

Loop-Mediated Isothermal Amplification as a Fast Noninvasive Method of *Helicobacter pylori* Diagnosis

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Background: *Helicobacter pylori* infection is etiologically associated with some important health problems such as gastric cancer. Because of the high clinical importance of *H. pylori* infection, development of a non-invasive test for the detection of *H. pylori* is desirable. **Methods:** In this study, a loop-mediated isothermal amplification (LAMP) targeted *ureC* of *H. pylori* was evaluated on 100 stool specimens and compared with a stool antigen test. Culture and rapid urease test were considered as gold standards.

Results: The overall detection rate of the fecal antigen test and LAMP was 58% and 82%, respectively. The analytical sensitivity of the fecal antigen test and LAMP was 500 and 10 *H. pylori* cells/g and 10 fg DNA/reaction, which is equal to six *H. pylori* genome. **Conclusion:** LAMP technique has been characterized by high sensitivity and low detection limit for the detection of *H. pylori* in stool specimen. Clinical diagnostic performance of LAMP was better than the stool antigen test. *J. Clin. Lab. Anal.* 00:1–7, 2015. © 2015 Wiley Periodicals, Inc.

Key words: clinical performance; *Helicobacter pylori*; limit of detection; loop-mediated isothermal amplification; sensitivity; stool antigen test

INTRODUCTION

Helicobacter pylori is a curved, microaerophilic, and Gram-negative rod bacterium. This bacterium is the most common human pathogen such that over half of the world's population is infected with this bacterium (1). In developed and developing countries, about 50% and 90% of adults, respectively, are infected with the bacteria (2). This bacterium is considered as the main cause of gastritis, gastric and duodenal ulcers, gastric adenocarcinoma, and MALT lymphoma (3).

In 1994, the International Agency for Research on Cancer introduced *H. pylori* as a class A carcinogens (definite carcinogen) (4). Gastric cancer, as a consequence of chronic gastric infection by *H. pylori*, is the fourth most common cancer and the second leading cause of cancer-related death in the world (5, 6).

Several methods can be used to diagnose *H. pylori* infection, which are divided into invasive and noninvasive

methods based on the use of endoscopy. Histopathology, culture, and rapid urease test (RUT) are considered as the invasive tests. Fecal antigen test, urea breath test (UBT), and serology are known as the noninvasive tests (7, 8).

It is reported that mortality and morbidity rates at a single endoscopy were one in 2,000 and one in 200 people, respectively (9, 10). Therefore, great efforts have been performed to develop an accurate and reliable noninvasive method to diagnose *H. pylori*.

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acid technology for its detection in clinical, biological, and environmental samples have been developed (22, 26–34).

Stool specimen has been considered and used as a sample for the development of a direct method of *H. pylori* detection by many investigators because it is easy to collect by noninvasive access (35). A lot of studies have been conducted for detecting *H. pylori* in stool samples based on PCR with target sequences such as *ureA*, *ureC*, and 16S rRNA genes from different populations. Different sensitivity and specificity have been reported ranging from 25% to 100% and 80% to 100%, respectively (33, 36–42). However, the result of these tests depend on the quality and amount of DNA recovered, the target sequences, differences in the specificity and sensitivity of the primers used, and the nature of the amplification protocol (43). As a consequence of the mentioned obstacles, the frequency of the *H. pylori* DNA detection in stool specimen also varies from 25% to 100%.

In addition to improved sensitivity and specificity, a diagnostic test for *H. pylori* detection must be rapid in performance, cost effective, and have potential of standardization and specially noninvasiveness. Since endoscopy as an invasive procedure is not necessary in many patients unless in patients with alarming symptoms, the development of diagnostic test for *H. pylori* detection has traditionally focused on a noninvasive test (11).

LAMP, as a novel nucleic acid isothermal amplification technique, has been described by Notomi et al. (44), and nowadays known as a rapid, specific, sensitive, cost-effective, easy-operating, and most promising molecular diagnostic test for infectious pathogens (23, 30, 44, 45).

The aim of this study was to evaluate the LAMP reaction as a fast noninvasive method and its diagnostic value for the detection of *H. pylori* in stool of patients.

The overall detection rate of the fecal antigen test was 58% (58/100). The sensitivity, specificity, PPV, and NPV of the fecal antigen test were comparable to other studies, which were 79.3%, 71.4%, 79.3%, and 71.4%, respectively. The sensitivity and specificity of the fecal antigen test, which have been reported in previous studies, range from 67% to 100% and 83% to 99%, respectively. While the sensitivity and specificity were significantly higher when the monoclonal fecal antigen test has been used (43, 46, 47), it must be emphasized that monoclonal kits are not affordable in terms of price (48, 49). In addition, the sensitivity of the stool antigen test will decrease in frozen fecal sample. The analytical sensitivity of the stool antigen test kit used in this study was 500 bacteria per gram of feces, whereas in other studies the analytical sensitivity was not checked.

The overall detection rate of the LAMP method was 82% samples (82/100). The sensitivity, specificity, PPV, and NPV were 100%, 42.8%, 70.7%, and 100%, respectively. The LAMP test was positive in 24 stool specimens, which were negative in the fecal antigen test. This is be-

cause the *H. pylori* is present at a low number in fecal samples and only a test with sufficient limit of detection or high analytical sensitivity can detect it. The LAMP method has a detection limit as low as ten *H. pylori* cell per gram of feces, which is 50 times more sensitive than the fecal antigen test. In addition, the inhibitors, which are present in extracted DNA from stool, were tolerated by Bst DNA polymerase in the LAMP reaction (35).

However, the analytical sensitivity was not evaluated in many studies, but the analytical sensitivity of the LAMP for detection of *H. pylori* in stool samples achieved in this study was more than earlier studies using other molecular tests (50).

The sensitivity and specificity that have been reported in previous studies ranged from 42.6% to 93.7% and 92.3% to 100%, respectively (42, 48, 50).

For example, in a study using a real-time PCR technique the sensitivity and specificity of *H. pylori* detection in feces have been reported as 69% and 100%, respectively (33). These values for EIA were 88.9% and 94.6% (50). Low specificity of the mentioned methods is due to low limit of detection or high analytical sensitivity. Thus, it is suggested that the gold standard for *H. pylori* diagnosis should be revised.

The limit of detection was also determined using pure DNA. The minimum amount of the pure *H. pylori* DNA, which was detected by the LAMP method, was 10 fg. This amount of DNA is approximately equal to six *H. pylori* genome. This means the limit of detection of pure DNA was equal to the one achieved by 16SrRNA-PCR (29, 42) and ten times more sensitive than the earlier study (30).

CONCLUSIONS

LAMP technique has been characterized by high sensitivity and low detection limits for the detection of *H. pylori* in stool specimens, and the clinical diagnostic performance was better than the stool antigen test.

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CONFLICT OF INTEREST

The authors have declared no conflict of interest.