Comparison of Four Invasive Methods for Diagnosis of *Helicobacter pylori* Infection: Fluorescence in situ Hybridization, Histology, Culture, Rapid Urease Test

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Abstract—**Background:** *Helicobacter pylori* (*H. pylori*) is one of the most prevalent pathogenic bacteria globally. Choosing reliable methods will lead to a correct diagnosis of infection. The aim of this study was to evaluate four *H. pylori* infection diagnostic methods from dispeptic patients. **Methods:** In this descriptive cross-sectional study, 165 antrum biopsy specimens were obtained from dyspeptic patients referred to the endoscopy unit of Shariati Hospital, Isfahan, Iran, and collected in 2018. Four diagnostic methods of *H. pylori*, namely histology, culture, rapid urease test (RUT) and fluorescence in situ hybridisation (FISH) were tested for each patient. The gold standard of the study was for positive confirming one of the two tests, RUT or histology. **Results:** According to the predefined criteria, the prevalence of *H. pylori* infection was 55.2%. Among the four diagnostic methods, the most sensitive ones were FISH and RUT, respectively (95.7 and 92.3%). Despite the high specificity of the histological examination (100%), its NPV was lower than the other methods (88%). The kappa coefficient of agreement between the gold standard and the tested techniques was perfect ($P < 0.001$). **Conclusion:** FISH and histology are recommended in combination with diagnosis of *H. pylori* infection, which can manage its complications in the most optimal manner.

INTRODUCTION

Helicobacter pylori (*H. pylori*) is a Gram-negative and microaerophilic spiral shaped bacterium [1]. *H. pylori* infection is recognized as the major cause of chronic gastritis in the human stomach of more than half of the world population [2]. This microorganism is associated with the development of gastric cancer, peptic ulcer and gastric mucosa-associated lymphoid tissue (MALT) lymphoma, which is reported worldwide [3]. Gastric adenocarcinoma is the second resulting cause of cancer death worldwide [4]. The prevalence of acute infection rate varies among regions, seeming to be highly prevalent in developing (70−90%) rather than developed countries (25−50%) [5]. In Iran, the prevalence of gastric infection with *H. pylori* is known to be high, with the reported prevalence ranging from 36 to 90% over different geographic areas [6].

H. pylori infection can be diagnosed by invasive and noninvasive tests. In invasive methods such as histology, culture and molecular methods, endoscopic biopsy of gastric tissue is required, and noninvasive tests include UBT (Urea Breath Test) and Serology and Stool Antigen Test (SAT) which are independent of endoscopic surgery. The major limitation in invasive methods is the patchy distribution of *H. pylori* infection and the low bacterial density on the gastric mucosa [7]. Over the recent years, the use of molecular techniques such as fluorescence in situ hybridization (FISH), enzyme immunoassay and real-time polymerase chain reaction have become popular widely available for microbiologists. These techniques are able to detect pathogens directly from a gastric biopsy specimen over a brief period of time, and can be used as appropriate epidemiological screening tools [8].

Exact detection of infection is an important part of managing the eradication of *H. pylori* and treatment plan. Accordingly, specific and accurate assays are required for the most optimal management in clinical practice. However, each of the above mentioned methods has limitations and, despite the wide variety of conventional diagnostic tests, there is no general consensus as to using only one gold standard. Thus, it

is recommended that a combination of two or more techniques be employed for a more valid confirmation [9].

The aim of this study was to compare the efficacy of four different methods, namely histology, rapid urease test (RUT), culture and FISH, regarding the detection of *H. pylori* infection in gastric biopsy specimens of dyspeptic patients.

MATERIALS AND METHODS

Study Design

A total of 165 patients with dyspeptic symptoms were admitted to the outpatient Gastroenterology Clinic and Endoscopy Unit in Shariati Hospital in Isfahan, Iran. These patients underwent gastric endoscopy from April, 2018 to July, 2018. The tissue samples were assessed for gastritis and *H. pylori* infection.

Patients who had received antibiotics, H2-receptor blockers, proton-pump inhibitors (PPIs) and non-steridal anti-inflammatory drugs (NSAIDs) within 15 days prior to endoscopy were excluded.

Patient Sampling

Three antrum biopsies were obtained from each patient: one set of antrum was used for RUT; a second set was fixed and transported in 10% buffered formalin for histopathological examination. Finally, the last set of biopsies was placed in sterile Eppendorf tubes, containing 1 mL sterile physiological solution (0.9% NaCl), and immediately transported to the microbiology laboratory.

