Appropriate Use of Special Stains for Identifying *Helicobacter pylori*

Recommendations From the Rodger C. Haggitt Gastrointestinal Pathology Society

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Abstract: *Helicobacter pylori* is a major cause of gastroduodenal injury, gastric cancer, and lymphoma, and, thus, there is great interest in its detection and eradication. Several detection methods are available, including histochemical and immunohistochemical stains. Application of these stains in clinical practice is heterogenous, to say the least. Although they were developed to enhance *H. pylori* detection, changing practice models, financial considerations, and a perceived need for rapid case turnaround have led to their widespread use in routine staining studies ordered reflexively on all gastric biopsies. Emerging data suggest that most of these stains are not needed to establish a diagnosis of *H. pylori* infection, and their added value when biopsies show minimal, or no, inflammation is not clear. In this manuscript, the Rodger C. Haggitt Gastrointestinal Pathology Society puts forth recommendations regarding ancillary stain usage for *H. pylori* detection based upon critical literature review and collective experience. Pathologists rarely, if ever, detect *H. pylori* in "normal" biopsies, but readily observe them in optimally stained hematoxylin and eosin sections from infected patients. Therefore, we suggest that use of ancillary stains is appropriate when biopsies show chronic, or chronic active, gastritis without detectable *H. pylori* in hematoxylin and eosin-stained sections, but performing them "up front" on all gastric biopsies is generally unnecessary. Application of these stains to nongastric biopsies and polyps is appropriate in an extremely limited set of circumstances. It is our hope that recommendations provided herein will provide helpful information to gastroenterologists, pathologists, and others involved in the evaluation of patients for possible *H. pylori* infection.

Key Words: reflex testing, gastritis, immunohistochemistry, ancillary, up front

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BACKGROUND

The link between *Helicobacter pylori* infection and gastritis and peptic ulcer disease was established in the seminal publication by Marshall and Warren in 1984.[1] The monumental nature of that discovery fully justified the authors' Nobel Prize in Physiology or Medicine in 2005. It is now inarguably clear that *H. pylori* is the dominant cause of gastritis worldwide. Major disease associations include duodenal and gastric ulcers, chronic gastritis, atrophic gastritis, iron deficiency, mucosa-associated lymphoid tissue (MALT)-type lymphomas of the stomach, and gastric adenocarcinoma.[2] Indeed, *H. pylori* is considered a class 1 carcinogen by the World Health Organization.[3]

Eradication of *H. pylori* is commonly recommended when it is identified.[4] Strong recommendations for treatment are made if active peptic disease, untreated confirmed history of peptic ulcer disease, low-grade gastric MALT-type

lymphoma, or locally excised early gastric cancer are present. The value of *H. pylori* eradication in other, more common, situations is less clear. Benefits of eradication among patients with nonulcer dyspepsia, unexplained iron deficiency, nonsteroidal anti-inflammatory drug use, gastroesophageal reflux disease (GERD), and populations at low risk for gastric cancer have not been clearly demonstrated.[5] Indeed, infected patients do not necessarily have any symptoms or endoscopic evidence of mucosal injury. Some investigators consider the organism to be commensal in this situation and do not recommend its eradication.[6,7]

Despite its variable clinical and endoscopic features, *H. pylori* infection induces some degree of chronic inflammation of the gastric mucosa in virtually all cases: the presence of at least mild chronic inflammation is 100% sensitive for *H. pylori* infection.[8] Severity of inflammation varies depending on the duration of infection and the presence or absence of bacterial virulence factors, such as CagA.[9] Progressively more intense inflammation is increasingly predictive of underlying infection. Greater than 90% of gastric biopsies that contain *H. pylori* show moderate to severe chronic gastritis characterized by a superficial, band-like infiltrate rich in plasma cells and other mononuclear cells, often in combination with neutrophilic inflammation (ie, chronic active gastritis) (Fig. 1).[10] Moderate chronic gastritis (ie, large confluent groups of chronic inflammatory cells identifiable at low magnification) is 97% sensitive and 98% specific for *H. pylori* infection, with a negative predictive value of 98%.[8,10] Other helpful features include intraepithelial neutrophils in gastric pits, pit abscesses, and lymphoid aggregates with germinal centers. Chronic gastritis without neutrophils (ie, chronic inactive gastritis) less commonly reflects ongoing *H. pylori* infection, although it may be seen in incompletely treated patients with *H. pylori* gastritis or in those who receive antibiotic therapy with partial efficacy against the organism. Persistent inflammation leads to intestinal metaplasia and atrophy of the gastric mucosa, which is associated with a decreased likelihood of *H. pylori* detection.[10] Successful *H. pylori* eradication causes a fairly rapid disappearance of neutrophils and gradually diminishing chronic inflammation, but intestinal metaplasia may not resolve.[11]

