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Original Article

Comparison between phenol red chromo-endoscopy and a stool rapid immunoassay for the diagnosis of *Helicobacter pylori* in patients with gastritis



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ABSTRACT

Helicobacter pylori infection is a widespread problem all over the world. Non-invasive techniques are demanded for rapid diagnosis and treatment follow up. The aim of this study was to compare the diagnostic value of phenol red chromo-endoscopy and stool (Rapid Strip HpSA) for *H. pylori* detection with reference to histopathology as the gold standard. A total of 80 adult patients with dyspepsia were enrolled on this study. Patients underwent phenol red chromo-endoscopy. Multiple Gastric biopsies were taken and examined for *H. pylori* detection. Stool sample was collected from every patient for Rapid Strip HpSA test. The study included 38 males (47.5%) and 42 females (52.5%) with their ages ranged between 19 and 56 years. According to histopathology, 71 patients (88.8%) were *H. pylori* positive and 9 (11.2%) were negative, most of biopsies showed inflammation with variable degree of activity, which showed significant statistical correlation with the density of *H. pylori* ($P < 0.05$). Phenol red chromo-endoscopy had 90.1% sensitivity, 88.9% specificity, 98.5% positive predictive value (PPV), 53.3% negative predictive value (NPV) and 90% accuracy. Rapid Strip HpSA had a sensitivity 93%, 77.8% Specificity, 97.1% PPV, 58% NPV and 91.3% accuracy. In conclusion; Phenol red chromo endoscopy was more specific and less sensitive than the rapid stool Rapid Strip HpSA[®] test regarding the detection of *H. pylori* infection with reference to histopathology as a gold standard, yet both showed high diagnostic accuracy; thus they can be used as reliable diagnostic tools for *H. pylori* infection in cases contraindicated for gastric biopsy.

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1. Introduction

The prevalence of *Helicobacter pylori* (*H. pylori*) shows large different geographical variations. In various developing countries, more than 80% of the population is *H.*

pylori positive, even at young ages [1]. Infections are usually acquired in early childhood in all countries. The higher prevalence among the elderly reflects higher infection rates when they were children rather than infection at late ages [2]. Presence of *H. pylori* is known to be associated with a wide range of gastrointestinal disorders including peptic ulcer, gastric carcinoma, and mucosa-associated tissue lymphoma, thus the diagnosis and eradication of the pathogen is crucial for the management of these diseases [3].

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A variety of highly sensitive and specific tests are available to diagnose *H. pylori* infection; one of them is an invasive method based on endoscopy and gastric biopsies. Histopathology has been considered to be the gold standard test for detection of *H. pylori* infection [4]. Although achieving sensitivity and specificity of >95% in *H. pylori* diagnosis, false negative results are unavoidable due to the uneven distribution of the organism across the gastric mucosa [5]. Furthermore, the detection of *H. pylori* by histopathology relies upon a number of issues including the site, number, and size of gastric biopsies, method of staining, and the level of experience of the examining pathologist [6].

Another method is chromo-endoscopy, which refers to the topical application of stains at the time of endoscopy in an effort to enhance tissue characterization, differentiation, or diagnosis [7], it has been used in the evaluation of various gastrointestinal lesions as Barrett's esophagus, gastric metaplasia and adenocarcinoma. Phenol red is a pH indicator; it detects alkaline pH by a color change from yellow to red, the urease produced by the bacterium catalyzes hydrolysis of urea to NH_3 and CO_2 , resulting in an increase in pH. As a result, *H. pylori* can be observed in red stained mucosa after phenol red chromo-endoscopy [8]. The use of phenol red for the diagnosis of *H. pylori* infection was initially described and used by Kohli et al. to assess infection distribution in the gastric mucosa [9].

There are other noninvasive methods for detection of *H. pylori* infection; including serology, urea breath tests (UBTS) and stool antigen test which is based on a rapid immunoassay (Rapid Strip HpSA[®]) that utilizes a monoclonal *H. pylori* antibody with sensitivity 96.1% (Meridian Bioscience Europe). In the last few years, more interest has been paid for the noninvasive techniques [10].

The availability of various tests for detection of gastric *H. pylori* infection with variable efficiency raises the question of which of these methods is more efficient? Thus the current study was done to compare between the diagnostic value of phenol red chromo-endoscopy in *H. pylori* detection and the rapid immunoassay in stool specimens (Rapid Strip HpSA[®]) with reference to histopathology as the gold standard.

