Are Routine Ancillary Stains Required to Diagnose *Helicobacter* Infection in Gastric Biopsy Specimens?

An Institutional Quality Assurance Review

Douglas J. Hartman, MD,[1] *and Scott R. Owens, MD*[2]

Key Words: *Helicobacter*; Immunostain; Giemsa; Warthin-Starry; Quality assurance study

DOI: 10.1309/AJCPD8FFBJ5LSLTE

Abstract

Gastric biopsies are often done to evaluate for Helicobacter *gastritis. Given the oncogenic association with* Helicobacter *gastritis and the relative ease of therapy, it is important for pathology departments to identify all positive cases. We describe an institutional quality assurance study of an institutional method for the diagnosis of* Helicobacter *gastritis. We reviewed 356 gastric biopsy specimens from a 4-week period at 1 institution. Approximately half were evaluated by 4 methods, H&E stain, Giemsa stain, Warthin-Starry stain, and* Helicobacter *immunostain, while the remainder were stained only with H&E and* Helicobacter *immunostains. There were 30 cases of* Helicobacter *gastritis diagnosed; about 83% of cases were diagnosed on the initial H&E-stained slides. Our study highlights a quality assurance study and a head-to head comparison of 4 methods not previously reported and supports the use of ancillary stains at the discretion of the sign-out pathologist.*

Since Marshall and Warren's[1] landmark Nobel Prize–winning work recognizing *Helicobacter pylori* (HP) as the common cause of gastritis and peptic disease of the duodenum, several laboratory methods have been proposed to identify patients with HP infection of the stomach, including breath tests, polymerase chain reaction–based assays, serologic studies, enzyme-linked immunosorbent assay, fluorescence in situ hybridization, brush cytology, and gastric biopsy.[2-7] Chronic HP infection causes a characteristic lymphoplasmacytic chronic antral gastritis with active (neutrophilic) inflammation in the mucous neck region of the mucosa and is associated with an increased risk for gastric adenocarcinoma and mucosa-associated lymphoid tissue lymphomas.[8-11] Although there is no agreed-on "gold standard" for diagnosis, gastric biopsies are often done during endoscopic examination to rule out HP infection by histologic evaluation.

Endoscopic grading systems have been developed to select appropriate patients for high clinical concern for HP infection.[12] On routine tissue biopsy specimens, several methods have been proposed as ways to enhance the diagnosis of HP infection, such as Giemsa stain, Warthin-Starry stain, polyclonal immunostain, and others.[13,14] Many observers, however, assert that routine H&E-stained sections are sufficient to diagnose most cases of HP gastritis.[15-17]

The clinical practice at the University of Pittsburgh Medical Center is to perform immunohistochemical analysis on gastric biopsy specimens if the characteristic chronic (often at least focally active) gastritis is present but HP organisms cannot be identified by routine H&E stains. In contrast, in other pathology departments, routine special stains or immunostains for HP may be performed on all or a select subset

of gastric specimens (such as cases with a request to "rule out *Helicobacter*"). This study was performed to determine the usefulness of routine ancillary stains for HP in patients at a tertiary care center in whom gastric biopsies are done. A secondary end point was to examine whether requests from clinicians to rule out *Helicobacter* correlate with HP infection.

Materials and Methods

We obtained 356 consecutive gastric biopsy specimens from 335 unique patients in a 4-week period at 1 institution (UPMC Presbyterian Campus, Pittsburgh, PA). In addition to routine H&E stains, 130 of the biopsy specimens from 130 unique patients were stained with Warthin-Starry and Giemsa cytochemical stains and polyclonal *Helicobacter* immunostain (polyclonal *Helicobacter* antibody, Cell Marque, Rocklin, CA), and the remaining 226 biopsy specimens from 205 unique patients were additionally stained with the polyclonal *Helicobacter* immunostain only. The study was approved by the institutional quality assurance board.