TECHNIQUES FOR *H. PYLORI* DETECTION

Methods for detection of *H. pylori* can be divided into noninvasive and invasive techniques on the basis of whether or not tissue is obtained by biopsy. Although the focus of this manuscript is on histochemical and immunohistochemical stains once tissue has been obtained, a brief discussion of the various means of detecting *H. pylori* is appropriate as the results of such testing could influence stain utilization.

Noninvasive Tests

Anti-*Helicobacter* Antibodies

Anti-*Helicobacter* antibodies form in almost all patients with *H. pylori* infection. They are detectable by readily available serologic tests that show a very high sensitivity (90% to 97%) for *H. pylori* infection.[12] The likelihood of finding *H. pylori* by invasive means in a patient with known negative serologic studies is very low. Only 2% of *H. pylori*-infected patients are seronegative for both IgG and IgA.[13] Specificity for *H. pylori* infection is also reasonably high, but antibodies persist for a considerable period of time after successful eradication, meaning that serologic positivity does not necessarily imply ongoing infection. The likelihood of finding active *H. pylori* infection in patients with positive serologic studies depends on epidemiologic factors. Positive *H. pylori* serologies are highly associated with current infection in regions where infection is endemic and treatment is either largely unavailable or not clinically indicated. The likelihood of a serology-positive individual having *H. pylori* infection is much lower in nonendemic areas and those in which antibiotic therapy is readily available, such as the United States.

Helicobacter Antigen Stool Assay

Stool antigen testing utilizes polyclonal or monoclonal antibodies directed against bacterial antigens in an enzymatic immunoassay. These assays detect *H. pylori* in nearly 95% of patients with active infection.[14] Treatment with proton pump inhibitors does not decrease their sensitivity.[15]

Urease Breath Test

These assays are based on the principle that urease activity is present in the stomachs of *H. pylori*-infected individuals. Patients ingest urea labeled with a carbon isotope (^{13}C or ^{14}C), which is cleaved by urease to produce labeled carbon dioxide that is detected on the exhaled breath. The urease breath test detects active infection with high sensitivity (>95%) and specificity (>95%), although treatment with proton pump inhibitors does decrease its sensitivity.[15,16] Stool assays and urease breath tests are the preferred methods of detecting ongoing *H. pylori* infection by noninvasive means.[12]

Invasive Tests

Rapid Urease Test

A number of commercially available rapid urease, or *Campylobacter*-like organism, tests are available. These assays rely on the urease activity of *H. pylori* to change the color indicator of a substrate to which tissue biopsy fragments are directly applied. The tests are inexpensive once the biopsy has been obtained and have high specificity and sensitivity, although sensitivities are lower when *H. pylori* organisms are present in small numbers.[12] These assays do not allow assessment for morphologic disease patterns of the mucosa (eg, atrophic gastritis, malignancy) when used independent of histologic examination and are probably redundant when performed in combination with histologic biopsy interpretation.

