Sueptrakool Wisessombat et al., J Gastroint Dig Syst 2014, 4:3
http://dx.doi.org/10.4172/2161-069X.S1.021

OMICS Group Conferences
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3rd International Conference on
Gastroenterology & Urology
July 28-30, 2014 DoubleTree by Hilton Hotel San Francisco Airport, USA

Multiplex PCR for identification of *Helicobacter pylori* and simultaneous detection of cag A gene Crohn's disease

Sueptrakool Wisessombat[1], Chatruthai Meethai[2], Souvalak Hamgo[2], Beebah Bendusoh[2], Sudarat Wongkidakam[2] and **Apisara Wannapruk[2]**
[1]Walailak University, Thailand
[2]Prince of Songkla University, Thailand

Helicobacter pylori, Gram-negative microaerobic bacterium is associated with human gastritis, gastric ulcer and gastric cancer. Conventional culture methods for identification of *H. pylori* are complex, labor intensive, and time-consuming. The objective of the present investigation was to establish a multiplex PCR for the identification of *H. pylori*. Reference strains including *H. pylori* LMG 8775 and NCTC 11638 were used for the validation in this study. *H. pylori* were cultured on Brain Heart Infusion blood agar at 37°C for 48 h at microaerobic atmosphere. Bacterial DNA was extracted and purified by QIAamp DNA Mini Kit. Multiplex PCR detected three genes of 16S rRNA, *cag*A encoding for virulence factor cytotoxin-associated gene A, and ureC for housekeeping *urease* gene C. The best combination of primers and the annealing temperature of multiplex PCR were examined. The result indicated that multiplex PCR with annealing temperature at 57°C was able to effectively amplify specific products. In total, 55 gastric biopsies from dyspeptic patients were comparatively studied using rapid urease test (CLO test) and multiplex PCR. The results revealed that CLO test detected *H. pylori* in 7 gastric biopsies (12.73%). Whereas, 6 specimens evaluated by the multiplex PCR were identified as *H. pylori* (10.91%). In addition, *cag*A gene was detected in all positive samples. This system is useful for the detection of the presence of *cag*A gene that is responsible for toxin activity in *H. pylori*.

sueptrakool.wi@wu.ac.th