For H&E stains, Harris hematoxylin and Eosin Y Alcoholic were used (Anatech, Battle Creek, MI) with standard staining procedures (staining times 3:30 minutes for hematoxylin and 1:00 minute for eosin). Briefly, for the Warthin-Starry stain, a microwave method was used. Slides were placed in silver nitrate 1% solution and microwaved for 20 seconds at 80% power for 3 rounds with 30 seconds for the final round. After cooling for 5 minutes, slides were placed in gelatin, 5% solution, and microwaved for 10 seconds at 80% power; then developer solution was added according to instructions. Slides were then rinsed and coverslipped. For Giemsa staining, deparaffinized slides were placed into methanol for 2 changes (3 minutes each), stained in Jenner solution for 7 minutes, stained in working Giemsa solution for 45 minutes, rapidly dipped in 1% glacial acetic acid, and coverslipped. For immunostaining, 4-μm slides from paraffin-embedded, formalin-fixed gastric biopsy specimens were dried and loaded onto the Ventana BenchMark system (Ventana Medical Systems, Tucson, AZ) using the Iview procedure. Examples of the H&E, Giemsa, and Warthin-Starry stains and *Helicobacter* immunostain are shown in **Image 1**.

For the first part of the study, the slides were independently reviewed by both of us in a retrospective, blinded manner, searching for HP organisms on all 4 slides for each case. We were blinded to each other's results, and independent note was made of whether HP organisms were identified in each stain. Chronic gastritis in the mucosal biopsy specimens was noted, and the intensity of the gastritis, if present, was semiquantitatively graded as mild, moderate, or severe.[12] Chronic gastritis graded as mild generally ranged from only a few mononuclear cells to small groups in the lamina propria and was best seen at relatively high microscopic power (×10-×20), while moderate gastritis contained large groups of mononuclear cells and was appreciable at low-power (×4) examination. Examples of moderate and mild chronic gastritis are shown in **Image 2A** and **Image 2D**. Severe gastritis had dense, diffuse, and uniform lymphoplasmacytic inflammation that filled the superficial lamina propria. In addition, it was noted whether there was a clinical request to rule out *Helicobacter*. For the second half of the study, only an immunostain for HP was performed on all cases, and it was noted whether there was a clinical request to rule out *Helicobacter*.

For statistical analysis, a diagnosis of HP gastritis by any method (usually by routine H&E or HP immunostain) was considered to represent a true-positive. Statistics were compiled on the cases, and sensitivity and specificity values for the different staining methods were calculated.

Results

The patient demographics and specimen characteristics are listed in **Table 1**. Of all biopsy specimens included in the study, 283 contained antral mucosa, and 73 did not. In 25 biopsy fragments (8.8%) containing antral mucosa, positivity was found for HP gastritis; 5 biopsy fragments (7%) without antral mucosa were positive for HP gastritis. In total, there were 187 cases that were received with a mandate to rule out *Helicobacter* on the requisition sheet or the specimen bottle; of these, 21 (11.2%) had HP gastritis. Nine biopsy specimens with HP gastritis were received without a request to rule out the organism.

For the first half of the study, of the 128 biopsy specimens, 87 were from the gastric antrum, 19 from the gastric body or fundus (oxyntic mucosa), 21 contained a mixture of antral and oxyntic mucosa, and 1 was from the gastric cardia. There were 13 cases of HP infection found using immunohistochemical analysis, 10 of which had been diagnosed as HP gastritis on first review using only H&E. In 5 of the 13 cases positive by immunohistochemical analysis, the organisms could not be seen on Giemsa or Warthin-Starry stain (ie, they were visible only using immunohistochemical analysis). Of the 13, 10 were found in biopsy specimens that contained antral mucosa or a mixture of antral and oxyntic mucosa, and 3 were found in biopsy specimens that contained only oxyntic mucosa. Examples of positive cases are shown in Image 2A, **Image 2B**, and **Image 2C**. Of the 3 cases in which the diagnosis of HP was not initially made and organisms were found with the immunostain used for the study, 2 had been previously immunostained at the time of diagnosis. Of these, 1 immunostain was positive in retrospect (ie, the organisms had been overlooked on first review) and 1 was negative (ie, a few organisms appeared on the second immunostain, but the