2. Patients and methods

This study was done at the Gastrointestinal Endoscopy Unit; Ain Shams University Hospital in the period between Jan and Jun 2014. The hospital ethical committee approved the study protocol. Informed consent was obtained from each patient enrolled on the study.

80 patients were included in this study. All patients were >18 years old and suffered of dyspepsia, they were referred for upper gastrointestinal endoscopy. Any patient received *H. pylori* eradication therapy up to 6 months before the endoscopic procedure, or those with gastric surgery were excluded.

Upper gastrointestinal endoscopy was carried out for all patients using Olympus XQ-30 endoscope (Olympus Co., Tokyo, Japan) with forward vision. Gastric juice was

aspirated to improve visibility during endoscopy. Chromo-endoscopic staining, using a spray type catheter (PW-5L-1; Olympus Co., Tokyo, Japan) was done. 20 ml of phenol red at 0.1% concentration were instilled over the mucosa of the gastric cavity in a homogeneous way in all patients. After a minute the reaction of the mucosa to the application of the dye was visualized. Red staining of the mucosa either diffuse or focal indicated a positive reaction while yellow staining meant negative.

Multiple biopsy samples of both the antrum, corpus, lesser and greater curvatures of gastric mucosa were taken, using standard biopsy forceps. The samples were fixed in 10% formalin and routinely processed. After staining with hematoxylin and eosin, the slides were examined by experienced pathologist who was unaware of the red phenol stain results. Adequacy of the biopsies was evaluated; 77 cases were adequate, in the remaining three cases the biopsy procedure was repeated. The histopathological examination was done to detect the presence and extent of activity (neutrophilic infiltrate), chronic inflammation (lymphocytes and plasma cells infiltrate), surface epithelial atrophy, the intestinal metaplasia and the density of *H. pylori* organism infection. The changes were scored semi-quantitatively as (0 for absent, 1+ for mild, 2+ for moderate or 3+ for severe) according to Sydney' system [11]. Geimsa stain was used in suspicious cases.

H. pylori stool antigen test (HpSA[®]) was done for every patient; a fresh stool sample was collected from each case and delivered to an expert technician who was unaware of the phenol red stain results; a small portion was emulsified with diluents in a test tube by using an applicator stick. A diluted patient stool sample is dispensed into the sample port of the test device (containing *H. pylori* monoclonal antibody) and the appearance of a pink-red line in the reading window next to the letter T after 5 minutes of incubation at room temperature (20°–26° C) indicated a positive result. The test was considered negative when only one blue colored band (control band) appeared across the white central area of the reaction strip (Meridian Bioscience; Inc.).

3. Statistical analysis

Data was collected and statistically analyzed using SPSS v.18.0 (IBM Corp., Armonk, NY, USA). Quantitative and semi-quantitative variables were described as mean \pm SD, while qualitative variables were described as frequency and percentage. Also sensitivity, specificity, positive and negative predictive values (PPV) (NPV), accuracy, positive and negative likelihood ratios, and diagnostic odds ratio were calculated. Concordance correlation coefficient (kappa coefficient) and Kappa index for agreement test were done to estimate the correlation between the two diagnostic methods (phenol red chromo-endoscopy and stool antigen test) with reference to histopathology as the gold standard. Chi-Square test was used to assess the statistical difference between the semi-quantitative histopathological variables and density of *H. pylori* infection. *P* value <0.05 was used to indicate statistical significance.

Table 1
The study population characteristics.

Parameter	Findings	
Age (years)		
Range	19–56	
Mean \pm SD	35.8 \pm 8	
Sex		
Male	38	47.5%
Female	42	52.5%
Endoscopic findings		
Normal	22	27.5%
Gastritis	49	61.2%
Duodenal ulcer	1	1.3%
Gastric ulcer	2	2.5%
Hiatus hernia	2	2.5%
Gastro-eosophageal reflux disease	4	5%
Histopathological examination (<i>H. pylori</i>)		
Positive	71	88.8%
Negative	9	11.2%
Phenol red chromoendoscopy		
Positive	65	81.2%
Negative	15	18.8%
Stool antigen test		
Positive	68	85%
Negative	12	15%

SD, standard deviation.

4. Results

A total of 80 patients were included in this study. Thirty-eight patients were males (47.5%) and 42 patients were females (52.5%). Patients' ages ranged between 19 and 56 years with a mean age of 35.8 \pm 8 years (Table 1).