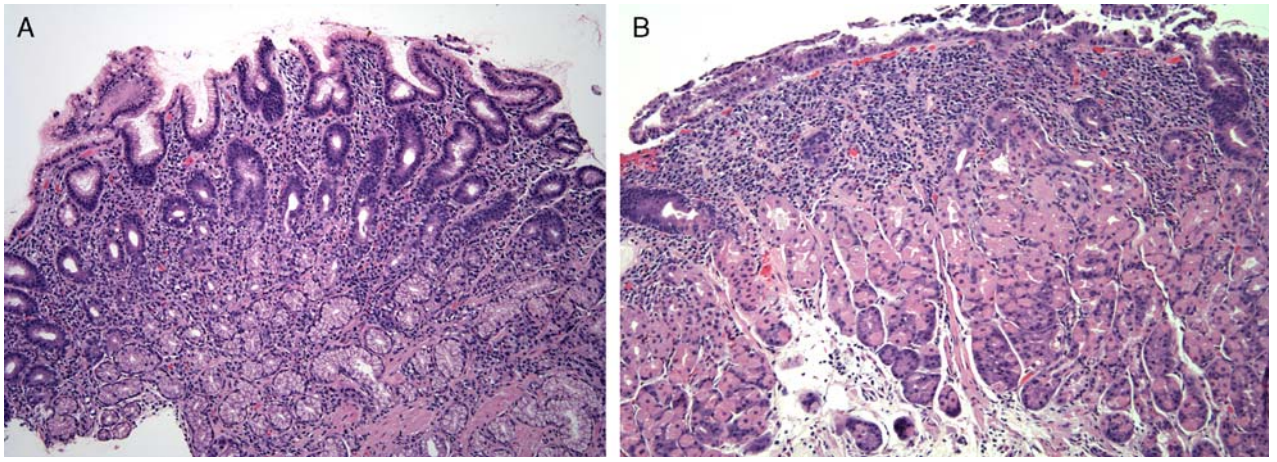

FIGURE 1. A, Chronic active *H. pylori*-associated gastritis diffusely involves the antral mucosa. Sheets of plasma cells and lymphocytes are present between the gastric pits and show relative sparing of the deeper mucosa. B, *H. pylori* infection of the proximal stomach produces a superficial chronic gastritis with a band of mononuclear cell-rich inflammation subjacent to the foveolar epithelium.

Culture for Organisms

Cultures for *H. pylori* can be performed directly from biopsy samples and have essentially 100% specificity for *H. pylori* infection. Several issues preclude their widespread use. First, they have lower sensitivity compared with other assays. Second, tissue samples obtained for culture require immediate attention because *H. pylori* organisms are fragile outside their native environment.[12] Third, cultures are similar to the *Campylobacter*-like organism test in that they do not allow morphologic assessment of the mucosa.

Molecular Testing For *H. pylori*

H. pylori can be detected by in situ hybridization or polymerase chain reaction. Although their performance characteristics are high, several factors detract from the utility of these methods. Both tests detect organisms, regardless of whether they are alive or dead, and are susceptible to false-positive results due to contamination.[12] These assays are also expensive and are not generally used for routine evaluation of patients.

Histologic Examination

Hematoxylin and eosin (H&E) staining protocols are not standardized across practices, and organisms may be difficult to detect in some laboratories, but optimized staining protocols detect *H. pylori* infection with sensitivities approaching 100%. Ancillary histochemical and immunohistochemical stains enhance detection even further in specific situations. The former utilize dyes that directly stain the organisms, whereas the latter involve indirect staining of organisms: a primary antibody directed against *H. pylori* binds to a secondary antibody that is conjugated to an enzyme. A host of histochemical stains have been used to identify *H. pylori*, including Wright-Giemsa, toluidine blue, thiazine, "Genta," Warthin-Starry, and Alcian yellow. Immunohistochemical staining has become the gold standard for *H. pylori* detection and show near 100% sensitivity and specificity in some studies. The latter are more costly than histochemical stains, and their availability in remote and third world areas is limited. For these reasons, there is still interest in the application of various histochemical stains to gastric mucosal biopsies for *H. pylori* detection.