DOI: 10.1309/AJCPD8FFBJ5LSLTE

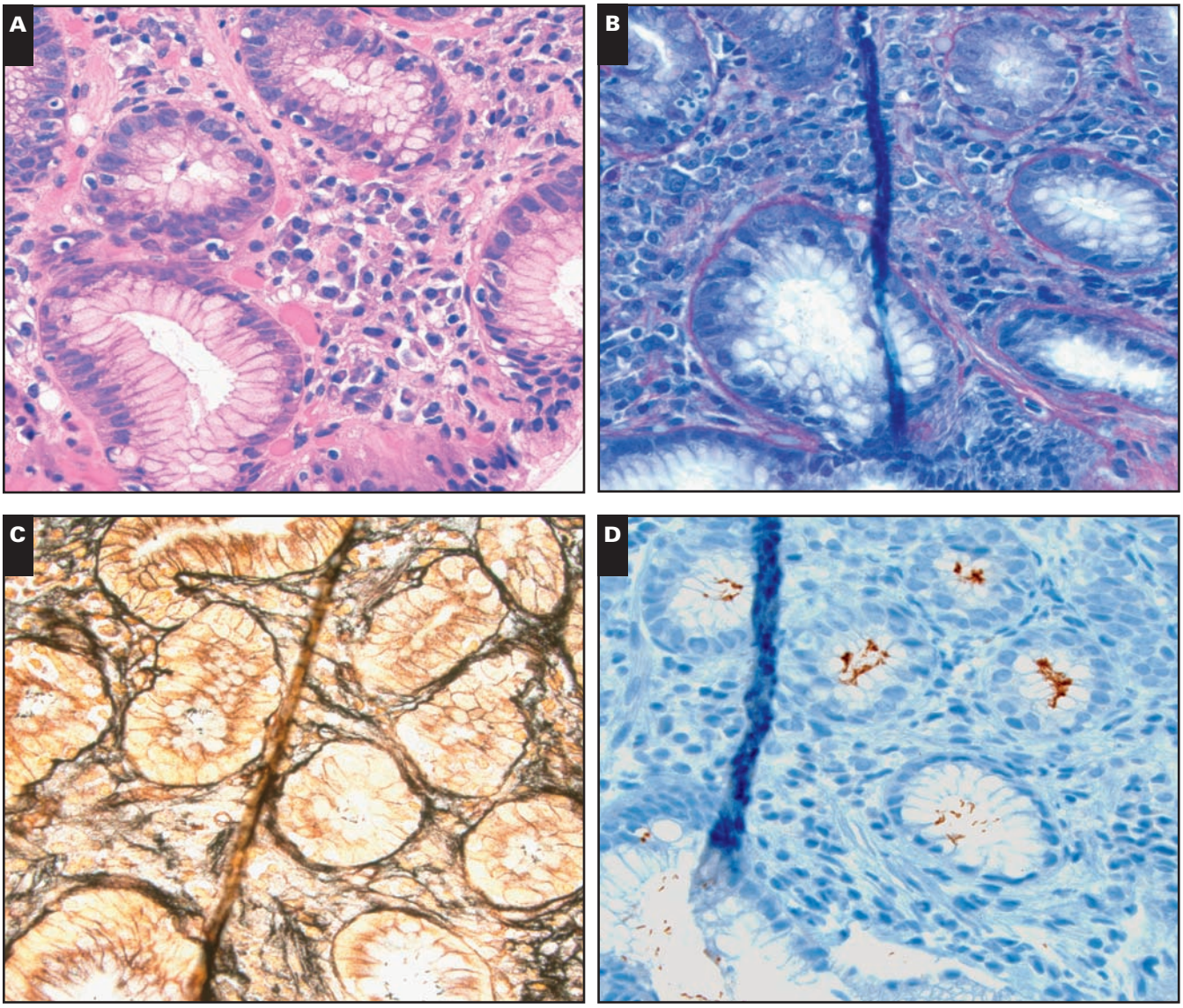

Image 1 **A**, *Helicobacter* organisms (H&E, ×600). **B-D**, Same section stained by Giemsa (**B**, ×600), Warthin-Starry (**C**, ×600), and polyclonal *Helicobacter* immunostain (**D**, ×600). Note characteristic superficial chronic gastritis characterized by numerous plasma cells.

first was negative). The third had only mild chronic inactive gastritis, and no immunostain was ordered initially, but the biopsy specimen contained only oxyntic mucosa. The remaining 12 cases with HP gastritis had moderate or severe chronic gastritis. None of the cases without HP had any more than mild chronic gastritis.

