
GEN-ETI-K DEIA	94.4	81.0	89.3	90.4	85.9	88.2
Pylori-prob	85.4	50.0	89.3	85.0	87.9	82.6
PCR ELISA	80.5	54.0	86.0	66.0	80.2	68.8

[a] Based on the cutoff values obtained with the ROC curves with biopsy specimens from untreated patients and applied to the different situations encountered in the clinical laboratory. Cutoff values were 0.350, 0.450, and 0.350 for the GEN-ETI-K DEIA, Pylori-prob, and PCR ELISA, respectively.

When specificity was tested with other urease-positive organisms, the ODs obtained were lower than the cutoff value chosen for each assay, confirming the specificity of the colorimetric hybridization method. A 100% specificity was obtained by Bass et al. for the detection of *Chlamydia trachomatis* in endocervical specimens with the Amplicor kit (3).

This type of assay allows screening and confirmatory tests to be performed together in situations in which the PCR band is doubtful, because a hybridization step is included in the protocol. Hybridization is a critical factor in increasing the specificity of the method.

However, in our study, when the DEIAs were compared to other methods with gastric biopsy specimens a lack of specificity of the colorimetric hybridization assays was noted. This could, in fact, be explained by the lack of sensitivity of the reference methods used. Despite the fact that culture is the reference method for *H. pylori* detection in biopsy specimens, this method has certain limitations which can contribute to a decrease in its sensitivity, such as demanding conditions for transportation. Histological examination can also fail in *H. pylori* detection, especially if there are few bacteria present.

Recently, several studies have been performed with colorimetric hybridization assays, mainly for the diagnosis of viral infections but also some bacterial infections. This technique proved to be as sensitive for the detection of amplified products as traditional Southern hybridization but faster and more convenient, because it does not require radioisotopes, as demonstrated for *Mycobacterium leprae* (33), hepatitis B virus (17), and human immunodeficiency virus type 1 (24). For bacteria, similar techniques have been used to detect different species of *Mycobacterium* (*M. tuberculosis* [8, 23, 37], *M. leprae* [33], and *M. xenopi* [13]), as well as *Bordetella pertussis* (5), *Salmonella* species (6), *C. trachomatis* (3), and enterotoxigenic *Escherichia coli* (28). These studies have shown that colorimetric hybridization is an effective method for routine diagnosis. For *C. trachomatis*, a commercial PCR detection kit that includes a colorimetric step has been developed (3).

The methodology evaluated here incorporates a colorimetric detection of amplification products by hybridization on a solid phase, an innovation that makes this new technology feasible in clinical laboratories. With this procedure, detection of PCR products is rapid (4 h), easy, and objective. It does not require an electrophoresis apparatus or a UV light source. Furthermore, the use of ethidium bromide is avoided, and its similarity to the widely used enzyme-linked immunoassays is a characteristic that makes the colorimetric hybridization assay familiar to any clinical microbiology laboratory. These points make this assay suitable for automation and the analysis of large numbers of samples.

The colorimetric hybridization assay can be used not only for *H. pylori* detection in gastric biopsy specimens but also for *H. pylori* detection in any kind of potentially infected specimen, such as saliva, feces, gastric juice, and dental plaque. Another potential application is the detection of pathogenicity markers as described by O'Meara et al., who detected the heat-labile toxin-encoding gene of enterotoxigenic *E. coli* (28). This technique could be readily performed on biopsy specimens in parallel with a PCR designed to detect the presence of *H. pylori*. Currently, the best candidate is *cagA*, a marker for the Cag pathogenicity island (6), which has been found to be associated with more severe inflammation and diseases, e.g., peptic ulcer and gastric cancer. Another application which has already been proposed (19) is the detection of *vacA* gene mosaicism (1).

Finally, another potential use of colorimetric hybridization is the possibility of quantification of the PCR-amplified fragments as shown by Chevrier et al. for the detection of *Salmonella* (7) and by van der Vliet et al. for the detection of *M. leprae* (33). This application must be developed in future studies and can potentially contribute to the evaluation of the number of microorganisms present per infected cell.

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