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Comparison of Five PCR Methods for Detection of *Helicobacter pylori* DNA in Gastric Tissues

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Five different PCR methods for the detection of *Helicobacter pylori* were evaluated. The results of this study indicate that of the five PCR methods examined, the *ureC* (*glmM*) gene PCR is the most sensitive and specific for the detection of *H. pylori* in gastric biopsy specimens.

Helicobacter pylori is an etiologic agent of chronic active gastritis and gastric and duodenal ulcers (6, 12). Many PCR 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 (17), the 26-kDa species-specific antigen (SSA) gene (7, 14), the urease A (*ureA*) gene (3), and the urease C (*ureC*) gene (1). The *ureC* gene has been shown to encode the phosphoglucosamine mutase, which is unrelated to urease production, and was renamed the *glmM* gene (4). To determine which PCR method is most appropriate to use, we compared the sensitivities and the specificities of five different PCR methods for the detection of *H. pylori* in gastric biopsy specimens.

The specimens used for this study were gastric biopsy samples from patients who had undergone endoscopy for diagnosis of abdominal pain or discomfort. Three pieces of gastric tissue were taken from each patient. The use of these specimens for research was approved by the internal review board of the Tri-Service General Hospital, Taipei, Taiwan. No histopathology was performed on these tissues in this study. These three pieces of tissue were pooled and ground together. An aliquot of the tissue homogenate was used for culture, and the remain-

TABLE 1. Conditions for the five different PCR methods

Target (reference), nucleotide (nt) positions amplified, and size of PCR products	Primer names and sequences	PCR conditions
16S rRNA (8)		
nt 407–853, 446 bp	Hp1, 5′-CTGGAGAGACTAAGCCCTCC-3′ Hp3, 5′-AGGATCAAGGTTTAAGGATT-3′	95°C, 30s; 55°C, 30s; 72°C, 30s (30 cycles)
nt 635–744, 110 bp	Hp1, 5′-CTGGAGAGACTAAGCCCTCC-3′ Hp2, 5′-ATTACTGACGCTGATTGTGC-3′	95°C, 30s; 62°C, 30s; 72°C, 30s (30 cycles)
Random sequence (17), nt 4835–5041, 207 bp	CAM-2, 5′-TAACAAACCGATAATGGCGC-3′ CAM-4, 5′-CATCTTGTTAGAGGGATTGG-3′	95°C, 1 min; 42°C, 30s; 72°C, 1 min (40 cycles)
26-kDa SSA gene (7), nt 474–776, 303 bp	Primer 3, 5′-TGGCGTGTCTATTGACAGCGAGC-3′ Primer 4, 5′-CCTGCTGGGCATACTTCACCAG-3′	98°C, 10 min (1 cycle); 92°C, 30s; 68°C, 1 min (37 cycles); 92°C, 30s; 68°C, 1 min; 72°C, 2 min (6 cycles)
Urease A gene (3), nt 304–714, 411 bp	HPU1, 5′-GCCAATGGTAAATTAGTT-3′ HPU2, 5′-CTCCTTAATTGTTTTTAC-3′	94°C, 1 min; 45°C, 1 min; 72°C, 1 min (35 cycles)
glmM gene (1), nt 784–1077, 294 bp	Forward primer, 5′-AAGCTTTTAGGGGTGTTAGGGGTTT-3′ Reverse primer, 5′-AAGCTTACTTTCTAACACTAACGC-3′	93°C, 1 min; 55°C, 1 min; 72°C, 1 min (35 cycles)

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TABLE 2. Results of five PCR methods for the detection of *H. pylori* in 50 gastric biopsy specimens

No. of specimens	Culture	PCR				
		16s rRNA gene	Random sequence	SSA gene	*ureA* gene	*ureC* (*glmM*) gene
7	+	+	+	+	+	+
11	+	+	−	+	+	+
2	+	+	+	+	−	+
4	+	+	−	+	−	+
1	−	+	+	+	+	+
10	−	+	−	+	−	−
2	−	+	−	−	−	−
13	−	−	−	−	−	−

ing was used for PCR. *H. pylori* culture and identification were performed as described previously (9).

For PCR, DNA was isolated from 100 μl of tissue homogenate by using the Puregene DNA isolation kit (Gentra Systems, Inc., Minneapolis, Minn.) according to the manufacturer's instructions. Ten microliters (100 ng) of DNA was used as the template for each PCR. Each sample was examined by five different PCR methods. The PCRs were performed as described previously (1, 3, 7, 8, 17). The primer sequences, conditions, and sizes of these PCR methods are summarized in Table 1.