For the second, prospective part of the study, the routine use of immunohistochemical analysis for all stomach biopsy specimens was evaluated. Of 226 biopsy specimens (205 unique patients), 17 (from 15 patients) showed HP gastritis. Of the 17 biopsy specimens, 15 could be diagnosed without the use of any special stains. Nearly all of the biopsy specimens (16/17) contained antral-type mucosa, and all had at least moderate chronic gastritis. Five biopsy specimens contained moderate or severe chronic gastritis but did not show HP organisms by immunostains. Overall sensitivity and specificity for organism identification by H&E only, each of the cytochemical and immunohistochemical stains, and for identification of infection by the presence of at least moderate and at least mild chronic gastritis are given in **Table 2**.

Discussion

Gastric biopsies to rule out HP gastritis are commonly encountered in the practice of gastrointestinal pathology, and the accurate diagnosis of HP infection is important because of the association of longstanding infection with the development

DOI: 10.1309/AJCPD8FFBJ5LSLTE

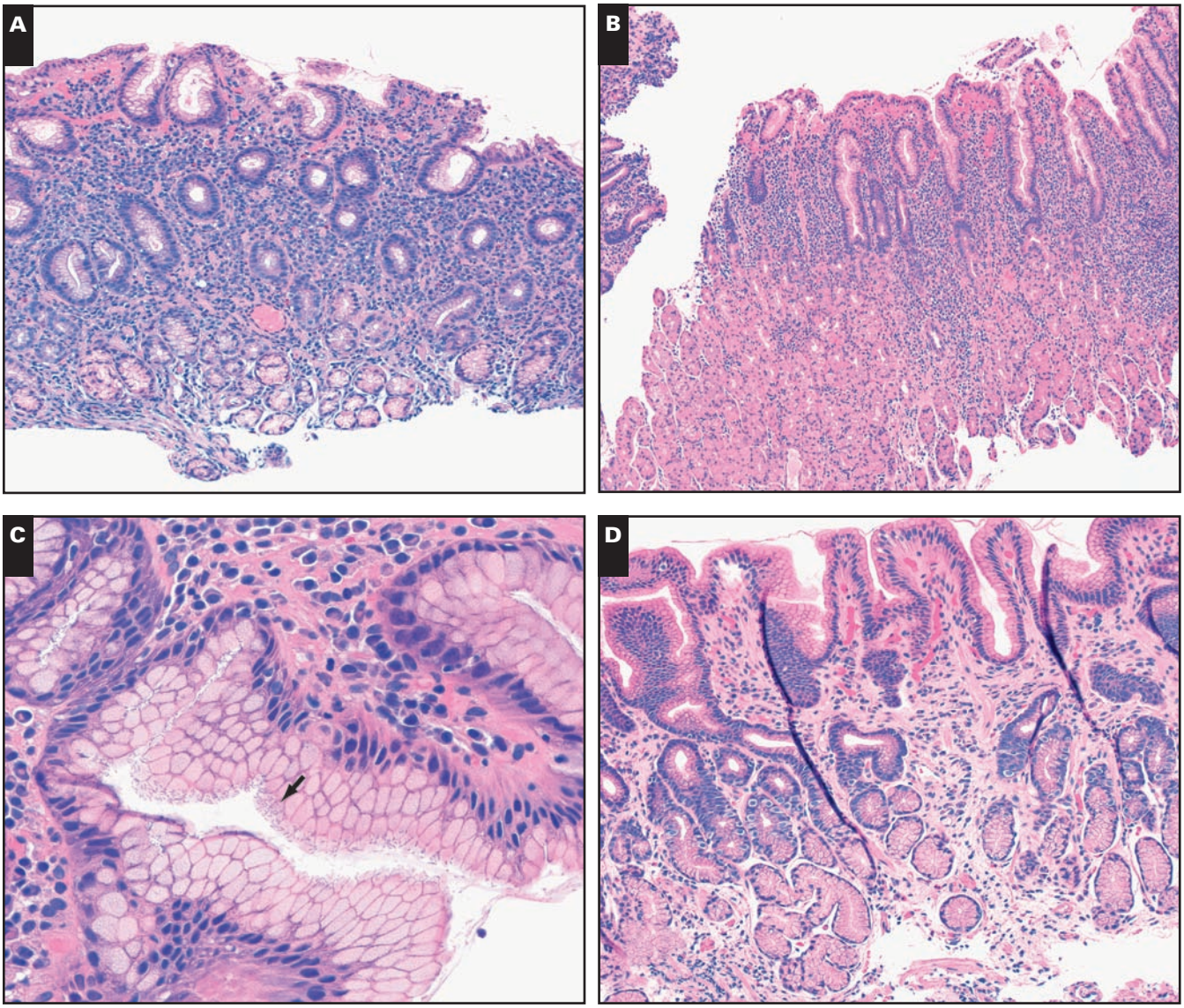

Image 2 **A**, Antral biopsy specimen with moderate gastritis (H&E, ×200). **B**, Oxyntic mucosa with moderate gastritis (H&E, ×100). **C**, *Helicobacter* organisms (indicated by arrow) (H&E, ×600). Both the antral and body/fundus biopsy specimens were positive for *H pylori*. **D**, Antral biopsy specimen with mild gastritis (H&E, ×200).

Table 1
Population Characteristics*

Variable	Result
Mean (range) age (y)	
Males (n = 131)	55.72 (17-88)