Assessing the optimal choice of special stains requires some historical context. In the pre–proton pump inhibitor era, large numbers of *H. pylori* organisms were present in the mucus lining of the foveolar surface and showed a predilection for the antrum (Fig. 2). This distribution of organisms is still typical in many parts of the world today. Comparisons among various histochemical stains (ie, H&E, Wright-Giemsa, toluidine blue, thiazine, "Genta", Warthin-Starry, and Alcian yellow) and between histochemical and immunohistochemical stains show very little difference in sensitivity and specificity when organisms are abundant.[17] However, the widespread availability of proton pump inhibitors has clearly altered the features of *H. pylori* infection. Organisms are present in smaller numbers, are more likely to be located in the deeper pits and glands, may "migrate" to the proximal stomach, and display coccoid and intracellular forms in patients who receive acid suppression (Fig. 3).[18] In this setting, most histochemical stains, including H&E, have sensitivities in the 60% to 90% range compared with immunohistochemistry, which is why many authors advocate use of immunostains rather than histochemical stains if ancillary testing is indicated (Table 1).[8,13,19,20]

Not all bacteria in the stomach are *H. pylori*. The differential diagnosis includes oral flora contaminants, bacteria in gastric contents of patients with hypochlorhydria, and *H. heilmannii* infection. In most cases, non-*Helicobacter* bacteria are easily distinguished from *H. pylori* and do not pose substantial diagnostic challenges. The former appear as "clouds" of cocci and/or bacilli that are thicker and larger

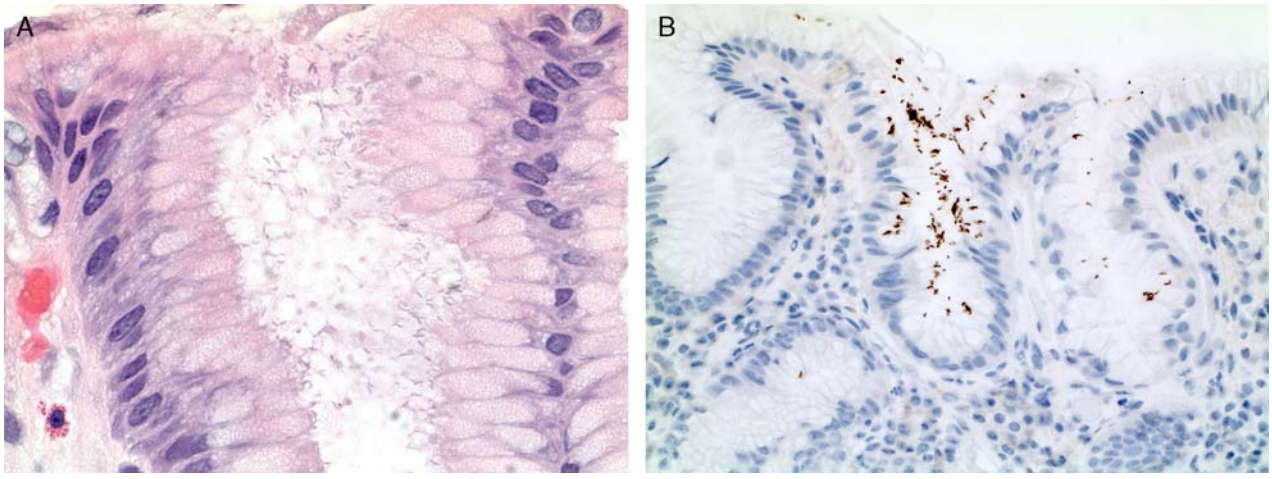

FIGURE 2. A, Innumerable curvilinear *H. pylori* organisms are present within the mucus layer adherent to foveolar epithelium. B, Organisms show strong immunopositivity with the *H. pylori* antibody. Although use of immunostains in this situation may be useful for teaching purposes, they are of no added value to H&E-stained sections in the management of patients.

