Identification of *Helicobacter pylori* by different conventional staining techniques and its comparison with polymerase chain reaction

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ABSTRACT

الأهداف: تقييم كشف هيليكوباكتر بلوري عن طريق أساليب التلطيخ النسيجية ولمقارنتها مع لطخة غرام وتفاعل البلمرة التسلسلي.

الطريقة: أجريت دراسة مستعرضة من 436 مريضاً، وحضور "المستشفى الجراحي العميق"، أناند، ولاية غوجارات، الهند خلال الفترة من فبراير 2008م حتى أكتوبر 2011م. وقد أجريت الدراسة في قسم علم الأحياء المجهرية، أناند، الهند. وتعرضت الخزعات النسيجية لتلطيخ باستخدام الهيماتوكسيلين ويوزين، وبلطخة بالغميزا، ولطخة ستاري وارثن، ولطخة غرام. تم إجراء تفاعل البلمرة التسلسلي على 71 عينات الخزعة.

النتائج: كانت الحساسية والقيم التنبؤية سلبية في جميع البقع النسيجية، كانت لطخة ستاري وارثن، والهيماتوكسيلين ويوزين، ولطخة بالغميزا ممتازة. أظهرت لطخة غرام نتائج ممتازة خصوصاً لدى قيم الحساسية، والقيمة التنبؤية الإيجابية. كانت حساسية تفاعل البلمرة التسلسلي أقل بشكل ملحوظ بالمقارنة مع الأساليب الأخرى.

خاتمة: كانت حساسية البقع النسيجية أفضل من تفاعل البلمرة التسلسلي. يمكننا أن نستنتج أنه عندما لا يتوفر تفاعل البلمرة التسلسلي لدى مختبر متواضع فأن الأنسجة النسيجية تعتبر أفضل لتشخيص هيليكوباكتر بلوري. ومع ذلك لطخة غرام هو الوسيلة المفضلة تماماً وبأسعار معقولة وموثوق بها وبسيطة لتحديد هيليكوباكتر بلوري مقارنة بجميع الأنسجة وتفاعل البلمرة التسلسلي.

Objectives: To evaluate *Helicobacter pylori* (*H. pylori*) detection by histological staining methods, and to compare with those of Gram staining and polymerase chain reaction (PCR).

Methods: This is a cross-sectional study conducted at the Department of Microbiology, Shree P. M. Patel Paramedical College, Anand, Gujarat, India on 436 patients attending the Deep Surgical Hospital, Anand, Gujarat between February 2008 and October 2011. Biopsies were subjected to histological staining using Hematoxylin & Eosin (H&E), Giemsa, and Warthin-Starry stains, as well as with Gram staining. The PCR was performed on 71 biopsy samples.

Results: Sensitivity and negative predictive values of all 3 histological stains (Warthin-Starry, H&E, and Giemsa) were excellent. Gram staining showed excellent results pertaining to sensitivity, specificity, positive predictive value, and accuracy. Sensitivity of PCR was remarkably low compared to all the staining methods. The sensitivity of all histological stains was found better than PCR.

Conclusion: From the findings in our study, we conclude that in a mediocre laboratory, where PCR facility is not available, histological stain can be a better substitute for the diagnosis of *H. pylori*. Our findings also confirm the assertion that Gram staining is a preferred stain, affordable, reliable, and simple means for identifying *H. pylori* compared with both histology and PCR.

