Detection of the *glmM* Gene in *Helicobacter pylori* Isolates with a Novel Primer by PCR[▽]

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A novel reverse primer (GLM MR1) was designed for detection of the *glmM* gene in *Helicobacter pylori* by PCR. The percentage of amplification in clinical isolates using GLM MR1 was 100% for detection of the *glmM* gene and 86.36% for the *ureA* gene. The primer designed is useful for the identification of *H. pylori*.

Helicobacter pylori is the human-specific and etiologic agent of chronic active gastritis as well as gastric and duodenal ulcers (2, 6). Several assays have been developed to diagnose *H. pylori*, including microbiologic culture, histological examination, rapid gastric biopsy urease tests, urea breath tests, serologic tests, and detection by DNA and RNA probe technology (9).

PCR-based methods have been developed to detect the organism directly in clinical specimens. The targets of these PCR methods include the 16S rRNA gene (8), the random chromosome sequence (15), the 26-kDa species-specific antigen (SSA) genes (7, 11), the urease (*ureA*) gene (3), and the *glmM* (*ureC*) gene (1).

The *ureC* gene encodes for a phosphoglucosamine mutase; this gene is unrelated to urease production, so it was renamed *glmM*. This gene is considered a “housekeeping” gene, and it participates directly in cell wall synthesis (4).

The aim of this work was to design a reverse primer for detecting the *glmM* gene by PCR from clinical strains of *Helicobacter pylori* isolated from gastric biopsy specimens to confirm the presence of the bacterium.

The *ureA* gene was amplified in this work for use as a positive control, since this gene is widely used for identifying *H. pylori* by PCR (3, 9). This reverse primer (GLM MR1; 5'-GCA TTC ACA AAC TTA TCC CCA ATC-3') for detecting the *glmM* gene from *H. pylori* was designed because the original primers described by Tomasini et al. (13) were used in other studies of our research group for the amplification of this gene as well as *vacA*, *cagA*, and others in a PCR multiplex (data not shown), but when these primers were used, we observed nonspecific bands. These bands were also found in a non-multiplex assay using the original *glmM* primers, as shown in Fig. 1 (5).

GLM MR1 was based on sequences found in the GenBank database at the National Center of Biotechnology Information (NCBI) (accession no. AE001446). A bioinformatic analysis was done using bioinformatics programs (DNAMAN v. 5 and

Bioedit v. 7.0.9) to confirm that there were no interactions between GLM MR1 and the GLM MF primer reported by Tomasini et al. (13) (Table 1). The two primers did not show complementarities or other types of interactions. GLM MR1 amplifies a product of 140 bp, as shown in Fig. 2.

To validate the design of the GLM MR1 primer, the product of PCR was sequenced. PCR was done using *Helicobacter pylori* ATCC 43504 DNA; the product was purified with a purification kit (Marligen Bioscience) as described by the manufacturer, and it was sequenced at the Unidad de Síntesis y Secuenciación IBT of the Universidad Nacional Autónoma de México (UNAM), Cuernavaca-Morelos, Mexico. The alignment of the sequences resulting from sequencing was done at the National Center for Biotechnology Information (NCBI) database with the Basic Local Alignment Search Tool (BLAST). The results of the alignment showed a high alignment score (>200) of the primers, and they aligned only with *H. pylori* strains (data not shown).

PCR using *glmM* (GLM MR1 and GLM MF) and *ureA* primers was applied to 101 *H. pylori* clinical isolates obtained from gastric biopsy specimens from 14 pediatric patients who had undergone endoscopy for diagnosis of abdominal recurrent pain or discomfort at the Department of Pediatric Endoscopy of the Hospital General Centro Médico La Raza of the

FIG. 1. Product from PCR amplification of *glmM* gene. Primers described by Tomasini et al. (13); nonspecific bands (BI) are shown. Lane M, 100-bp ladder; lane 1, no-template control; lanes 2 and 3, amplicon for *glmM* gene.