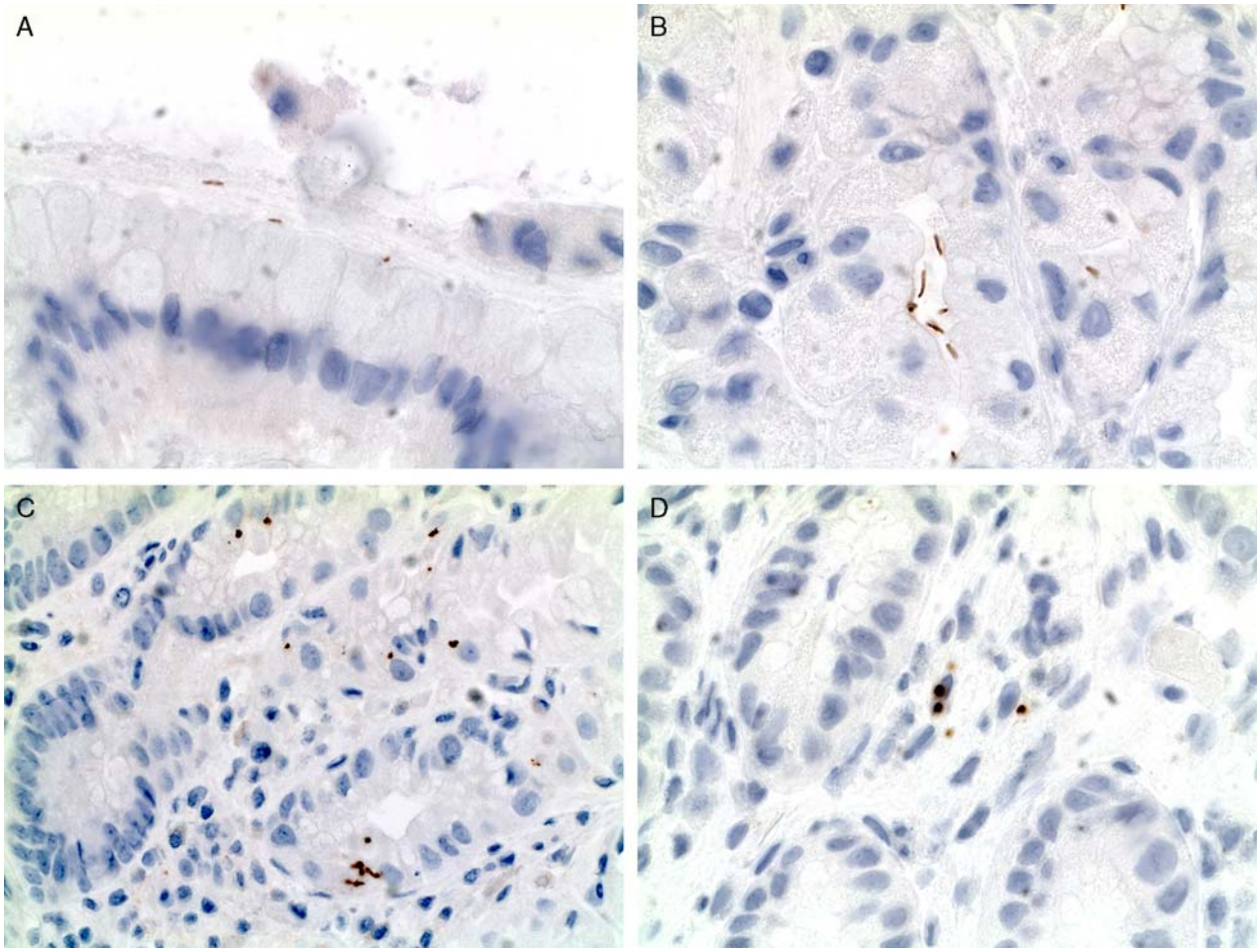

FIGURE 3. Patients who have been incompletely treated for *H. pylori* infection or received proton pump inhibitor therapy have far fewer bacteria in biopsies that may require immunohistochemical stains for detection. Rare bacteria are present in the mucus layer (A) and pit lumina (B). Infrequent coccoid forms are present in patients receiving proton pump inhibitor therapy (C), many of which are intracellular (D).