Rapid Urease Test

The antrum biopsy specimen was placed in tubes containing a urea solution. Then two drops of 1% (V/V) indicator phenol red was added. The test was considered positive when the indicator solution changed from yellow to pink. The results were recorded in less than 24 h.

Histology

The biopsy tissues in 10% formalin were processed for histopathology using an automated tissue processor (ATP). Formalin-fixed paraffin-embedded (FFPE) tissue blocks were sectioned using a microtome, cut into 3–4 micrometer sections and dewaxed and used for histopathological Staining. Slides were stained with Hematoxylin-Eosin (H&E) and Giemsa by routine protocols. Giemsa stain helps to demonstrate the presence of the *H. pylori*.

Gastritis was established according to the Updated Sydney System. All samples were evaluated by a pathologist who was not aware of the results of the other tests.

Culture

Biopsy specimens were sent to the Clinical Microbiology Lab within half an hour of sampling in sterile tubes. Then, biopsies were homogenized in saline and inoculated on selective medium Columbia Agar (Gibco, USA) supplemented with 7% sheep blood and 10% fetal calf serum (FCS) and campylobacter selective supplement (Merck, Germany). Also, a part of the homogenized tissue was inoculated into a blood agar plate. The plates were incubated for 5–10 days at 37°C in a microaerophilic environment (Anoxomat; MART Microbiology BV, Drachten, The Netherlands).

H. pylori was identified based on colony morphology, Gram stain as a gull wing-shape bacteria and also by positive reactions for oxidase, catalase, and strong urease activity. Molecular identification was carried out by PCR amplification of a ureC (glmM) fragment. The primer sequences used were:

F: (5'-TGGGACTGATGGCGTGAGGG-3') and R: (5'-AAGGGCGTTTTTAGATTTTT-3') PCR was performed as described by Nafisi et al. [10].

FISH

Paraffin-embedded antrum biopsy sections were examined by FISH. Briefly, for the hybridization of the samples, each slide of the tissue sections was covered with 40 μL of hybridization buffer (0.9 M NaCl, 20 mM Tris-HCl, pH 8, 0.01% SDS, 20% formamide) containing 5 ng/μL of Fluorescein isothiocyanate-labeled oligonucleotide Hpy-1.

Probe Hpy-1 (5'-CACACCTGACTGACTATC-CCG-3') targeted to a 16S rRNA position was used to specifically identify *H. pylori* [11].

Then the slides were put separately into a moisture chamber and incubated at 46°C for 90 min for the hybridization step. Stringent washing was carried out in washing buffer (20 mM Tris-HCl, pH 8, 0.01% SDS, 225 mM NaCl) at 48°C for 15 min.

The slides were then stained with 1 μg/mL DAPI (4',6-diamidine-2'-phenylindole dihydrochloride) for 5 min. DAPI nonspecifically stains the DNA of any cell, including bacteria, blue. Finally, the slides were washed with PBS, left to air dry, covered with fluorescent mounting medium (DAKO, Denmark), and examined with an epi-fluorescence microscope (Japan) equipped with different filters.

Statistical Aanalysis

Sensitivity (Se), specificity (Sp), positive predictive value (PPV), negative predictive value (NPV), and 95% confidence intervals (95% CI) were calculated for each of the testing methods by using histology or RUT as the gold standard [12]. The agreement between different diagnostic tests was evaluated by calculating Cohen's Kappa confidence. The chi-square test was used to compare the qualitative variables. McNemar's

Table 1. The number of cases in each group of pathological diagnosis specimens

Pathological diagnosis	All patients ($n = 165$)	
Chronic gastritis	73%	44%
Chronic gastritis with intestinal metaplasia	5%	3%
Chronic active gastritis	50%	30%
Chronic active follicular gastritis	4%	2.4%
Chronic active gastritis with intestinal metaplasia	6%	3.6%
Erosive gastritis	17%	10.3%
Normal	8%	4.8%
Cancer	2%	1.2%

test was also applied. A *P* value of less than 0.05 was considered statistically significant. Analyses were performed in SPSS version 20.

RESULTS

A total of 165 patients, consisting of 84 males and 81 females, enrolled in this cross-sectional study. The mean age was 50.3 ± 15.5 years old with the age ranging from 15 to 83 years. In endoscopic diagnosis, 78 (47.3%) patients were identified as chronic gastritis (CG) and 60 (36.4%) as chronic active gastritis (CAG), and related pathological changes such as chronic follicular gastritis and chronic gastritis with intestinal metaplasia were also observed. Based on these results, gastritis was the most common finding in the patients (94%). (Table 1).