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TABLE 1. Oligonucleotide sequence of primers and PCR product sizes

Amplified gene	Primer	Sequence (5'-3')	PCR product size (bp)	Reference
<i>ureA</i>	URE AR	CTC CTT AAT TGT TTT TAC	411	12
	URE AF	GCC AAT GGT AAA TTA GTT		
<i>glmM</i>	GLM MR	GCA TTC ACA AAC TTA TCC	140	13
	GLM MF	CCA ATC ^a GGA TAA GCT TTT AGG GGT GTT AGG GG		

^a Primer designed in this work.

Instituto Mexicano del Seguro Social (IMSS), Mexico City, Mexico. All of the clinical isolates (101) were rapid urease test (RUT) and culture positive.

H. pylori ATCC 43504 DNA was used to standardize both PCRs, as well as DNA from *Proteus mirabilis* as a negative control and no template as a negative-control reaction. DNA from 101 pure clinical isolates was obtained using the thiocyanate guanidine method (10). Primer sequence, sizes of the products from PCR, and references are summarized in Table 1. Briefly, the template DNA from the reference strain (80 ng) and 5.0 pM of each primer (*glmM* and *ureA*) were added to a PCR mixture tube containing 1 U of *Taq* DNA polymerase (Invitrogen), 2.5 mM each deoxynucleoside triphosphate, 1.5 mM Tris-HCl (pH 8.3), and 1.5 mM MgCl₂; the volume was adjusted with distilled water to 25 µl. Reaction mixture was subjected to 35 amplification cycles of 10 min at 94°C, 1 min at 94°C, 1 min at 45°C (*ureA* [12]) and 58°C (*glmM*; this work), and 1 min at 72°C, followed by a 10-min final extension at 72°C (model 9600 Thermocycler; Perkin-Elmer Cetus). The amplicons were visualized with ethidium bromide after electrophoresis was done.

The results of PCR amplification of *ureA* (*ureAPCR*) and *glmM* (*glmMPCR*), isolation of clinical strains (culture), and rapid urease testing (RUT) of clinical isolates are shown in Table 2; the *ureAPCR* amplified 86.36% (94 of 101) even though all clinical isolates were positive when the rapid urease test (RUT) was done from culture. Moreover, *glmMPCR* fully amplified in the 101 clinical isolates (100%) and the presence

TABLE 2. Diagnosis of *H. pylori* from biopsy specimens and clinical isolates

Test	% diagnosed (no. of samples diagnosed/total no.)
Culture	100 (101/101)
RUT.....	100 (101/101)
<i>ureAPCR</i>	86.36 (94/101)
<i>glmMPCR</i>	100 (101/101)

of nonspecific bands or other products was not observed in *P. mirabilis* DNA amplification, confirming the high specificity of the primer for amplification by PCR. We highly recommend the use of the *glmM* gene, as well as the GLM MR1 primer together with GLM MF, described by Tomasini et al. (13), for the diagnosis and identification of *H. pylori* in clinical isolates from gastric biopsy specimens.

As shown by our results, the “gold standard” method for diagnosis of *H. pylori* infection is still culture of the microorganism, but this method is complex, tedious, and expensive. The development of molecular biology techniques for the PCR detection of DNA from several fastidious microorganisms such as *H. pylori* has been highly recommended (3). The development of specific primers based on “housekeeping genes” such as 16S rRNA, *glmM*, and *ureA* is useful (15). In the present work, both genes used are housekeeping genes; however, the amplification of the *ureA* gene (86.36%) was lower than that of the *glmM* gene (100%); this result may be due to sequence polymorphism in the *ureA* loci or to the diversity of clones within the same patient (1, 14, 15). The *glmM* gene is essential for the development of the cell wall in bacteria as well as for the growth of the microorganism, and this gene has been extensively used for confirming the presence of *H. pylori*. We recommend the use of the *glmM* gene and the GLM MR1 primer for the detection of *H. pylori* by PCR because of the high degree of specificity obtained in this study. It would be interesting to develop assays using these primers with samples such as biopsy specimens or saliva rather than just with pure-culture strains.

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FIG. 2. Amplification of *glmM* with the primers GLM MR1 (this report) and GLM F (13). There are no nonspecific bands; a product of 140 bp was obtained. Lane M, 100-bp ladder; lanes 1 and 2, no-template controls; lane 3, amplicon of *glmM* gene.

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