Females (n = 204)	54.84 (18-96)
Clinical mandate to "rule out *Helicobacter pylori*" (335 patients)	
Yes	187 (55.8)
No	148 (44.2)
Antral mucosa (335 patients)	
Present	276 (82.4)
Absent	59 (17.6)
Moderate or severe gastritis (356 specimens)	
Present	36 (10.1)
Absent	320 (89.9)

* Data are given as number (percentage) unless otherwise indicated.

of neoplasia and the fact that infected patients can be treated with combination therapy including proton pump inhibitors and antibiotics.[18,19] While many staining modalities can be used to identify HP in gastric biopsy specimens, our local practice experience and several studies have suggested that routine H&E staining is typically sufficient for identification of the organism, in contrast with the practice advocated by some for routine ancillary staining for all gastric biopsy specimens.[13,20-22] H&E staining can vary between laboratories, however, and it is prudent for an individual laboratory to evaluate the ability of its H&E stain to detect HP on positive cases by using special stains.

Our findings indicate that routine ancillary staining of all gastric biopsy specimens for HP is not indicated in our

DOI: 10.1309/AJCPD8FFBJ5LSLTE

Table 2
Sensitivity and Specificity Values for H&E, Giemsa, Warthin-Starry, and Immunohistochemical Stains and for the Presence of at Least Moderate and Mild Chronic Gastritis in the Diagnosis of *Helicobacter pylori* Gastritis

					Chronic Gastritis	
	H&E	Giemsa	Warthin-Starry	Immunohistochemical Staining	At Least Moderate	At Least Mild
Sensitivity (%)	83	62	62	97-100	97	100
Specificity (%)	100	97	98	100	98	81