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The discovery of *Helicobacter pylori* (*H. pylori*) species not only introduced the whole new group of the bacteria to science but also revolutionized our concept of gastroduodenal pathology and diverted the world wide attention from pH (H^+ ion concentration) to *H. pylori*. The *H. pylori* are thought to represent a significant etiopathogenic factor in diseases of the upper gastrointestinal tract. It seems, therefore, important to elaborate effective techniques for its detection. Microbiological diagnosis of *H. pylori* would be considered as a superlative method to all, however, it is quite demanding to perform as it requires specialized enrichment media with complicated incubation techniques.[1] Histological diagnosis of biopsies for identification of infectious organism is considered as a gold standard, particularly when culture is difficult to perform.[2] Attempts to rapidly identify the organism in the tissue include various staining procedures.[3] Antral biopsy specimen processed for histology would therefore provide an easier and cost effective alternative means of diagnosing *H. pylori* infection, and moreover is considered to be the "gold standard" test for *H. pylori*.[4] Numerous staining techniques have been concocted to identify *H. pylori* in histological sections but their sensitivity and specificity vary significantly.[5] Although a heavy bacterial load is readily apparent on routine Hematoxylin-Eosin (H&E) stained sections but to enhance the detection of the presence of low density of organism, especially after therapy requires special staining techniques such as Gram, Giemsa, Genta, Dieterle, or Warthin-Starry stain is required.[5] Giemsa stain has also been preferred by many researchers as it is easy to perform and its accessibility in most of the histopathology laboratory.[6] Warthin-Starry is considered to be the most superior and sensitive, but technically is more demanding and frequently not reproducible, and the granular appearance of the organism may be perplexed with silver precipitate.[7] Although various methods have their own strength and weakness, none have been shown to be superior to other in terms of cost, convenience, and sensitivity.[7] Studies using molecular biological techniques are of particular significance, since they enable a rapid and precise diagnosis to be made. Numerous molecular techniques like dot blot hybridization, deoxyribonucleic acid-ribonucleic acid (DNA-RNA) hybridization assay, and restriction fragment length polymorphism (RFLP) methods have also been developed. All these require lengthy processing time, and hence, not suitable as a routine procedure. In recent years, it has been shown that the presence of bacteria can be detected by polymerase chain reaction (PCR) using various primers.[8] The PCR is thought to represent one of most precise techniques in the diagnosis of *H. pylori* in biopsies of the gastric mucosa. The PCR has been demonstrated to be a reliable and highly sensitive tool for detection of bacterial gene sequences in a variety of clinical specimens. This innate sensitivity could be important if rigorous transport procedures from surgery to the diagnostic laboratory are difficult to implement. The aim of the present study was to compare the histological staining methods using H&E, Giemsa, and Warthin-Starry stained sections with Gram staining method and PCR, with special emphasis on the factors that influence their general usefulness including availability, rapidity, sensitivity, specificity, accuracy, reproducibility, and cost.

Methods. This is a cross-sectional study of 436 consecutive symptomatic patients attending the Endoscopy Unit of Deep Surgical Hospital, Anand, Gujarat, India between February and October 2008. There were 267 males and 169 females with age range of 15-90 years. Subjects were diagnosed as having gastritis (217), duodenitis (24), duodenal ulcer (21), gastric ulcer (2), and reflux esophagitis (172). Patients having a history of previous gastric surgery, recent or active gastrointestinal bleeding, patients taking aspirin or non-steroidal anti-inflammatory drugs (NSAIDS) in the past 4 weeks, or are on proton pump inhibitors (PPI), patients with previous therapy to eradicate *H. pylori*, or if the informed consent was not obtained were excluded from the study. Approval of the Human Research Ethics Committee of H. M. Patel Center for Medical Care and Education, Pramukh Swami Medical College, Karamsad was obtained prior to initiation of the work. This study was conducted according to the principles of Helsinki Declaration. Dully filled consent form was obtained from all patients participating in the study.

Detection of H. pylori. The *H. pylori* status was determined by performing various invasive and non-invasive tests. Four fragments of antral biopsy/lesion were obtained from each patient and divided into 4 parts, each for rapid urease test (RUT), culture, Gram staining, and histopathology. For Gram staining, the biopsy sample was crushed and smears were prepared and stained by routine protocols. Presence of spiral gram negative microorganism embedded in the tissue cells was diagnostic for *H. pylori*.[9] For histological diagnosis, one piece of antral biopsy was fixed in 10% buffered formalin for 24 hours, processed, and then embedded in paraffin. A 5 μm sections were cut from

each specimen, and 3 slides were prepared from each block, which were subsequently stained by Giemsa and H&E as recommended by the manufacturers, and the third slide was stained by Warthin-Starry stain.

Warthin-Starry stain. Commercially available Warthin-Starry kit is very expensive (82,000 Indian rupees/100 slides, varies from country to country), and is not appropriate for routine use in the laboratory, additionally escalating the cost to patients, and considering this fact, the techniques for reagent preparations were standardize in our laboratory.