The specificities of the five PCR methods were first examined for 15 different bacteria: *Staphylococcus aureus* ATCC 25923, *Staphylococcus epidermidis* ATCC 12228, *Streptococcus pyogenes* ATCC 19615, *Streptococcus agalactiae* ATCC 13813, *Enterococcus faecalis* ATCC 29212, *Haemophilus influenzae* ATCC 35056, *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853, *Pseudomonas maltophilia*, *Klebsiella pneumoniae* ATCC 13883, *Serratia marcescens*, *Enterobacter cloacae* ATCC 23315, *Proteus mirabilis* ATCC 7002, *Shigella sonnei* ATCC 25931, and *Salmonella typhi* ATCC 6539. The *H. pylori* DNA was used as the positive control.

Bacterial DNA was also isolated by using the Puregene DNA isolation kit (Gentra Systems). None of the PCR methods produced any PCR products from these 15 different bacteria. To determine whether these negative PCR results were false due to the presence of PCR inhibitors, these bacterial samples were examined by the bacterial universal PCR (11) with primers U1 (5′-CGGTTACCTTGTTACGACTT-3′) and U2 (5′-CCTTGTACACACCGCCCGTC-3′). All 15 bacterial samples were positive in this universal PCR.

All 24 culture-positive specimens were positive in the 16S rRNA gene, the SSA gene, and the *ureC* (*glmM*) gene PCRs. Only 18 of the 24 culture-positive specimens were positive in the *ureA* gene PCR, and 9 were positive in the random chromosome sequence PCR. One of the 26 culture-negative specimens was positive in all five PCRs, indicating that this specimen was false negative in culture. Twelve of the remaining 25 culture-negative specimens were positive in the 16S rRNA gene PCR, and 10 were positive in the SSA gene PCR. All of these 25 culture-negative specimens were negative in the *ureC* (*glmM*) gene, the *ureA* gene, and the random chromosome sequence PCRs (Table 2).

To determine the sensitivities of these PCR methods, a 10-fold serial dilution, from 10 ng to 1 fg, of a purified *H. pylori* DNA was made. Each dilution was examined by all five PCRs. The 16S rRNA gene PCR was determined to have a sensitivity of 0.01 pg of *H. pylori* DNA, which corresponds to approximately 5 organisms. The sensitivity of the other four PCR methods was found to be 10-fold (0.1 pg) lower than that of the 16S rRNA gene PCR. This is conceivable since the 16S rRNA gene PCR is a seminested PCR and the other four methods are single-step PCRs. However, the 16S rRNA gene PCR has a very poor specificity. It produced positive results with 13 of the 26 culture-negative biopsy specimens as described above. This finding is consistent with the previous report that the 16S rRNA gene PCR nonspecifically amplifies human DNA (2). Unfortunately, the 16S rRNA gene PCR has been the most widely used method for the detection of *H. pylori* in clinical specimens (10, 11, 13, 15, 18). The SSA gene PCR was also found to have a problem with specificity in this study. Although this PCR did not amplify any of the other bacterial DNAs, it amplified 10 of the 25 *H. pylori* culture-negative biopsy specimens. It is highly unlikely that all 10 samples were false negative by culture. The reasons for this poor sensitivity remain to be investigated.

The *ureA* gene and the random sequence PCRs appeared to be specific for *H. pylori*, but the sensitivities of these two methods were unsatisfactory. The random sequence PCR amplified only 38% (9 of 24) of *H. pylori* culture-positive biopsy samples, and the *ureA* gene PCR amplified 75% (18 of 24) of them. This low sensitivity may be due to sequence polymorphism in these two loci (5, 16, 17).

The *ureC* (*glmM*) gene PCR amplified all 24 *H. pylori* culture-positive biopsy specimens (positive predictive value, 100%) (Table 3) and produced only one false-positive result on 26 *H. pylori* culture-negative specimens (negative predictive value, 96%) (Table 3) or other bacterial DNA. However, this specimen was very likely false negative in culture because it was positive in all five PCRs, as mentioned above, and the patient had symptoms typical of *H. pylori* gastritis according to endoscopic examination. Although the sensitivity of the *ureC* (*glmM*) gene PCR was found to be 10 times lower (50 organisms) than that of the 16S rRNA gene PCR, the results of this study suggest that it has sufficient sensitivity for clinical applications. We therefore consider the *ureC* (*glmM*) gene PCR to be the most appropriate of the five different PCR methods examined for detection of *H. pylori* organisms in clinical specimens.

TABLE 3. Positive and negative predictive values of the five different PCR methods

Value	Results [% (no. of samples with value/total no.)] for PCR method				
	16S rRNA gene	Random sequence	SSA gene	*ureA* gene	*ureC* (*glmM*) gene
Positive predictive[a]	100 (24/24)	75 (18/24)	100 (24/24)	38 (9/24)	100 (24/24)
Negative predictive[b]	46 (12/26)	96 (25/26)	54 (14/26)	96 (25/26)	96 (25/26)

[a] Compared with 24 culture-positive samples.
[b] Compared with 26 culture-negative samples.

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