

# Detection of *Helicobacter pylori* by polymerase chain reaction: A comparison in sample preparation

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Gastric biopsy samples obtained from 14 patients with upper abdominal pain, clinically diagnosed as acid peptic disease, were analysed for the presence of *Helicobacter pylori* (*H. pylori*) by Polymerase Chain Reaction (PCR) using partially (template A) and completely purified DNA (template B). Antigen specific primer was used to analyse the sample by PCR method. The presence of *H. pylori* in the samples was confirmed by running a positive control. The presence of *H. pylori* was also detected by urease method using standard protocol. Among the 14 samples studied, 8 showed the presence of *H. pylori* with both templates A and B. Among these 8 samples only 3 showed positive for the presence of *H. pylori* with urease method. The present work discusses the results obtained in the detection of *H. pylori* in template A and B by PCR method.

**Key Words:** *H. pylori*, Gastritis, Duodenal ulcer, Polymerase Chain Reaction, Antigen specific primer

## Introduction

*Helicobacter pylori* (*H. pylori*), a micro aerobic spiral gram-negative bacterium, known to be associated with active chronic gastritis and peptic ulcer.<sup>[1]</sup> Approximately two third of the world population are infected with *H. pylori*.<sup>[2]</sup> Early detection of *H. pylori* infection may help to prevent gastric related disease.<sup>[3,4]</sup> It has been reported that *H. pylori* can also cause carcinoma of the stomach.<sup>[5,6]</sup> A number of diagnostic tests have been developed for the detection of *H. pylori*. This includes cultur-

ing of gastric biopsy specimen,<sup>[7]</sup> rapid urease test<sup>[8]</sup> and staining and histology technique.<sup>[9]</sup> Culturing of *H. pylori* requires 5 to 7 days of incubation and has been shown to give rise to a false negative value in a number of samples.<sup>[10]</sup> Urease test although a rapid method but less sensitive in the detection of *H. pylori* and the results show both false positive and false negative values.<sup>[11]</sup> Histological examination of stained biopsy smears is also a non-specific test and has a low sensitivity for detection of this organism.<sup>[12]</sup>

Several molecular techniques have been developed for the detection of *H. pylori* in gastric biopsy specimen such as dot blot hybridization, DNA-RNA hybridization assay and RFLP methods.<sup>[13]</sup> All these methods require lengthy processing time<sup>[13]</sup> and hence not suitable to adopt as a routine procedure in clinical laboratories. In recent years it has been shown that the presence of bacteria in biopsy material can be detected using Polymerase Chain Reaction (PCR).<sup>[14]</sup> Using this method *H. pylori* has been detected with various primers and the results have been compared with the conventional methods. Most of the studies have shown that the sensitivity of detection of *H. pylori* by PCR method was better compared to other methods.<sup>[10]</sup> This method though sensitive to detect *H. pylori* has not been used for diagnosis in most of the hospitals. The initial preparation of sample such as purification of DNA and the cost of the reagents

might be the inhibiting factors to use this technique in the hospitals. It was, therefore, of interest to detect the presence of *H. pylori* in samples obtained after partial and complete purification of DNA using antigen specific primer. The present work discusses the results obtained in samples with partial and complete purification of DNA.

## Materials and Methods

Gastric biopsy samples were collected from ten male and four female patients at DAE Hospital, Kalpakkam, Tamilnadu. Among these 14 patients five were suffering from duodenal ulcer, three from non-ulcer dyspepsia, two from gastritis, two from antral gastritis and two from duodenitis. Age of the patients varied between 17 and 50 with an average of 34.5.

Antigen specific forward 5' (TGG CGT GTC TAT TGA CGA CGA GC) 3' and reverse 5' (CCT GCT GCG CAT TCA CCA TG) 3' primers were procured from Genei (Bangalore GENEI, India).

Detection of *H. pylori* by urease test was followed by the method described elsewhere.<sup>[8]</sup> The biopsy material was placed in a solution containing 10% urea, 0.005% potassium dihydrogen phosphate and phenol red indicator (pH 6.8). The change of colour was observed between 30 min and 24 hr. If the colour changes from yellow to pink it indicates the presence of *H. pylori*.