The most common endoscopic change in the studied patients was gastritis in 49 patients (61.2%), while 22 patients (27.5%) had normal endoscopic findings (Table 1).

According to histopathological examination, 71 patients (88.8%) were *H. pylori* positive and 9 patients (11.2%) were negative. Phenol red chromo-endoscopy examination revealed that 65 patients (81.2%) were positive for *H. pylori*, while 15 patients (18.8%) were negative, meanwhile, stool antigen test showed 68 patients (85%) were positive for *H. pylori* and 12 (15%) were negative (Table 1).

The commonest microscopic findings of the gastric biopsies were gastric chronic inflammation in 79 cases

Table 3
Performance of phenol red chromo-endoscopy and stool antigen test for *H. pylori* detection and their agreement.

Statistical results	Phenol red test	Stool antigen test
Area under the ROC curve (AUC)	0.895	0.854
Standard error	0.0583	0.0751
95% confidence interval	0.806–0.953	0.757–0.923
z statistic	6.773	4.712
Significance level P (area = 0.5)	<0.0001	<0.0001
True positive (TP)	64	66
True negative (TN)	8	7
False positive (FP)	1	2
False negative (FN)	7	5
Sensitivity (95% CI)	90.1 (80.7–95.9)	93.0(84.3–97.7)
Specificity (95% CI)	88.9 (51.8–99.7)	77.8 (40.0–97.2)
Positive predictive value (PPV)	98.5	97.1
Negative predictive value (NPV)	53.3	58
Likelihood ratio positive (95% CI)	8.11 (1.3–51.6)	4.18 (1.2–14.2)
Likelihood ratio negative (95% CI)	0.11 (0.05–0.2)	0.09 (0.04–0.2)
Diagnostic accuracy	90.0	91.3
Kappa index for agreement (95% CI)	0.612 (0.373–0.851)	0.617 (0.360–0.875)

(98.8%), followed by *H. pylori* infection in 71 patients (88.8%) and neutrophilic infiltration in 68 cases (85%), while mucosal atrophy was found in only in 27 patients (33.8%) and intestinal mataplasia was not encountered in any case. The semi-quantitative assessment of *H. pylori* infection showed a significant statistical correlation with activity, inflammation and mucosal atrophy ($P < 0.05$) (Table 2) (Fig. 1).

With histopathology as gold standard, phenol red chromo-endoscopy test had one false positive result, 7 false negative results, 8 true negative results and 64 true positive results. However, stool antigen test had 66 true positive, 2 false positive and 7 true negative and 5 false negative results (Table 3).

Phenol red chromo-endoscopy had 90.1% sensitivity, 88.9% specificity, 98.5% and 90% accuracy. As regard stool antigen test; it had a sensitivity of 93.0%, 77.8% specificity and 91.3% accuracy. Kappa index for agreement (95% CI) was (0.612, 0.617) for phenol red chromo endoscopy and stool antigen test respectively as shown in Table 3 (Figs. 2 and 3).

Table 2
Histopathological data using Sydney scoring system.

The microscopic findings	0 Number (%)	1+ Number (%)	2+ Number (%)	3+ Number (%)	Mean \pm SD	Chi-sq	P*
Activity (Neutrophilic infiltrate)	12 (15%)	39 (48.7%)	26 (32.5%)	3 (3.8%)	1.25 \pm 0.75	10.2	0.02
Chronic inflammation	1 (1.2%)	23 (28.8%)	38 (47.5%)	18 (22.5%)	1.9 \pm 0.75	11.3	0.01
Epithelial atrophy	53 (66.2%)	16 (20%)	11 (13.8%)	0	0.48 \pm 0.73	54.8	0.00
Intestinal metaplasia	80 (100%)	0	0	0	0 \pm 0		
<i>Helicobacter pylori</i>	9 (11.2%)	23 (28.8%)	40 (50%)	8 (10%)	1.56 \pm 0.84		

P* is the statistical correlation value between the scores of different parameters and the density of *Helicobacter pylori* infection. SD, standard deviation.