Procedure. A 1 liter distilled water was acidulated with 0.1 g citric acid until a pH of 4.0 was achieved. Deparaffinized and rehydrated sections were rinsed in distilled water and immersed in 1% silver nitrate ($AgNO_3$) solution preheated in 50°C water bath. Silver was allowed to impregnate the slide for 30 minutes, meanwhile 2% $AgNO_3$, 5% gelatin, and 0.15% hydroquinone was warmed at 54°C water bath with closed lid (to prevent oxidation), and while the slide is impregnated, the developer was prepared by adding 2% $AgNO_3$ - 1.5 ml, 5% gelatin - 3.75 ml, 0.15% hydroquinone - 2 ml (concentration of developer for staining 5 slides), the slides were then removed from the impregnator and was flooded with warm developer until they show light brown or yellow color, then the slides were quickly washed with hot tap water (50°C), rinsed in distilled water, dehydrated in 95% alcohol (one minute), then absolute alcohol (10 seconds), cleared with xylene (2 changes) by placing the slides in 2 jars for 5 minutes each, and mounted with dibutylphthalate xylene (DPX). Slides stained by Warthin-Starry, H&E, Giemsa, and Gram were carefully examined by a technologist, the slides were independently reviewed in a retrospective, blinded manner, searching for *H. pylori* organisms on all 4 slides for each case. Each stain was assessed on a different day without referring to the results of the other stain, as well as the outcome of culture, or RUT. The PCR was performed on 71 biopsy specimens.

The DNA extraction from biopsy sample. The DNA was isolated from the homogenate using of QIAamp DNA kit (QIAGEN, Hilden, Germany, CAT NO: 51304) according to the manufacturer's instruction and DNA was stored at -20°C until analysis.

The PCR conditions.[10] The PCR was carried out with primers specific to *H. pylori*. The primer selected was of cag A gene (cg1-GAT AAC AGG CAA GCT TTT GAGG 3, cg 2-CTG CAA AAG ATT GTT TGG CAGA)and vacA gene (vc 1-ATG GAA ATA CAA CAA ACA CAC, vc 2-CTG CTT GAA TGC GCC AAAC). The PCR was performed under the following conditions: one µl of each oligonucleotide primer was placed (50 picomole/µl for each primer) in an Eppendorf tube, and 5 µl of extracted DNA; 5 µI of 10x PCR buffer (500 mM KCl, 100 mM Tris-Cl, 15 mM $MgCl_2$, 0.1% gelatin [pH: 8.3]); 8 µl of deoxynucleoside triphosphate mixture (final concentration, 1.25 mM each dATP, dCTP, dGTP, and dTTP (B'Genei, India); 2.5 U of Taq DNA polymerase (B'Genei); and molecular biology-grade distilled water were added to make a final reaction volume of 50 µl. The mixture was briefly spinned in a microcentrifuge, and placed in a thermal cycler (ESCO mini thermo cycler, United Kingdom). Forty cycles were employed and each cycle comprised 5 minute pre-denaturation at 95°C, one minute denaturation at 95°C, one minute annealing at 60°C, and one minute extension at 72°C. After 35 cycles, the reaction mixture was further extended for 7 minutes at 72°C, and the mixture was subsequently refrigerated at 4°C before analysis. Subsequently, 8µl of PCR product were run on a 2.5% agarose gels, containing ethidium bromide, and photographed under an ultraviolet (UV) light. A 349-bp band was considered a positive PCR for cagA gene and 286-bp band for vacA gene.

Statistical analysis. Sensitivity, specificity, positive predictive values, and negative predictive values, and accuracy for the test were analyzed in comparison with true positive criteria. The chi-square test was used for statistical analysis of *H. pylori* infection and clinical outcomes. A $p<0.05$ was considered statistically significant.

Results. Of the 436 patients, 376 were originally negative in all the tests, whereas 60 were positive using a combination of 3 different tests (serology, RUT, and culture).