Figure 1 shows the percentage of *H. pylori* positive patients in each test. The results of RUT and culture were approximately identical. Among the four tests, FISH showed a higher positive rate compared to other tests. Overall, according to the gold standard of study (histology and/or RUT), the prevalence of *H. pylori* infection in Isfahan was 55.2% (91/165). Among 91 *H. pylori* positive patients, 81% were positive by both RUT and histology.

The mean age of infected and non-infected patients was 50.1 and 50.3, respectively ($p = 0.93$). There was no difference between males and females concerning the prevalence of *H. pylori* infection ($p = 0.60$).

Table 2 shows the sensitivity, specificity, PPV, NPV and diagnostic accuracy of each method. FISH test was the most sensitive method (95.7%), followed by RUT (92.3%). Despite the high specificity and PPV of histology (100%), NPV of this method was lower than that of the other three methods (88.1%). Agreement was found between the results of the diagnostic tests and the gold standard. All four methods produced very similar results ($p < 0.001$). However, there were significant differences in the sensitivity of some methods compared to the gold standard. McNemar's test revealed a significant difference in the sensitivity value comparing histology to the gold standard ($p = 0.002$), as well as between culture and the gold standard ($p = 0.008$). Comparison of RUT to the gold standard showed a significant difference ($p = 0.02$). When FISH was compared to the gold standard, there was no significant difference between the results ($p = 0.37$). FISH demonstrated the highest accuracy (96.9%).

DISCUSSION

Helicobacter pylori (*H. pylori*) is a common pathogenic bacterium that plays an important role in the development of gastroduodenal diseases through causing chronic and persistent infections. In clinical settings, a reliable diagnosis is essential for patients with *H. pylori* infection in each geographical region [13]. The present study aimed to compare the results of four different invasive diagnostic methods of *H. pylori*
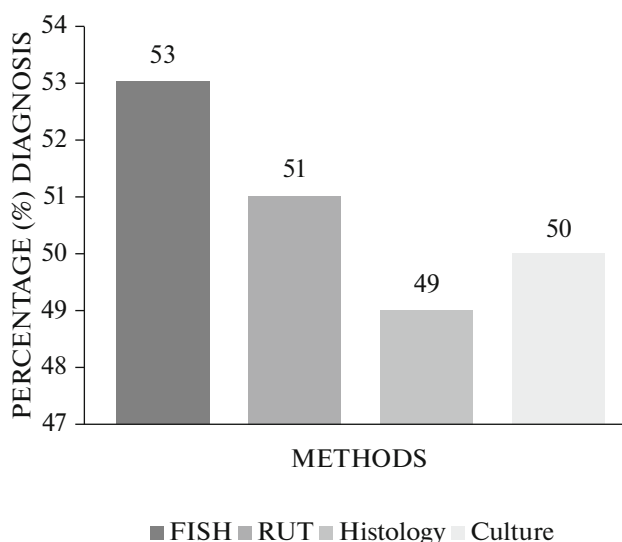

Fig. 1. *H. pylori* positive cases in gastric mucosal biopsies by the four methods.

Table 2. Comparison of four methods for diagnosis of *H. pylori* infections by histology and or RUT as gold standard

	Sensitivity, %	Specificity, %	PPV, %	NPV, %	Kappa value, %	*P*-value	Accuracy, %
Histology	89	100	100	88.1	0.87	<001	93
RUT	92.3	100	100	91.4	0.91	<001	95.7
Culture	91.2	100	100	90.2	0.90	<001	95.1
FISH	95.7	98.6	98.9	94.8	0.93	<001	96.9

RUT: rapid urease test; FISH: fluorescence in situ hybridization; PPV: positive predictive value; NPV: negative predictive value.

infection in an Iranian population with gastrointestinal disorders.

In the FISH method, 88 slides of positive *H. pylori* were found with the highest number of positivity among other methods. In the histological examination, positive *H. pylori* had the lowest positivity among the studied methods using specific Giemsa staining. The consistency of each method with the gold standard was calculated in the comparative analysis using kappa statistics. All four methods were completely consistent with the gold standard, but histology had the lowest percentage.