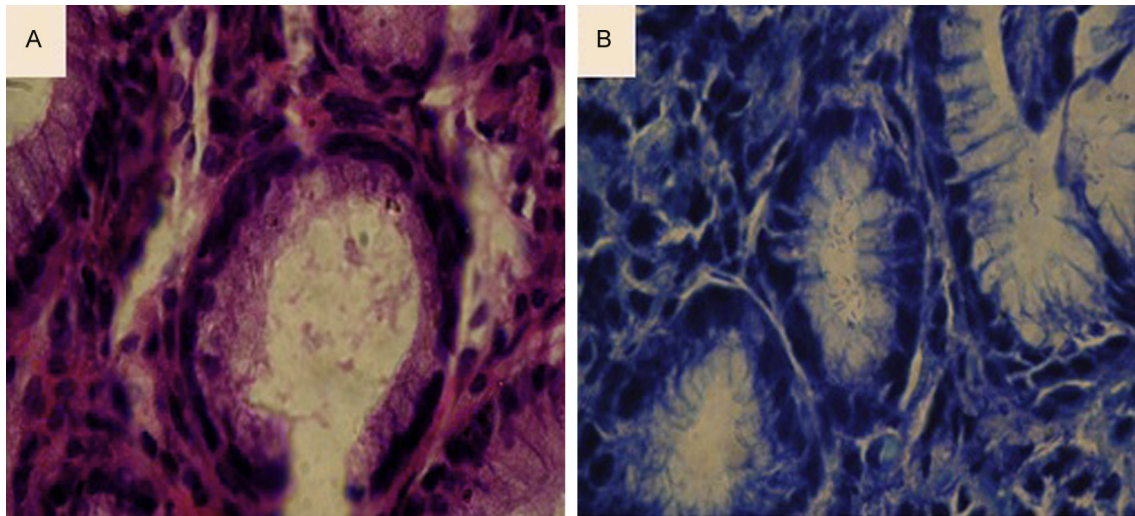


Fig. 1. (A) Gastric glands contain curved thread like structures close and some are adherent to the epithelial surface (H&E \times oil immersion). (B) The *Helicobacter pylori* bacilli are more clearly visible inside the glandular lumen (Geimsa stain \times oil immersion).

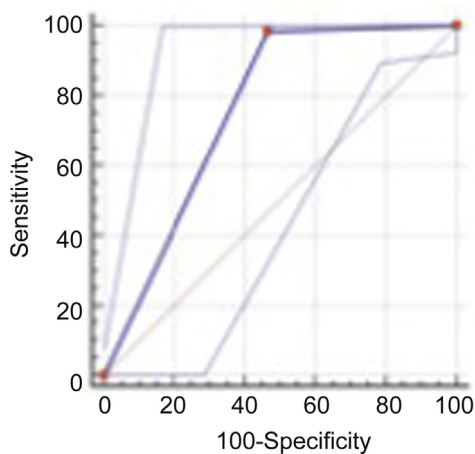


Fig. 2. Phenol red chromo-endoscopy ROC curve.

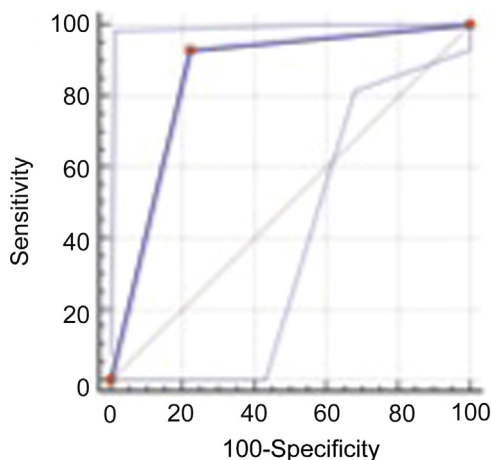


Fig. 3. Stool antigen test ROC curve.

5. Discussion

We observed that phenol red chromo-endoscopy was more specific and less sensitive than the rapid stool antigen test (Rapid Strip HpSA[®]) regarding the detection of *H. pylori* infection with reference to histopathology as a gold standard; phenol red chromo endoscopy had 90.1% sensitivity, 88.9% specificity, while Rapid Strip test (HpSA[®]) had a sensitivity of 93.0% and 77.8% specificity. Overall, both showed high diagnostic accuracy of 90% and 91.3% for phenol red chromo-endoscopy and Rapid Strip test (HpSA[®]) respectively. They represented valuable ways of diagnosis of *H. pylori* infection without compromising accuracy of detection.

The commonest microscopic findings of the gastric biopsies beside *H. pylori* were inflammation with variable grades of activity, while the least common was mucosal atrophy. Intestinal metaplasia was not found in any studied case.

One of the limitations of this study is the small number of the patients free of *H. pylori* infection (9 patients) in comparison to the positively infected cases (71 patients); this is due to the fact that the study group was confined to patients with dyspepsia. Another limitation is the lack of correlation between the endoscopic findings and the Sydney Scoring system; as the taken biopsies were not topographically labeled.