Criteria for true positive result for H. pylori. Subjects were classified as having current infection with *H. pylori* if RUT was positive within 4 hours, or if the *H. pylori* were cultured from the biopsy specimen, or if ELISA is positive along with any positive invasive tests (RUT, culture). If only serology was positive then it was considered to be past infection.[11]

Assessment of all staining methods for H. pylori detection. After consensus examination of the 60 Gold standard positive cases, all the 60 samples were confirmed positive by Warthin-Starry, H&E, and Giemsa stains showing 100% sensitivity, and negative predictive value (NPV), while 55 were confirmed by Gram staining method, specificity of H&E was little better (87.9%) than Warthin-Starry (84.5%), and Giemsa (84.5%). The H&E was found the most accurate (90.1%) method for detecting *H. pylori*

Table 1 - Comparison of sensitivity, specificity, positive- (PPV) and negative predictive value (NPV), and accuracy of various histological staining techniques with Gram staining found in a study conducted at the Endoscopy Unit of Deep Surgical Hospital, Anand, Gujarat, India.

Stains	Sensitivity*	Specificity*	PPV*	NPV*	Accuracy(%)
Warthin-Starry	100.0 (75.2-100.0)	84.5 (78.9-84.5)	59.1 (44.4-59.1)	100.0 (93.4-100.0)	(87.3)
H&E	100.0 (75.2-100.0)	87.9 (82.5-87.9)	65.0 (49.1-65.0)	100.0 (93.8-100.0)	(90.1)
Giemsa	100.0 (75.2-100.0)	84.5 (78.9-84.5)	59.1 (44.4-59.1)	100.0 (93.4-100.0)	(87.3)
Gram stain	92.3 (71.1-92.3)	100.0 (95.3-100.0)	100.0 (77.0-100.0)	98.3 (93.6-98.3)	(98.6)

*95% (confidence interval), H&E - Hematoxylin-Eosin

Table 2 - Inter-observer agreement and Kappa statistics for all staining techniques utilized in a study conducted at the Endoscopy Unit of Deep Surgical Hospital, Anand, Gujarat, India.

Stains	Agreement (%)	Kappa coefficient	*P*-value
Warthin-Starry			
H&E	(97.2)	0.932	0.047*
Giemsa	(100.0)	1.00	0.000†
Gram stain	(85.9)	0.624	0.102
H&E			
Giemsa	(97.2)	0.932	0.047*
Gram stain	(88.7)	0.683	0.100
Giemsa			
Gram stain	(85.9)	0.624	0.102

*indicates significance at 5% level, †indicates significance at 1% level, H&E - Hematoxylin-Eosin

Table 3 - Evaluation of histopathology with polymerase chain reaction (PCR) observed in a study conducted at the Endoscopy Unit of Deep Surgical Hospital, Anand, Gujarat, India (n=71).

Method	Sensitivity	Specificity	PPV	NPV	Accuracy
Histopathology	(100)	(73.3)	(40.7)	(100)	(77.4)
PCR	(61.5)	(75.8)	(36.3)	(89.7)	(73.2)

(%), PPV - positive predictive value, NPV - negative predictive value

Table 4 - Comparison of histopathology and polymerase chain reaction (PCR) with various endoscopic findings observed in a study conducted at the Endoscopy Unit of Deep Surgical Hospital, Anand, Gujarat, India (n [%]).

Diseases	Histopathology		PCR	
	Negative	Positive	Negative	Positive
Duodenal ulcer	2 (22.3)	7 (77.7)	5 (65.6)	4 (44.4)
Duodenitis	1 (50.0)	1 (50.0)	2 (100.0)	0 (0.0)
Gastritis	27 (79.5)	7 (20.5)	24 (70.6)	10 (29.4)
Reflux esophagitis	14 (53.8)	12 (46.2)	18 (69.2)	8 (30.8)
Total	**44 (62.0)**	**27 (38.0)**	**49 (69.0)**	**22 (31.0)**

infection compared to other histological methods (**Table 1**). High number of false positive results by Warthin-Starry (57), Giemsa (57), and H&E (47) stains resulted in its decreased specificity. On the contrary, Gram staining did not showed any false positive result owing to 100% specificity. Gram staining was found to be the most accurate method (98.6%) amongst all the staining methods. Whereas comparing histological stains, we found H&E to be the most accurate method (**Table 1**).