In most hospitals, histology is the most common method of diagnosing *H. pylori* in suspected patients with upper gastrointestinal symptoms, and in regions with high prevalence. This microscopic technique diagnoses the apparent morphology of bacterium and its spiral form. Upon requesting this test, the gastroenterologists receive the complete information about the presence of acute or chronic inflammation in the gastric mucosa and its association with pathological changes such as metaplasia, cancer, and gastric atrophy [14, 15]. In the present study, the sensitivity of histology was 88%, and 10 patients were false negative. McNemar's test indicated that the sensitivity of histology was more significant compared with the gold standard. Kocsmar et al. observed a sensitivity of 83% [16], and Aftab et al. gained 86% sensitivity by histology [17], which confirms the results of the present study.

The sensitivity of histology is often influenced by number, site, and size of the collected biopsies. Low bacterial density in biopsy samples and the patchy bacterial colonization in the stomach tissue lead to sampling errors and false negative results [18]. For a variety of reasons such as antibiotic use and PPI, the conversion of spiral form to *H. pylori* coccoid form in tissue makes the pathologists unable to differentiate this specific bacterial morphology from other cocci in the microscopic observation. Other limitations of this method are the high cost, long access time of the results and dependence on the pathologists' skills and experience [5, 7]. In our center, some patients suffered from chronic follicular gastritis and chronic gastritis with intestinal metaplasia. Long-term complications of *H. pylori* infection such as chronic gastric ulcers, metaplasia and gastric cancer reduce the number of bacteria in the stomach. As bacterial density decreases, inflammatory activity is also reduced, and

bacteria are not detectable in antral biopsy specimens, or there are only in small numbers in the body area [16]. Lack of awareness and misuse of antibiotics on the part of some patients are further potential causes of false negative results in histology. Therefore, it is necessary for physicians to interpret histology results according to patients' clinical symptoms along with at least one other method.

RUT is applicable as a diagnostic screening test for *H. pylori* infection in epidemiological studies. It is a popular and common method for most researchers owing to its easy procedure, low cost, and availability for more rapid results in the endoscopy unit [19]. In our study, RUT had a sensitivity of 92% with 7 false negatives, and there was a statistically significant difference between its sensitivity and the gold standard. Consistent with the present study, Khalifehghooli et al. observed a method sensitivity of 95.6% [5] and Ramis et al. recorded a sensitivity of 100% [20]. False negative result in the RUT occurs due to the irregular distribution of bacteria in gastric mucus, the use of antimicrobial drugs, bismuth compounds, PPI consumption, stomach bleeding, consumption of H2-receptor antagonists, and intestinal metaplasia. Sensitivity of the RUT method also depends on the bacterial density, such that at least 10^5 bacteria are necessary for a positive RUT [7, 12]. Gastric urease-positive *Helicobacter* spp. and non-*Helicobacter* species can also cause false positives, yet their prevalence is <1% in the gastric biopsies. Oral colonization with *Helicobacter heilmannii* also interferes with testing [12, 21]. In the present study, the specificity of our method was 100% and there was no false positive. The addition of more biopsy samples, especially if taken from both antrum and corpus areas, increases the precision of procedure.

The culture is another invasive diagnostic method performed only in cases where empirical antibiotic treatment fails due to technical difficulties in isolation; this method determines the antibiotic sensitivity in order to prescribe correct drugs for patients. Since some strains are resistant to first-line antibiotics of treatment, the success of culture enables diagnostic centers to routinely perform antibiotic sensitivity tests and be effective in eradicating infection [7, 22]. False negative results occur due to many reasons such as low bacterial count in the sample, contamination, viable but non culturable state (VBNC) or coccoid, inappropriate sample transfer conditions, and use of antimi-

crobial drugs [12]. In the present study, suitable conditions were provided in order to transfer and minimize sampling interval until cultivation. The incubation period was extended to 10 days under microaerophilic conditions, and a non-selective medium such as blood agar was employed since at least 5% of strains did not grow in the selective media with antibiotics. Therefore, the culture sensitivity of study was 91.2%, which is higher than other studies [12, 20]. Further reported were eight false negatives. However, culture method is time consuming, and requires special temperature conditions (cool temperature) prior to culture, microaerophilic conditions, and enriched culture media that are not routinely available at diagnostic centers, hence the fact that physicians prefer histology.