Strengths of the study include the reference of histopathology as the gold standard, together with the use of special stains to confirm the diagnosis. To the best of our knowledge this is the first study that compares phenol red chromo-endoscopy with stool antigen test for *H. pylori* detection.

Our findings regarding phenol red chromo-endoscopy (90.1% sensitivity, 88.9% specificity, a positive predictive value (PPV) of 98.5%, negative predictive value (NPV) of 53.3% and accuracy of 90%) were in agreement with Iseki et al. [8] who showed that dye's sensitivity and specificity to detect *H. pylori* were 95% and 92%, respectively in sixty-five

patients with early gastric cancer before their operations. Also Ahumada et al. [12] study included 160 patients; they claimed that phenol red chromo-endoscopy had a sensitivity of 91% and specificity of 89%.

On the other hand, Mitsuhashi et al. [13] reported a lower sensitivity of 74.3% and a higher specificity of 100%, with positive and negative predictive values of 100% and 72.7% respectively in a sample of 82 surgically resected stomachs with early gastric carcinomas. Such controversy might be due to the difference in sampling technique; as our study the sampling was endoscopic, while they obtained the biopsies after the operation with free access to every part of the stomach. Also Hernández-Garcés et al. [14] study in Cuba on 195 patients showed a phenol red chromo-endoscopy sensitivity of 72.6% and specificity of 75.5% with Kappa coefficient of 0.4, with a positive predictive value of 89.8% and negative predictive value of 48.1% with diagnostic accuracy of 73.3%.

Furthermore, in our study with the histopathology as gold standard, a rapid immunoassay for *H. pylori* antigen detection in stool specimen (Rapid Strip HpSA[®]) was found to have sensitivity of 93%, 77.8% specificity, 97.1% positive predictive value (PPV), 58% negative predictive value (NPV) and 91.3% accuracy. Such results matched with the results of Cheng and Hu [15] who studied 80 patients; the sensitivity, specificity, and accuracy of HpSA test were 100%, 93.2%, and 96.3%, respectively. Also Lu et al. study of one hundred-twenty patients showed that the stool strip HpSA[®] test relative to the confirmed results had sensitivity, specificity, and accuracy 96.8%, 82.8% and 90%, respectively [16].

The microscopic examination of the gastric biopsies showed that *H. pylori* infection was significantly correlated with neutrophilic infiltrate, chronic inflammation and mucosal atrophy ($P < 0.05$), this is in agreement with Xu et al. [17] who studied 315 patients with dyspepsia, he found that neutrophilic infiltration may be recognized as a sign and a sensitive indicator of *H. pylori* infection. Also Tanko et al. [18] who examined gastric biopsies of 100 patients with dyspepsia, he found a significant correlation between the intensity of *H. pylori* infection and neutrophilic infiltration, chronic inflammation and intestinal metaplasia, in their study the mucosal atrophy was found in 38% of the studied patients. This was in agreement with our study, in which the mucosal atrophy was represented in 33.8% of cases. The absence of intestinal metaplasia in our study might be attributed to the nature of the studied cases; as they were newly complaining patients of dyspepsia.

Our findings suggest that phenol red chromo-endoscopy is a useful valid method for *H. pylori* detection with its advantages that include immediate reading, ability to detect focal and scattered infection which can be missed by random biopsy taking, so it is a reliable diagnostic test in cases contraindicated for the biopsy as in diseases of coagulopathy. Its value is nearly similar to that of stool antigen test (Rapid Strip HpSA[®]), which can be used for the follow up of treatment of *H. pylori* infection.

Further researches are needed in the field of gastritis associated with *H. pylori* infection, with further

correlation between the endoscopic findings and the Sydney histopathological scoring system. This will give a clue of whether the endoscopy can be used as a gold standard for the diagnosis of *H. pylori* infection.

6. Conclusion

Phenol red chromo-endoscopy was more specific and less sensitive than the rapid stool antigen test (Rapid Strip HpSA[®]) regarding the detection of *H. pylori* infection with reference to histopathology as a gold standard. Yet both showed high diagnostic accuracy; thus they can be used as reliable diagnostic tools for *H. pylori* infection in cases contraindicated for gastric biopsy.