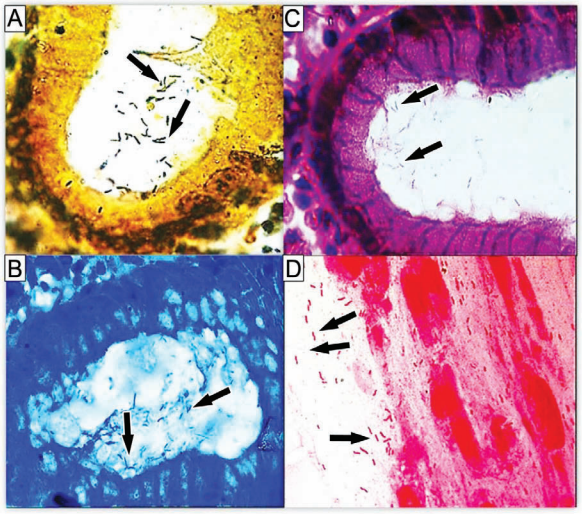

Figure 1 - Comparison of different histological staining techniques for *Helicobacter pylori* (*H. pylori*) detection such as: **A**) Warthin-Starry stain showing *H. pylori* in the gastric lumen (arrows, magnification 1000x); **B**) methylene blue stain showing *H. pylori* in the gastric lumen (arrows, magnification 1000x); **C**) Hematoxylin and Eosin stain showing *H. pylori* in the gastric lumen (arrows , magnification 1000x); and **D**) Gram staining showing *H. pylori* (Gram negative bacilli) in the gastric lumen (arrows, magnification 1000x).

Agreement between all the staining techniques. **Table 2** shows that Warthin-Starry showed the highest agreement with Giemsa (100%, p=0.000), and then with H&E (97.2%, p=0.047). The H&E showed good agreement with Giemsa (97.2%, p=0.047).

Comparison between histological stains and PCR (n=71). Out of 71 biopsy samples, 27 were detected positive by histology, and 22 were detected positive by PCR. Sensitivity of histological stains was found to be excellent (100%) compared to PCR (61.5%), while the specificity and accuracy of both were almost analogous (**Table 3**). When we compared the detection rate of *H. pylori* in various gastroduodenal diseases, we found that in patients with duodenal ulcer, the detection rate of histopathology was higher (77.7%) than PCR (44.4%). In reflux patients, also the detection rate of histopathology was higher (46.2%) than PCR (30.8%),

but in gastritis patients the difference was not that significant (**Table 4**).

Comparison of cost and technicalities of the method. Warthin-Starry stain showed remarkable result because the organisms are coated with the silver stain, and therefore looks larger, making the identification easy (**Figure 1A**). We found the technique to be quite demanding because the glassware had to be thoroughly cleaned to prevent precipitation, and the working solution has to be freshly prepared, overall, it took one hour to produce satisfactory result. However, the method was found to be exceedingly reliable, and no variations were noted, and therefore no repeats were required. Reagents were also widely available and simple to prepare. We found excellent sensitivity (100%) of the method for detecting *H. pylori*, but cost wise it was quite expensive to the patient (approximately 40 Indian rupees/slide) compared to H&E and Giemsa stains, but still not comparable to the cost of the commercial kit.

Giemsa stain. **Figure 1B** is very straightforward, inexpensive, and takes approximately 5 minutes to perform, and is easily reproducible. We found excellent sensitivity (100%) of the stain, but the specificity was low (84.5%). The H&E method (**Figure 1C**) is very simple and economical method. It takes 3 minutes of technical time, despite the fact that in both H&E and Giemsa stains, it does not provide any disparity to trace the organisms, and we found it to be the excellent sensitive method (100%). Cost wise also, both stains are very affordable (15 Indian rupees/slide) to the patients. Gram staining too is undemanding to execute and inexpensive. It takes approximately 5 minutes of technical time and provides a good result, nevertheless it does not produce any contrast with other gram negative organisms, *H. pylori* being typical spiral bacilli can be easily discriminated from the other contaminating organisms (**Figure 1D**). We found the method to be highly sensitive (92.3%) and specific (100%) compared to histological staining methods with the minimum cost of 10 Indian rupees/slide.

Discussion. Pathologists have been trying to find the perfect stain for detection of *H. pylori* in gastric biopsies for decades. Histological examination is currently accepted as "gold standard" by most researchers,[12] and other methods are also compared with it. A correct and reliable histological diagnosis of *H. pylori* gastritis has a great influence on clinical practice as an indicator for therapy. The sensitivity and specificity of histological stain for detection of *H. pylori* depends not only on the number and site of the biopsies, but also on the staining technique and experience of the pathologist and high inter-observer variation has been reported.[12] Our results confirm that in the hands of an experienced histopathologist, the difference between the 3 histological methods (H&E, Giemsa, and Warthin-Starry) for demonstrating the presence of *H. pylori* in gastric antral biopsies is not significant.