FISH is a fast technique with up to 3 h of time to access results; this test does not need specialized laboratory equipment, and can be performed at a relatively affordable cost; therefore, the limitations on sample transfer and antibiotics are ineffective as a result of testing. This method is able to directly detect pathogens in the blocks prepared for histological studies, frozen biopsies, and isolated colonies without any need for DNA preparation [12, 23]. The detection of mutations related to clarithromycin resistance and coccoid forms are among other advantages of this test [24]. In the present study, the FISH was more sensitive compared with other conventional methods for the diagnosis of *H. pylori*. FISH sensitivity in the current study was higher than the research by Demiray [12]. FISH specificity in the present research was 98.7%, consistent with Samarbaf-Zadeh et al. [25]. In the McNemars test, FISH sensitivity had no significant difference with the gold standard of study ($p = 0.37$). There were 4 false negative cases in FISH test. The low level of *H. pylori* colonization in the examined biopsy and poor binding of specific probe to the target gene can lead to error in microscopic diagnosis and false negative results. A positive case was also reported in FISH method which was negative in other methods. In this patient, the spiral form of bacterium was probably transferred to coccoid form, which was not diagnosed by other three methods; in FISH method, on the other hand, it was hybridized with a specific probe.

In the present study, sampling was only done on gastric antrum, which was a research limitation.

CONCLUSIONS

The present study indicated that FISH method has the highest percentage of consistency with the gold standard. As far as the authors of the present study are concerned, most medical centers of the studied geographical region only use histology to diagnose *H. pylori* infection. However, it seems that combining this method with a molecular technique such as FISH can lead to a reduction in false negative results and improve the accuracy of the *H. pylori* infection for a

better management of complications such as adenocarcinoma.

COMPLIANCE WITH ETHICAL STANDARDS

Conflict of interests. The authors declare that they have no conflict of interest.

Statement of compliance with standards of research involving humans as subjects. The study was approved by the Ethics Committee of Isfahan University of Medical Sciences, and the patients declared their consent.

REFERENCES

1. Kalali, B., Formichella, L., and Gerhard, M., Diagnosis of *Helicobacter pylori*: Changes towards the future, *Diseases,* 2015, vol. 3, pp. 122−135.

2. Mandal, A.K., Kafle, P., Puri, P., Chaulagai, B., Sidhu, J.S., and Hassan, M., An association of *Helicobacter pylori* infection with endoscopic and histological findings in the Nepalese population, *J. Fam. Med. Primary Care,* 2019, vol. 8, p. 1227.

3. Yantiss, R.K. and Lamps, L.W., To stain or not to stain... that remains the question, *Am. J. Clin. Pathol.,* 2012, vol. 137, pp. 343−345.

4. Contreras, M., Salazar, V., García-Amado, M.A., Reyes, N., Aparcero, M., and Silva, O., High frequency of *Helicobacter pylori* in the esophageal mucosa of dyspeptic patients and its possible association with histopathological alterations, *Int. J. Infect. Dis.,* 2012, vol. 16, pp. 364−370.

5. Khalifehgholi, M., Shamsipour, F., Ajhdarkosh, H., Daryani, N.E., Pourmand, M.R., and Hosseini, M., Comparison of five diagnostic methods for *Helicobacter pylori, Iran. J. Microbiol.,* 2013, vol. 5, p. e396.

6. Fakheri, H., Firoozi, M.S., and Bari, Z., Eradication of *Helicobacter pylori* in Iran: A review, *Middle East J. Dig. Dis.,* 2018, vol. 10, p. e5.

7. Talebi Bezmin Abadi, A., Diagnosis of *Helicobacter pylori* using invasive and noninvasive approaches, *J. Pathog.,* 2018, vol. 2018, pp. 1−13.

8. Pastukh, N., Binyamin, D., On, A., Paritsky, M., and Peretz, A., GenoType® Helico DR test in comparison with histology and culture for *Helicobacter pylori* detection and identification of resistance mutations to clarithromycin and fluoroquinolones, *Helicobacter,* 2017, vol. 22, p. e12447.

9. Patel, S.K., Pratap, C.B., Jain, A.K., Gulati, A.K., and Nath, G., Diagnosis of *Helicobacter pylori*: What should be the gold standard?, *World J. Gastroenterol.,* 2014, vol. 20, p. e12847.

10. Nafisi, M.R., Alipour, S.M., Karimi, A., Rahimian, G.A., and Imani, F.A., PCR and RFLP of ureC (glmM) gene for identification and typing of *Helicobacter pylori*

strains isolated from gastric biopsy specimens of gastric patients, *Kowsar Med. J.,* 2009, vol. 14, pp. 143−148.

11. Trebesius, K., Panthel, K., Strobel, S., Vogt, K., Faller, G., and Kirchner, T., Rapid and specific detection of *Helicobacter pylori* macrolide resistance in gastric tissue by fluorescent in situ hybridization, *Gut,* 2000, vol. 46, pp. 608−614.