practice. In contrast, immunostaining of cases with moderate or severe chronic gastritis is sufficient to diagnose nearly all cases of HP gastritis when the organisms cannot be seen on routine H&E stain, particularly when the gastric antrum has been sampled. It is interesting that 3 cases of HP gastritis were diagnosed on biopsy specimens containing only oxyntic mucosa. Two of these contained moderate gastritis, and the third was the only case in which HP was found in combination with mild gastritis, indicating that the organism should also be actively sought in this location. Furthermore, our findings suggest that a clinical suspicion of HP gastritis (as indicated by the surrogate finding of a clinical mandate to rule out *Helicobacter* on the requisition) should have essentially no role in the pathologic suspicion of HP infection. Rather, the presence of the characteristic gastritis of at least moderate severity should prompt a careful search for organisms and, when they cannot be found on H&E preparations, an order for HP immunostaining accompanied by a high pretest probability of infection. As a corollary, when at least moderate gastritis is found and no HP organisms can be found with the H&E stain, a careful search for organisms with an immunohistochemical stain is warranted to avoid overlooking subtle and/or scant positivity. Finally, the finding of 1 case in our series in which an initial HP immunostain was negative but a subsequent stain revealed scattered organisms suggests that when the index of suspicion is high based on the presence of at least moderate gastritis, repeating a negative stain may be prudent. In fact, the presence of the characteristic HP-type gastritis of at least moderate intensity may be sufficient to suggest the presence of the organism with high specificity (98% in our series), and a diagnosis of "*H pylori*–type gastritis" could potentially be sufficient for clinical treatment to be initiated.

Our findings confirm the assertion by other observers that H&E-stained sections are typically sufficient for diagnosis of HP gastritis. A recent study suggested that pathologists' ability to identify these distinct microorganisms was good no matter their training level.[16] In addition, nonimmunohistochemical methods of HP detection are less reliable than the immunohistochemical stain and will not allow dependable identification of subtle cases. Because they are not organism-specific, such cytochemical stains also highlight any bacteria in the surface mucus, meaning that the characteristic HP morphologic picture still needs to be carefully sought to avoid overdiagnosis. In contrast, the specificity of the immunostain is near 100%.[16] Thus, routine performance of Giemsa or Warthin-Starry staining, while less costly than immunohistochemical analysis, is not warranted either.

Our findings should be viewed in the light of the retrospective nature of the first part of the study, a relatively low disease prevalence in our local population, and the sample size. The overall rate of HP infection in our patient population was 8.4% (28 unique cases in 335 unique patients) during the 4-week period studied. Because all gastric biopsy specimens obtained at our institution are examined in 1 "center of excellence," this seems to be a true representation of the infection rate in patients biopsied endoscopically for our population, but it is lower than a recent report from the United States.[16] Nevertheless, we believe the relative sensitivity and specificity values to be a valid reflection of the usefulness of the methods of organism identification. Whether it may be sufficient to report *Helicobacter pylori*–type gastritis when at least moderate chronic gastritis is present, as suggested, will likely require additional study with a larger number of HP-positive cases.

A clinical request to rule out *Helicobacter* is not sensitive or specific for HP gastritis. Our institutional experience with regard to the use of ancillary stains in the evaluation of HP gastritis was validated. In addition, the presence of a moderate or severe gastritis is highly suggestive of HP gastritis, and these findings should be communicated in the pathology report.

From the Departments of Pathology, [1]University of Pittsburgh Medical Center, Pittsburgh, PA; and [2]University of Michigan, Ann Arbor.

Address reprint requests to Dr Hartman: Dept of Pathology, University of Pittsburgh Medical Center, 200 Lothrop St, A-610, Pittsburgh, PA 15213.

References

1. Marshall BJ, Warren JR. Unidentified curved bacilli in the stomach of patients with gastritis and peptic ulceration. *Lancet*. 1984;1:1311-1315.
2. Michel A, Waterboer T, Kist M, et al. *Helicobacter pylori* multiplex serology. *Helicobacter*. 2009;14:525-535.