Excised biopsy sample was transferred immediately into a micro-centrifuge tube containing 0.9% of NaCl (500 ml) and centrifuged at 12,000 g for 10 min. The pellet was resuspended in 300 µl of extraction buffer (20 mM Tris-HCl, pH 8.0; Tween-20, 5 µl/ml) and 3 µl of proteinase K (200 µg/ml) and kept at 55°C for 1-2 hr to ensure complete lysis of the tissue. This was further heated at 98°C for 10 min to inactivate the proteinase K. 2 ml aliquot of the above sample (template A) was removed at this stage for PCR amplification.<sup>[10]</sup> The remaining solution was extracted twice with a mixture of phenol: chloroform: isoamyl alcohol (25:24:1) and twice with chloroform: isoamyl alcohol (24:1) to get pure DNA. The purified DNA present in the aqueous layer following the above extraction was precipitated with absolute ethanol in the presence of one-tenth volume of 3 M sodium acetate at a pH 5.5 and left at -20°C for 1 hr. The

precipitated DNA was separated by centrifuging at 12000 g for 30 min at 4°C. The DNA pellet obtained was washed in 70% ethanol, dried at room temperature and suspended in 40ml of TE buffer (10 mM Tris, 1 mM EDTA). The purity of DNA was tested by measuring the absorption at 260 and 280 nm in a spectrophotometer (Hitachi, Japan). The purified DNA (template B) was then amplified using PCR.

The amplification of DNA was carried out in 0.2 ml reaction tubes by PCR using a thermal cycler (PERKIN ELMER 2400). A 50 µl reaction mixture consisted 1 x PCR buffer [10 mM Tris HCl (pH 8.3), 50 mM KCl, 1.5 mM MgCl<sub>2</sub> and 0.01% gelatin], four deoxynucleotide at concentrations of 200 µM each, forward and reverse primer each at a concentration of 0.1 µM and 2 µl of template (A) or 1 µl of template B. Prior to thermal cycle the genomic DNA was denatured at 94°C for 5 min. Following this the sample was allowed to undergo 40 cycle which consisted denaturation of DNA for 1 min at 94°C, annealing of primer for 1 min at 68°C and extension of primer for 1 min at 72°C. A final extension of DNA was carried out for 5 min at 72°C to ensure complete amplification. Distilled water served as a negative control and cultured *H. pylori* DNA served as positive control during the amplification. The amplified DNA product was analysed by electrophoresis using agarose prepared in TAE (Tris acetate-EDTA) buffer (2 g/100 ml). The size of the gel was 10 x 7 cm with a thickness of 0.3 cm. Samples were loaded on the gel and a current of 40mA was applied. 100-500bp DNA ladder was used as marker to know the size of the PCR product. The sample was allowed to run for 2 hr and the gel was stained with 0.5 mg /ml ethidium bromide for 30 min. The band was seen by illumination with UV light in a gel documentation system (Advanced American Biotechnology, AAB, USA).

## Results

Table 1 shows the results obtained in samples (template A and B) with PCR method. The table also shows the results obtained in biopsy samples by urease method. Among the 14 samples studied 3 showed positive with both PCR and urease method. In addition to these three, *H. pylori* was found to be positive in five

**Table 1: Clinical diagnosis and the results of the test carried out in patients with various gastric disorders for the detection of *H. pylori***

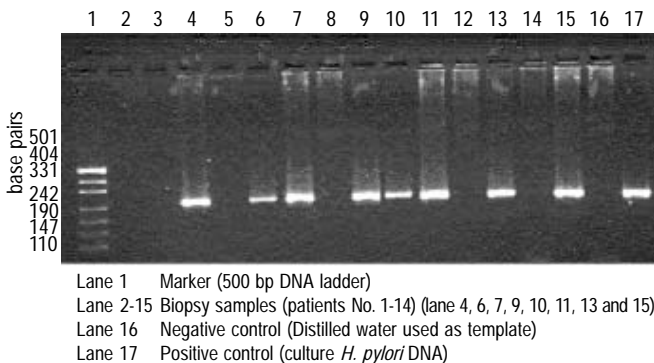
Case No	Age/ Sex	Diagnosis	Urease test	PCR amplification	
				Template (A)	Template (B)
1	40 / M	Antral Gastritis	-	-	-
2	48 / M	Gastritis	-	-	-
3	30 / F	Duodenitis	+	+	+
4	37 / M	Duodenal ulcer	-	-	-
5	17 / M	Non ulcer dyspepsia	-	+	+
6	39 / M	Duodenitis	-	+	+
7	21 / M	Duodenal ulcer	-	-	-
8	24 / F	Duodenal ulcer	+	+	+
9	45 / F	Non ulcer dyspepsia	-	+	+
10	42 / M	Non ulcer dyspepsia	+	+	+
11	50 / M	Duodenal ulcer	-	-	-
12	27 / M	Duodenal ulcer	-	+	+
13	23 / M	Gastritis	-	-	-
14	40 / F	Antral gastritis	-	+	+

Template (A): Partially purified DNA, Template (B): Completely purified DNA, '+': *H. pylori* positive, '-': *H. pylori* negative, M: Male, F: Female

cases by PCR method. The samples, which showed positive with PCR, were observed positive in both templates (A and B).