TABLE 1. GIPS Recommendations for Use of Ancillary Stains in Detection of *H. pylori*

Morphologic Findings	GIPS Recommendations For Special Stains*
Normal gastric mucosa	Not indicated
Chemical (reactive) gastropathy	Not indicated if chemical injury is only abnormality
	Appropriate if superimposed chronic gastritis is present
Chronic active gastritis	Not indicated if H&E demonstrates organisms
	Appropriate if H&E is negative for *H. pylori*
	Low yield if serologic studies are known to be negative
Chronic inactive gastritis	Not indicated if serologic studies are known to be negative, but probably justified in most other cases
	Appropriate if gastroduodenal ulcers are present
	Appropriate if gastric MALT-type lymphoma or adenocarcinoma is present
	Appropriate if duodenal lymphocytosis is present
	Appropriate in patients with prior *H. pylori* treatment
	Appropriate in high-risk demographic areas
Lymphocytic gastritis	Appropriate
Granulomatous gastritis	Unclear utility; no recommendation at this time
Eosinophilic gastritis	Unclear utility; no recommendation at this time
Isolated chronic active carditis	Appropriate
Isolated chronic inactive carditis	Not indicated, unless gastric biopsies are unavailable and/or serologic studies are positive
Fundic gland polyps	Not indicated
Hyperplastic polyps	Generally not indicated; ancillary stains may be considered if chronic inflammation is present and other biopsies are lacking
Isolated chronic active carditis	Appropriate
Isolated chronic inactive carditis	Not indicated, unless gastric biopsies are unavailable and/or serologic studies are positive
Barrett esophagus	Not indicated
Duodenal biopsies	Not indicated in overwhelming majority of cases

*We recommend use of immunohistochemistry as the preferred ancillary staining method.

than *H. pylori* organisms. Non-*Helicobacter* organisms are located in luminal mucin and show no specific relationship to the mucosa, whereas *H. pylori* organisms are seen in close proximity to the foveolar epithelium. *H. heilmannii* are nearly twice as long as *H. pylori* and are much thicker. They have a pronounced corkscrew appearance distinct from the curvilinear shape of *H. pylori* (Fig. 4). Histochemical stains do not distinguish *H. pylori* from other bacteria, and commercially available antibodies directed against *H. pylori* organisms cross-react with *H. heilmannii*, making ancillary stains less useful in this differential diagnosis.

Although characteristic inflammatory changes signifying *H. pylori* infection can prompt ordering of ancillary stains as needed, many laboratories use stains in a wide variety of other situations, including normal biopsies.

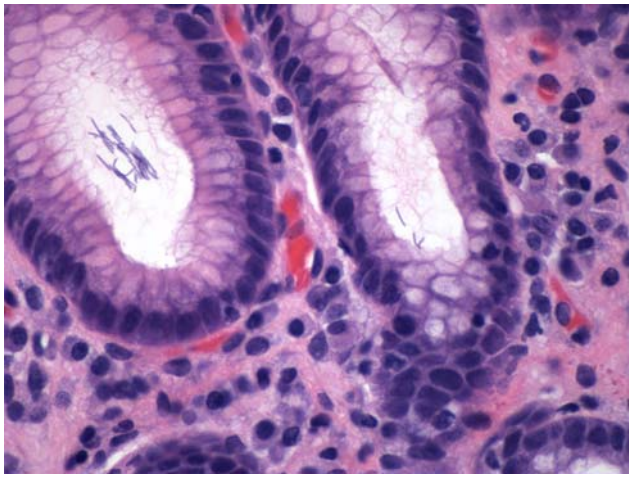

FIGURE 4. *H. heilmannii* are longer than *H. pylori* and have a pronounced corkscrew appearance. They are also positive with the commercially available *H. pylori* immunostain, and, thus, immunohistochemistry is of no value in distinguishing between these species.

Indeed, a recent survey of the Rodger C. Haggitt Gastrointestinal Pathology Society (GIPS) membership revealed that nearly 50% of pathologists with specific interest in gastrointestinal pathology use at least 1 ancillary stain for *H. pylori* reflexively (up front) on all gastric biopsies before review of initial sections. The added value of undirected ancillary to routine evaluation of H&E-stained sections has never been demonstrated, particularly when applied to cases that show minimal, if any, inflammation. The purposes of this manuscript are to critically evaluate the literature regarding the utility of ancillary stains for *H. pylori* detection and to propose practice guidelines for their use.