DOI: 10.1309/AJCPD8FFBJ5LSLTE

3. Al-Humayed SM, Ahmed ME, Bello CS, et al. Comparison of 4 laboratory methods for detection of *Helicobacter pylori*. *Saudi Med J*. 2008;29:530-532.
4. Akhter J, Shrestha HG. Rapid detection of *Helicobacter pylori* by endoscopic brush cytology and comparison with histopathology. *Kathmandu Univ Med J (KUMJ)*. 2007;5:387-390.
5. Tajbakhsh S, Samarbaf-Zadeh AR, Moosavian M. Comparison of fluorescent in situ hybridization and histological method for the diagnosis of *Helicobacter pylori* in gastric biopsy samples. *Med Sci Monit*. 2008;14:BR183-BR187.
6. de Martel C, Plummer M, van Doorn LJ, et al. Comparison of polymerase chain reaction and histopathology for the detection of *Helicobacter pylori* in gastric biopsies. *Int J Cancer*. 2010;126:1992-1996.
7. Palka M, Tomasik T, Windak A, et al. The reliability of ELISA in predicting *H pylori* infection in dyspeptic populations under age 45. *Med Sci Monit*. 2010;16:PH24-PH28.
8. Veldhuyzen van Zanten SJ, Sherman PM. *Helicobacter pylori* infection as a cause of gastritis, duodenal ulcer, gastric cancer and nonulcer dyspepsia: a systematic overview. *CMAJ*. 1994;150:177-185.
9. Uemura N, Okamoto S, Yamamoto S, et al. *Helicobacter pylori* infection and the development of gastric cancer. *N Engl J Med*. 2001;345:784-789.
10. NIH Consensus Conference. *Helicobacter pylori* in peptic ulcer disease. NIH Consensus Development Panel on *Helicobacter pylori* in Peptic Ulcer Disease. *JAMA*. 1994;272:65-69.
11. Morgner A, Bayerdorffer E, Neubauer A, et al. Malignant tumors of the stomach: gastric mucosa–associated lymphoid tissue lymphoma and *Helicobacter pylori*. *Gastroenterol Clin North Am*. 2000;29:593-607.
12. Dixon MF, Genta RM, Yardley JH, et al. Classification and grading of gastritis: the updated Sydney System: International Workshop on the Histopathology of Gastritis, Houston 1994. *Am J Surg Pathol*. 1996;20:1161-1181.
13. Genta RM, Robason GO, Graham DY. Simultaneous visualization of *Helicobacter pylori* and gastric morphology: a new stain. *Hum Pathol*. 1994;25:221-226.
14. Vartanian RK, Leung JK, Davis JE, et al. A novel alcian yellow–toluidine blue (Leung) stain for *Helicobacter* species: comparison with standard stains, a cost-effectiveness analysis, and supplemental utilities. *Mod Pathol*. 1998;11:72-78.
15. Sepulveda AR, Patil M. Practical approach to the pathologic diagnosis of gastritis. *Arch Pathol Lab Med*. 2008;132:1586-1593.
16. Wang XI, Zhang S, Abreo F, et al. The role of routine immunohistochemistry for *Helicobacter pylori* in gastric biopsy. *Ann Diagn Pathol*. 2010;14:256-259.
17. Faigel DO, Furth EE, Childs M, et al. Histological predictors of active *Helicobacter pylori* infection. *Dig Dis Sci*. 1996;41:937-943.
18. Talley NJ, Ormand JE, Carpenter HA, et al. Triple therapy for *Helicobacter pylori* in nonulcer dyspepsia. *Am J Gastroenterol*. 1991;86:121-123.
19. Rimbara E, Fischbach LA, Graham DY. Optimal therapy for *Helicobacter pylori* infections. *Nat Rev Gastroenterol Hepatol*. 2011;8:79-88.
20. Young DG. A stain for demonstrating *Helicobacter pylori* in gastric biopsies. *Biotech Histochem*. 2001;76:31-34.
21. Rotimi O, Cairns A, Gray S, et al. Histological identification of *Helicobacter pylori*: comparison of staining methods. *J Clin Pathol*. 2000;53:756-759.
22. El-Zimaity HM, Segura AM, Genta RM, et al. Histologic assessment of *Helicobacter pylori* status after therapy: comparison of Giemsa, Diff-Quik, and Genta stains. *Mod Pathol*. 1998;11:288-291.

DOI: 10.1309/AJCPD8FFBJ5LSLTE

HOLOGIC®

First and Only FDA Cleared
Digital Cytology System

Genius™ Cervical AI

Genius™ Review Station

Genius™ Digital Imager

Empower Your Genius With Ours

Make a Greater Impact on Cervical Cancer
with the Advanced Technology of the
Genius™ Digital Diagnostics System

Click or Scan
to discover more

genius™
DIGITAL DIAGNOSTICS