12. Demiray-Gürbüz, E., Yılmaz, Ö., Olivares, A.Z., Gönen, C., Sarıoğlu, S., and Soytürk, M., Rapid identification of *Helicobacter pylori* and assessment of clarithromycin susceptibility from clinical specimens using FISH, *J. Pathol.,* 2017, vol. 3, pp. 29−37.

13. Redéen, S., Petersson, F., Törnkrantz, E., Levander, H., Mårdh, E., and Borch, K., Reliability of diagnostic tests for *Helicobacter pylori* infection, *Gastroenterol. Res. Pract.,* 2011, vol. 2011, pp. 1−6.

14. Garza-González, E., Perez-Perez, G.I., Maldonado-Garza, H.J., and Bosques-Padilla, F.J., A review of *Helicobacter pylori* diagnosis, treatment, and methods to detect eradication, *World J. Gastroenterol.,* 2014, vol. 20, p. 1438.

15. Tzeng, J.-E., Lin, Y.-L., Chung, S.-M., and Chu, Y.-T., Comparison of four diagnostic methods for *Helicobacter pylori, Tzu Chi Med. J.,* 2005, vol. 17, pp. 339−343.

16. Kocsmár, É., Szirtes., I., Kramer, Z., Szijártó, A., Bene, L., and Buzás, G.M., Sensitivity of *Helicobacter pylori* detection by Giemsa staining is poor in comparison with immunohistochemistry and fluorescent in situ hybridization and strongly depends on inflammatory activity, *Helicobacter,* 2017, vol. 22, p. 12387.

17. Aftab, H., Yamaoka, Y., Ahmed, F., Khan, A.A., Subsomwong, P., and Miftahussurur, M., Validation of diagnostic tests and epidemiology of *Helicobacter pylori* infection in Bangladesh, *J. Infect. Dev. Countries,* 2018, vol. 12, pp. 305−312.

18. Tongtawee, T., Kaewpitoon, S., Kaewpitoon, N., Dechsukhum, C., Leeanansaksiri, W., and Loyd, A., Diagnosis of *Helicobacter pylori* infection, *Asian Pac. J. Cancer Prev.,* 2016, vol. 17, pp. 1631−1635.

19. Lopes, A.I., Vale, F.F., and Oleastro, M., *Helicobacter pylori* infection: Recent developments in diagnosis, *World J. Gastroenterol.,* 2014, vol. 20, p. e9299.

20. Ramis, I.B., Moraes, E.Pd., Fernandes, M.S., Mendoza-Sassi, R., Rodrigues, O., and Juliano, C.R.V., Evaluation of diagnostic methods for the detection of *Helicobacter pylori* in gastric biopsy specimens of dyspeptic patients, *Braz. J. Microbiol.,* 2012, vol. 43, pp. 903−908.

21. Mnena, E., Emmanuel, N., and Ega, R., Assessing the reliability of PCR, microbiological and histologic methods in detecting *Helicobacter pylori* in gastric biopsies, *J. Biomed. Sci.,* 2017, vol. 6, p. 32.

22. Peretz, A., On, A., Koifman, A., Brodsky, D., Isakovich, N., and Glyatman, T., An efficiency comparison between three invasive methods for the diagnosis of *Helicobacter pylori* infections: culture from stomach biopsy, rapid urease test (CUTest®), and histologic examination of gastric biopsy, *Ann. Clin. Lab. Sci.,* 2015, vol. 45, pp. 148−151.

23. Frickmann, H., Zautner, A.E., Moter, A., Kikhney, J., Hagen, R.M., and Stender, H., Fluorescence in situ hybridization (FISH) in the microbiological diagnostic routine laboratory: a review, *Crit. Rev. Microbiol.,* 2017, vol. 43, pp. 263−293.

24. Ciftci, I.H., Ugras, M., and Acarturk, G., Comparison of FISH, RFLP and agar dilution methods for testing clarithromycin resistance of *Helicobacter pylori, Turk. J. Gastroenterol.,* 2014, vol. 25, pp. 75−80.

25. Samarbaf-Zadeh, A.R., Tajbakhsh, S., Moosavian, S.M., Sadeghi-Zadeh, M., Azmi, M., and Hashemi, J., Application of fluorescent in situ hybridization (FISH) for the detection of *Helicobacter pylori, Med. Sci. Monit.,* 2006, vol. 12, pp. CR426−CR430.