METHODS FOR DEVELOPING RECOMMENDATIONS

A web-based survey regarding ancillary stain usage in the evaluation of gastrointestinal mucosal biopsies was distributed to the members of the GIPS. Data compiled from 171 respondents were presented at the GIPS forum during the 2012 United States and Canadian Academy of Pathology Annual Meeting in Vancouver, BC. The aims of the discussion were to identify and evaluate practice trends among gastrointestinal pathologists employed in various settings. Members of the GIPS were invited to participate in working groups charged with critically reviewing pertinent literature and developing practice guidelines for usage of ancillary stains commonly applied to gastrointestinal biopsies, particularly those that facilitate *H. pylori* detection. These working groups included pathologists practicing in both academic and nonacademic settings. Subsequent recommendations were vetted among members of the GIPS Executive Committee and made available to the GIPS members through the society website (http://usgips.com). Submitted comments were analyzed for validity and incorporated into the consensus recommendations before final review by a third

group of senior GIPS members, none of whom were authors of the manuscript or members of the GIPS Executive Committee. A compilation of literature findings and practice recommendations is enumerated below.

DETECTION OF *HELICOBACTER*: GENERAL CONSIDERATIONS

There is virtually no literature providing a bona fide cost analysis of the role of random gastric biopsies in *H. pylori* detection, nor are there any available data regarding financial costs of ancillary stains in the context of morbidity prevented and mortality delayed. No current evidence suggests that detecting organisms in patients with low bacterial load prevents *H. pylori*-related medical care expenses in the future, nor are there available data regarding special stain costs per year of life saved. Despite these limitations, many laboratories use at least 1 ancillary stain to detect *H. pylori* in all gastric biopsies, and a substantial subset of these groups use multiple stains on 1 or more biopsies from the same patient. The majority of these "up front" stains do not provide meaningful information beyond that which is apparent upon careful examination of H&E-stained sections.[8] Most *H. pylori*-positive gastric biopsies can be confidently diagnosed either by detecting infection in H&E-stained sections or by recognizing histologic features of infection that prompt evaluation with special stains. In subsequent sections, we outline the circumstances in which ancillary stains may facilitate histologic evaluation of gastric biopsies and propose recommendations regarding their use.

DETECTION OF *HELICOBACTER*: RECOMMENDATIONS FOR ANCILLARY STAIN USAGE

The purpose of this section is to provide data-based opinions regarding the appropriate and clinically indicated use of special stains when detecting *H. pylori* in tissue specimens. Although data describing the added value of ancillary stain usage for decreasing *H. pylori*-related morbidity and mortality are lacking, we can provide informed opinion regarding the scenarios in which special stains for *H. pylori* are appropriately applied. For the sake of clarity, we will present information in a question and answer format that outlines a number of clinical scenarios in which special stains for *H. pylori* might be ordered. We recommend the use of immunohistochemical, rather than histochemical, stains to facilitate *H. pylori* detection and will limit comments below to their use. However, we recognize that some pathologists may opt to use histochemical stains for reasons dictated by the natures of their practices.

How Often Should I Expect to Find *H. pylori* in Gastric Biopsies?

The frequency with which *H. pylori* gastritis is diagnosed in a population depends on whether or not patients are from endemic areas. Infection risk is linked to lower socioeconomic status; thus, *H. pylori* gastritis is more common in equatorial countries, urban areas, and regions with suboptimal sanitation, where infection rates approach 90%.[21] By comparison, 30% to 40% of the US population was infected with *H. pylori* in 2000, and a disproportionate number of these patients were elderly individuals who acquired *H. pylori* as children.[22] It is anticipated that the incidence of *H. pylori* infection in North America will decline as this group ages and *H. pylori* is systematically eradicated upon detection. Recent data also suggest that the prevalence of *H. pylori* infection in North America varies from state to state. Reported rates of infection range from a low of 3.9% in Kansas to a high of 31.7% in Puerto Rico, with relatively high prevalence noted in Louisiana (24%) and North Carolina (16%).[10,23,24] The prevalence of infection is higher among Medicaid patients compared with those with other types of insurance.[23]

Does Endoscopic Sampling Affect the Likelihood of Finding *H. pylori* if it is Present?