The *H. pylori* can be easily identified by H&E stain in most cases, but nevertheless the sensitivity is low, particularly when the number of bacterias are less. So, most laboratories use ancillary staining method in routine for the identification of the organisms. In this era of cost restraint, pathology laboratories must aspire to use the most cost effective, relatively cheap, easy to perform, and easy to interpret method in routine practice. Most of the laboratories found that the Warthin-Starry staining technique is costly and difficult to reproduce reliably, so we have attempted to develop the stain in our laboratory setting and compared it with other routine staining techniques. We found that Warthin-Starry is perfect (100%) in terms of sensitivity and NPV for detection of *H. pylori*. Our sensitivity results are in accordance with the study carried out by Jhala et al (100%)[13] in 2003, and Poddar et al (98%)[14] in 2007. We found the sensitivity of Warthin-Starry even higher than PCR, our results are consistent with other studies.[15] In fact in terms of sensitivity, all histological stains were perfect (100%). Our results do not associate with the review Hartman et al[16] in 2012 who substantiated that the routine performance of Giemsa or Warthin-Starry stain is not warranted. Our specificity with Warthin-Starry was 84.5%, slightly less than H&E (87.9%), as sometimes it may give a granular appearance of silver impregnation, which looks like the organism. These can lead to false positive biopsy readings. However, the accuracy of Warthin-Starry was exactly the same as Giemsa (87.3%). The Warthin-Starry showed highest agreement with Giemsa (100%, p=0.00), followed by H&E (97.2%, p=0.47).

From a practical point of view, we can say that identification is relatively easy with all the methods, but much easier with the Warthin-Starry method because the silver coating makes the organism larger. With Warthin-Starry, the *H. pylori* were visualized not only on the surface of the foveolar epithelium but also deep inside the gastric pits.[17] In most hospitals, H&E stain is considered to be the most popular practical diagnostic method for *H. pylori*.[18] It has been reported that the positive detection rate of *H. pylori* alone is 66% with many false positive and false negatives. We agree to this fact, as we got excellent sensitivity with H&E (100%),

and is in accordance with Tzeng et al[17] in 2005 who found H&E and Giemsa to be 98.5% sensitive, although the specificity (87.9%) was precisely analogous with Tzeng et al (92%).[17] The H&E showed 97% agreement with Giemsa stain and Warthin-Starry stain (p=0.047). The H&E showed highest accuracy (90%) compared to the other 2 stains. Thus, we are inclined to agree with those authors who consider H&E as the excellent (in terms of sensitivity and specificity), cost effective, easy, preferred stain for diagnosis even with low density of organisms. With the Giemsa stain, we also encounter excellent sensitivity (100%). Rotimi et al[19] in 2000 also found that the sensitivity of Giemsa stain is significantly high (that is, 98%) than H&E. Tzeng et al[17] in 1993 also got high sensitivity (98.5%) and specificity (89%). According to Orhan et al in 2008,[15] with H&E and Giemsa stains, curved bacteria are only detected when found in large numbers, and the sensitivity of H&E is low, especially when there are few bacteria. We disagree with this fact, as we did not encounter this problem, as with minimal density also careful examination revealed the organisms.

The *H. pylori* resides in, or beneath the surface of the mucus layer, and this area can be partially lost during the sample processing for histological examination, especially when a low bacterial count is encountered.[20] So in case with low bacterial count, Gram staining (direct smear) can be a good substitute. In our study, Gram staining showed 92% sensitivity, 100% specificity, 100% PPV with 98.6% accuracy, which has exceeded the results of all the 3 histological stains. We completely disagree to all authors who reported that sensitivity of Gram staining is generally poor and inferior to culture.[20,21]