Figure 1 shows the amplified DNA products by gel electrophoresis. Among the 17 lanes seen in Figure 1, lane 1 show the bands obtained with molecular weight marker, lane 2 to 15 correspond to the biopsy samples, lane 16 and 17 indicate negative and positive control respectively. Of these samples lane 4, 6, 7, 9, 10, 11, 13 and 15 showed the amplification of DNA.

Table 2 shows that 21.42% of the patients were positive by urease and 57.14% of the patients were positive by PCR method (both template A and B). With regards

**Figure 1: Agarose gel electrophoresis carried out in samples obtained with partially purified DNA after amplification using antigen specific primers****Table 2: Percentage of the samples positive with urease and PCR assay for *H. pylori***

Number of Patients	Percentage of cases positive with urease and PCR test	Percentage of cases positive with PCR alone
14	21.42 (3)	57.14 (8)

The numbers in the parenthesis indicate the number of patients.

to the concentration of  $\text{MgCl}_2$  1.5 mM was used for PCR study as we observed among the various concentrations of  $\text{MgCl}_2$  (1.5, 2.5 and 3.5 mM) tested during this work only 1.5 mM concentration of  $\text{MgCl}_2$  gave optimum amplification of DNA.

## Discussion

The present study has indicated that among the 14 patients, 8 showed the presence of *H. pylori* in both templates (A and B) by PCR method. As per the diagnosis among the 14 patients 11 had ulcer either duodenal or gastric and three had non-ulcer dyspepsia. The 8 patients who showed positive for the presence of *H. pylori* were from the 11 patients who were diagnosed for ulcer. Three patients with non-ulcer dyspepsia indicated absence of *H. pylori*. The 8 patients who showed the presence of *H. pylori* in both the templates indicate that the highly purified DNA was not required for the detection of *H. pylori*. Similar study carried out by Hammer et al<sup>[10]</sup> have shown that almost the 27 cases studied 19 showed positive by lysate (partial digestion) and only 11 showed positive with purified DNA. The authors have pointed out that among the 19 positive cases 8 would have become negative during the organic purification of DNA. This could be due to the addition of inhibitory materials or loss of DNA during the organic extraction.

Our study, however, showed positive with both templates indicating that a simple extraction of tissue material (template A) was sufficient to carry out the test. Among the 11 patients who showed ulcer condition by diagnosis only 8 showed positive by PCR method. The absence of *H. pylori* in the remaining 3 cases could be that the region from where the sample collected probably not infected with this organism, as it is known that this organism is present in patches in these patients. At the same time, it should be noted that the samples

analysed by PCR and urease test were collected from the same regions and thus the absence of *H. pylori* in five out of eight cases as analysed by urease test could be due to the poor sensitivity of detection and not due to the region of sample collection.

At present a number of primers were used to detect the *H. pylori* by PCR method such as species-specific antigen primer, urease gene, 16s rRNA gene.<sup>[15]</sup> The primer derived from the DNA sequence of a species-specific protein antigen has been shown to be present only in *H. pylori*. Antigen specific primer was selected in this study. O'Toole et al<sup>[16]</sup> have reported that the DNA sequence of this primer was specific to the antigen present only in *H. pylori* and not found in other bacteria.

Although PCR technique has been shown to be more sensitive in detecting *H. pylori* compared to urease test, PCR is not being used as a routine diagnostic procedure in many of the hospitals. This perhaps could be due to the cost of the instrument for initiating the work and also the cost of reagents used for the estimation. By considering the sensitivity of the technique in the detection of *H. pylori* and also its applications in various other diagnostic tests such as Leptospirosis, HIV, Hepatitis B etc the hospitals can justify the use of this technique.

In conclusion the present study has indicated that a simple extraction of tissue sample was sufficient to detect *H. pylori* which will reduce the time and cost of analysis.

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