

November 29, 2013

Title : Dual-Priming Oligonucleotide-based multiplex PCR (DPO-PCR) using Tissue Samples in Rapid Urease Test.

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1. Authors should state **the sensitivity and specificity** by using the dual-priming oligonucleotide-based multiplex polymerase chain reaction (**DPO-PCR**) in the **literature**.

....On the basis of the C¹³-urea breath test, rates of *H. pylori* detection by DPO-based multiplex PCR had a sensitivity of 87.5%, a specificity of 91.3%, a positive predictive value of 84.0%, a negative predictive value of 93.3%, and an accuracy of 90.0%.....

(Lee HJ, et al. J Infect Dis. 2013;208(7):1123-30.)

2. Authors stated that the diagnosis of *H. pylori* infection was made based on 1) histologic evidence of *H. pylori* in any of two specimens taken from antrum / corpus by silver stain or 2) positive results of CLO and serological test.

How about the **sensitivity and specificity in using these criteria as gold standard?**

... When *H. pylori* infection status was established by a concordance of test results with histology, a rapid urease test, serology and culture, the results of histology and a rapid urease test were revealed with good sensitivity (92-100%) and specificity (85-96%).

(Onders RP. Detection methods of Helicobacter pylori: accuracy and costs. Am Surg. 1997;63(8):665-8.)

3. Authors should clarify how **the diagnosis of HP** was made in their 35 patients.

Table 1.

Method of detection	Number of positivity	Number of <i>H. pylori</i> infection (%)
Silver stain – 1 st session	30	30 (85.7)
2 nd session	2	
CLOtest 1 st session	26	26 (74.3)
2 nd session	3	
DPO-PCR of gastric biopsy	37	34 (97.1)
DPO-PCR using tissue sample of CLO kit	34	33 (94.3)

4. **Clinical manifestations** were not mentioned, such as the usage of PPI or antibiotics before the endoscopy and *H. pylori* evaluation, correlations between types of ulcers and *H. pylori* infections, details in the treatment protocol.

... Patients were not permitted to take antibiotics and proton pump inhibitor (PPI) in the two months before the study enrollment. None of the patients had a history of *H. pylori* eradication or previous gastric surgery. Patients were also excluded if they had significant renal, hepatic, cardiovascular, metabolic or hematological disorders. Additionally, pregnant

or lactating women were excluded from our investigation....

.... If the result was *H. pylori* -negative on silver stain and CLO test, re-biopsy specimens under endoscopy was taken after 4 ~8 weeks of initial examination. Patients should not take PPIs for at least 2 weeks before endoscopy....

We revised the manuscripts.

5. Authors should clarify **how the rate of successful eradication** derived.

In results,

.... A total 28 patients with peptic ulcer disease were recommended with eradication therapy of *H. pylori* and follow-up was incomplete in 4 patients. Twenty four patients had completion of the standard eradication therapy for 7 days.....

.... *H. pylori* eradication (intention to treat) was successful in 23/28 (90.3%) and per protocol analyses were 95.8% (23/ 24).

6. Author didn't compare their results to those in the literature, we would like to know any **differences** were found.

In discussion,

... Previously, several PCR assays have been developed to detect *H. pylori* DNA in clinical specimens and have proved to be highly sensitive compared to the conventional assays. DPO-PCR is new technique and based on a multiplex PCR using a DPO patented technology. The structure of the DPO primers is fundamentally different from that of conventional primers....

... It was proven that the gastric biopsy specimens in an RUT gel can be used to confirm the

diagnosis of *H. pylori* infection despite having been stored at room temperature for 30 days. *H. pylori* DNA could be detected by PCR on gastric biopsy specimens obtained from the RUT kit. We combined the ideas and designed the study to determine the diagnostic accuracy of DPO-PCR test using tissue specimens from RUT kit...

7. Concerning about clarithromycin resistance, authors should **clarify the sensitivity and specificity of diagnosing resistance by using the 23S rRNA point mutation.**

In discussion,

... Clarithromycin is a key antibiotic of the standard triple therapy regimens for *H. pylori* infection. However, its resistance is a major determinant in a failure of eradication therapy. It has been demonstrated that A-to-G point mutations at positions 2142 and 2143 within domain V, or a T-to-C mutation at position 2717 within domain VI of the 23S rRNA gene cause clarithromycin resistance. Moreover, DPO - PCR was known as an alternative to culture and testing of clarithromycin resistance to *H. pylori*. Clinically, it was shown that the sensitivity of DPO-PCR was 97.7% and specificity was 83.1%, considering culture as the reference test....

(Lehours P, et al. BMC Gastroenterol. 2011;11:112-16.)

8. **Cost, time consuming, labor using, sensitivity, specificity and effectiveness** should be taken into account when using a new technique, authors should clarify in their study.

In discussion,

The cost of DPO- PCR is more expensive than that of rapid urease test. It is highly dependent on the activity and equipment of the laboratory in which the test is performed. However, the use of gastric biopsy specimens from the RUT kit can reduce the need of re-endoscopic examination with biopsy. Especially, when the RUT is negative and there is a suspicion of *H.*

pylori, our method will greatly lighten clinician and patient's burden. In addition, when clarithromycin is the first-choice drug or in countries with high prevalence of primary clarithromycin resistance, it will alleviate social and economic costs for medical assurance....

Dear Editor

Thank you very much for giving us an opportunity for revision.

Accurate and kind comments by the reviewer have been addressed. We also believe that these comments improved and clarified our manuscript. We responded to your suggestion by way of inserting memo in the attached file. Changes have been made by underlined in the revised manuscript to avoid any confusion.

We are looking forward to good response.

Thank you.

Sincerely,
Woo Chul Chung
Sung Hoon Jung