Histopathology versus PCR. Most authors find culture to be very demanding with low sensitivity and ascertains PCR as the best substitute. Despite this fact, we found culture (76%) to be more sensitive than PCR, and this discrepancy might be explained by the absence of microorganism in the biopsy piece used for PCR assay, or by the presence of in vivo Taq polymerase inhibitors.[21] While comparing PCR with histological stains, we also found the sensitivity of PCR (61.5%) was substandard to all the 3 stains used (100%), while the specificity of PCR (75.8%) was quite in sync with the specificity of the 3 stains (**Table 2**). The accuracy of histopathology (77.4%) and PCR (75.8%) was almost equivalent in our study. Different authors have different opinions regarding the comparison. A study from Mataram[22] stated that the PCR method has increased the positivity rates of *H. pylori* more than 4 times compared to the histological method. They found that out of 156 paraffin blocks of gastric biopsies, only 17 (10.9%) blocks were positive for *H. pylori* by histological examination. All of the 17 samples showed positive results on PCR method. Diarti study[22] has proved PCR superior to histological diagnosis, especially due to high false negative results with histology. Our result are analogous with the results of other studies that the sensitivity of histopathology is higher than PCR.[23-26] It is prudent to say that both histology and PCR are unrelated to any activity or viability of the cells, as histological detection is founded on visual observation of stained bacteria, and PCR permits specific amplification of bacterial DNA from the biopsy specimen. In the present study, the only constraint was a need to perform PCR on more samples so as to draw any firm assumption.

In conclusion, we can say that while comparing histological stains, the combination of Warthin-Starry coupled with Giemsa (κ=1.00, p=0.000) and H&E (κ= 0.93, p=0.047) proved superlative of all. The present study also confirms the usefulness of Giemsa and H&E stain. This techniques are relatively simple, economic, and easy to perform in any histopathological laboratory, may yield satisfactory diagnostic results in the hands of an experienced pathologist, and is as accurate as Warthin-Starry so it has been replaced in our laboratory. We also conclude that in a mediocre laboratory where PCR facility is not available, histological stain can be a better substitute for the diagnosis of *H. pylori*. Our findings also confirm the assertion that Gram staining is the preferred stain, affordable, reliable, technically not very intricate to perform, and convenient means for identifying *H. pylori* compared to both histology and PCR.

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References

1. Arora U, Agraval A, Singh K. Comparative evaluation of conventional methods and ELISA based IgG antibodies detection for diagnosis of *Helicobacter pylori* infection in cases of dyspepsia. ***Indian J Med Microbiol*** 2003; 21: 46-48.
2. Gupta E, Bhalla P, Khurana N. Histopathology for the diagnosis of infectious diseases. ***Indian J Med Microbiol*** 2009; 27: 100-106.
3. Taj Y, Essa F, Kazmi SU, Abdullah E. Sensitivity and specificity of various diagnostic tests in the detection of *Helicobacter pylori*. ***J Coll Physicians Surg Pak*** 2003; 13: 90-93.
4. Gomes CA Jr, Catapani WR, Mader AM, Locatelli A, Silva CB, Waisberg J. Antral exfoliative cytology for the detection of *Helicobacter pylori* in the stomach. ***World J Gastroenterol*** 2005; 11: 2784-2788.