Conflict of interest

The authors received no financial support for their research, and they report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

References

- [1] Pérez-Perez GI, Rthenbacher D, Brenner H. Epidemiology of *Helicobacter pylori* infection. *Helicobacter* 2004;9(1):1–6.
- [2] Kusters JG, van Vliet AH, Kuipers EJ. Pathogenesis of *Helicobacter pylori* infection. *Clin Microbiol Rev* 2006;19(3):449–90.
- [3] Georgopoulos SD, Papastergiou V, Karatapanis S. *Helicobacter pylori* eradication therapies in the era of increasing antibiotic resistance: a paradigm shift to improved efficacy. *Gastroenterol Res Pract* 2012;2012:757926.
- [4] van Ijzendoorn MC, Laheij RJ, de Boer WA, Jansen JB. The importance of corpus biopsies for the determination of *Helicobacter pylori* infection. *Neth J Med* 2005;63(4):141–5.
- [5] Cutler AF, Havstad S, Ma CK, Blaser MJ, Peres-Perez GI, Schubert TT. Accuracy of invasive and noninvasive tests to diagnose *Helicobacter pylori* infection. *Gastroenterology* 1995;109(1):136–41.
- [6] el-Zimaity HM. Accurate diagnosis of *Helicobacter pylori* with biopsy. *Gastroenterol Clin North Am* 2000;29(4):863–9.
- [7] ASGE Technology Committee, Wong Kee Song LM, Adler DG, Chand B, Conway JD, Croffie JM, et al. Chromoendoscopy. *Gastrointest Endosc* 2007;66(4):639–49.
- [8] Iseki K, Tatsuta M, Iishi H, Baba M, Ishiguro S. *Helicobacter pylori* infection in patients with early gastric cancer by the endoscopic phenol red test. *Gut* 1998;42(1):20–3.
- [9] Kohli Y, Kato T, Itoh S, Iwaki M, Yamazaki Y, Hata M. Endoscopic diagnosis of *Helicobacter pylori* distribution on human gastric mucosa in vivo. *J Kyoto Prefect Univ Med* 1991;100:219–25.
- [10] Kim JH, Kim HY, Kim NY, Kim SW, Kim JG, Kim JJ, et al. Seroepidemiological study of *Helicobacter pylori* infection in asymptomatic people in South Korea. *J Gastroenterol Hepatol* 2001;16(9):969–75.
- [11] Price AB. The Sydney system: histological division. *J Gastroenterol Hepatol* 1991;6:209–22.
- [12] Ahumada Trujillo JM, Martínez Carrillo MO, Cruz Parada MC, Gómez Peña Alfaro NS, Gutiérrez AR, Benítez Rodríguez, et al. Utilidad del rojo fenol en la detección endoscópica del *Helicobacter pylori* (phenol red utility for endoscopic *H. pylori* detection). *Endoscopia* 2007;19(1):112–3.
- [13] Mitsuhashi J, Mitomi H, Koizumi W, Kikuchi S, Okayasu I, Saigenji K. Spraying of phenol red dye as a screening test for *Helicobacter pylori* infection in surgically resected stomach specimens. *J Gastroenterol* 2003;38:1049–52.
- [14] Hernández-Garcés HR, Castellanos-González VV, González-Fabián L, Infante-Velázquez M, Peña K, Andraín-Sierra Y. Chromoendoscopy with red phenol in the diagnosis of *Helicobacter pylori* infection. *Rev Esp Enferm Dig* 2012;104:4–9.
- [15] Cheng H, Hu FL. The value of *Helicobacter pylori* stool antigen ImmunoCard STAT in diagnosis of HP infection and assessing of eradication of HP. *Zhonghua Yi Xue Za Zhi* 2004;84(14):1166–70.

- [16] Lu CY, Kuo FC, Wang SW, Lo YC, Wu IC, Chang LL, et al. The clinical applications and accuracy of 2 rapid near-patient tests in detecting *Helicobacter pylori* infection. *Diagn Microbiol Infect Dis* 2006;56(3):241–6.
- [17] Xu XQ, Wang ZH, Liao JX, Chen XY, Liu WZ, Xiao SD, et al. Predictive value of neutrophil infiltration as a marker of *Helicobacter pylori* infection. *World J Gastroenterol* 2012;36(18): 5101–5.
- [18] Tanko MN, Manasseh AN, Echejoh GO, Mandong BM, Malu AO, Okeke EN, et al. Relationship between *Helicobacter pylori*, inflammatory (neutrophil) activity, chronic gastritis, gastric atrophy and intestinal metaplasia. *Niger J Clin Pract* 2008;11(3):270–4.