5. Kaur G, Madhavan M, Basri AH, Sain AH, Hussain MS, Yatiban MK, et al. Rapid diagnosis of *Helicobacter pylori* infection in gastric imprints smears. ***Southeast Asian J Trop Med Public Health*** 2004; 35: 676-680.
6. Ciesielska U, Dziegiel P, Jagoda E, Podhorska-Okołów M, Zabel M. The detection of *Helicobacter pylori* in paraffin sections using the PCR technique and various primers as compared to histological techniques. ***Folia Morphol (Warsz)*** 2004; 63: 229-231.
7. Trakarnvanich V. Methylene blue staining of gastric tissue for the identification of *Helicobacter pylori*. ***Southeast Asian J Trop Med Public Health*** 2007; 38: 78-81.
8. Murugesan R, Bhattacharjee P, Vivek Kumar PR, Mohankumar MN, Nagarajan M, Jeevanram RK. Detection of *Helicobacter pylori* by polymerase chain reaction: A comparison in sample preparation. ***Indian Journal of Human Genetics*** 2005; 11: 35-38.
9. Vijaya D, Chandrashekhar N, Nagarantnamma T, Shivarudrappa AS. Simple stain for *Helicobacter pylori*. ***Journal of Clinical and Diagnostic Research*** 2012; 6: 664-666.
10. Wu CC, Chou PY, Hu CT, Liu ZC, Lin CY, Tseng YH, et al. Clinical Relevance of the vacA, iceA, cagA, and flaA genes of *Helicobacter pylori* strains isolated in Eastern Taiwan. ***J Clin Microbiol*** 2005; 43: 2913-2915.
11. Mishra RN, Bhagat M, Ahmed N. *Helicobacter pylori* in dyspepsia-antibiotic sensitivity and virulence pattern. ***Medical Journal Armed Forces India*** 2006; 62: 22-26.
12. Jemilohun AC, Otegbayo JA, Ola SO, Oluwasola AO, Akere A. Diagnostic accuracy of rapid urease test for the diagnosis of *Helicobacter pylori* gastric biopsies in Nigerian with dyspepsia. ***African Journal of Clinical and Experimental Microbiology*** 2011; 12: 62-66.
13. Jhala NC, Siegal GP, Klemm K, Atkinson BF, Jhala DN. Infiltration of *Helicobacter pylori* in the gastric mucosa. ***Am J Clin Pathol*** 2003; 119: 101-107.
14. Poddar U, Yachha SK. Helicobacter pylori in children: an Indian perspective. ***Indian Pediatr*** 2007; 44: 761-770.
15. Orhan D, Kalel G, Saltik-Temizel IN, Demir H, Bulun A, Karaağaoğlu E, et al. Immunohistochemical detection of *Helicobacter pylori* infection in gastric biopsies of urea breath test-positive and -negative pediatric patients. ***Turk J Pediatr*** 2008; 50: 34-39.
16. Hartman DJ, Owens SR. Are routine ancillary stains required to diagnose *Helicobacter* infection in gastric biopsy specimens? An institutional quality assurance review. ***Am J Clin Pathol*** 2012; 137: 255-260.
17. Tzeng JE, Lin YL, Chung SM, Chu YT. Comparison of four diagnostic methods for *Helicobacter pylori*. ***Tzu Chi Med J*** 2005; 17: 339-343.
18. Yantiss RK, Lamps LW. To stain or not to stain ….. That remains the question. ***Am J Clin Pathol*** 2012; 137: 343-345.
19. Rotimi O, Cairns A, Gray S. Histological identification of *Helicobacter pylori*: comparison of staining methods. ***J Clin Pathol*** 2000; 53: 756-759.
20. Sadeghifard N, Aslani MM, Ghasemi A. Comparison of different laboratory methods for diagnosis of *Helicobacter pylori*. ***Journal of Biological Sciences*** 2006; 6: 1146-1149.
21. Mégraud F, Lehours P. *Helicobacter pylori* detection and antimicrobial susceptibility testing. ***Clin Microbiol Rev*** 2007; 20: 280-322.
22. Diarti MW, Widita H, Soemohardjo S, Astuti W, Sumarno T, Jiwintarum Y, et al. The result discrepancies between histological and PCR method for detecting *H. pylori* in the patients with dyspepsia due to inappropriate preparation before endoscopy. ***The Indonesian Journal of Gastroenterology, Hepatology, and Digestive Endoscopy*** 2009; 10: 46-50.
23. Ghose C, Perez-Perez GI, van Doorn LJ, Domínguez-Bello MG, Blaser MJ. High frequency of gastric colonization with multiple *Helicobacter pylori* strains in Venezuelan subjects. ***J Clin Microbiol*** 2005; 43: 2635-2641.
24. Tang YL, Gan RL, Dong BH, Jiang RC, Tang RJ. Detection and location of *Helicobacter pylori* in human gastric carcinomas. ***World J Gastroenterol*** 2005; 11: 1387-1391.
25. Cesar ACB, Cury PM, Payao SLM, Liberatora PR, Silva AE. Comparison of histological and molecular diagnosis of *Helicobacter pylori* in benign lesions and gastric adenocarcinoma. ***Brazilian J Microbiol*** 2005; 36: 12-16.
26. Yakoob J, Jafri W, Abbas Z, Abid S, Islam M, Ahmed Z. The diagnostic yield of various tests for *Helicobacter pylori* infection in patients on acid-reducing drugs. ***Dig Dis Sci*** 2008; 53: 95-100.

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