There is a relationship between the number of gastric biopsy samples obtained and the rate of *H. pylori* identification. Mucosal atrophy is associated with a decreased likelihood of finding *H. pylori*, so multiple samples of antrum and body should be obtained when extensive intestinal metaplasia is present.[25] In the pre–proton pump inhibitor era, 1 sample of the antrum had 80% to 90% sensitivity for detection of *H. pylori*, and 2 samples (either both antral or antral and body) increased the sensitivity to 95% to 96%.[26] Proton pump inhibitors, however, may cause a shift of bacteria from the antrum to the more proximal stomach.[27] These agents also decrease the number of organisms in both antrum and body. Thus, the Sydney System recommendation of obtaining 2 samples from the antrum, 2 from the gastric body, and 1 from the incisura angularis seems particularly appropriate today.[28] The endoscopist may choose to place all of the biopsies in 1 container or submit them separately by anatomic site. Submitting in 1 cassette allows evaluation of both antrum and body in a single slide with 1 ancillary stain but may make it difficult to identify the anatomic site if atrophy of oxyntic glands is present. If multiple cassettes are used, immunohistochemistry for *H. pylori* should be performed judiciously, namely, limiting its use to the most inflamed biopsies that are more likely to contain detectable organisms. Multiple ancillary staining analyses performed on several different samples from the same patient are not normally justified, although they may be obtained in a serial manner, as needed (ie, immunohistochemical staining analyses performed on 1 block are initially negative for *H. pylori*, and subsequent staining analyses on additional tissue blocks are deemed necessary in the professional judgment of the pathologist).

How Effective is an H&E Stain for Detecting *H. pylori* Among Infected Patients?

The H&E stain is universally regarded as a very effective means of detecting *H. pylori* (Table 2). Sensitivities range from 70% to 95% and may be improved by prolonged

exposure to hematoxylin as well as fastidiousness and patience on the part of the examining pathologist.[8,10,24,29,30] One study demonstrated that evaluation of optimally stained H&E sections detects *H. pylori* with a sensitivity of 91% compared with immunohistochemistry, but requires assessment of approximately 5.75 high-power fields (range, 1 to 25) compared with a maximum of 3 high-power fields required to detect *H. pylori* by immunohistochemistry when organisms are present.[10] The specificity of H&E for *H. pylori* is very high, and there is usually no incremental value in performing special staining analyses when organisms are apparent in H&E-stained sections. Analysis of confirmatory immunohistochemical staining analyses may be indicated when diagnostic features are equivocal, such as cases in which organisms are rare or their morphologic appearance is altered. Histochemical stains are unlikely to detect rare coccoid forms, especially if they are present in deep glands or an intracellular location.

Is a Clinical Request of "Rule Out *H. pylori*" an Indication for Ancillary Staining Studies?

There is a poor correlation between the endoscopic appearance of the stomach and the presence of *H. pylori* or severity of inflammation. Endoscopic "gastritis" may yield histologically normal biopsies, whereas endoscopically normal stomachs may harbor a brisk *H. pylori* gastritis.[31,32] Data suggest no relationship between a clinician request to rule out *H. pylori* and histologic detection of organisms.[8] A request to evaluate for *H. pylori* when patients have a previously treated infection is probably reasonable, although treatment failures occur in a minority of instances. Patients with both successfully and unsuccessfully treated gastritis have persistent chronic inflammation for weeks to months after cessation of therapy, so this finding alone is of limited predictive value when assessing biopsies subsequent to eradication therapy.

Is it Appropriate to Perform Ancillary Staining if *H. pylori* Organisms Are Detected in Routine (H&E) Staining?

H. pylori organisms are, in most cases, readily seen with routine (H&E) stains when they are present, although conditions should be optimized for detection because formulations and staining methodologies can vary widely among institutions.[8,10,17,24,30] The specificity of H&E-stained sections for *H. pylori* is very high, and the organism can be easily distinguished from others in the differential diagnosis. Non-*Helicobacter* organisms encountered at the luminal surface, such as oral contaminants and bacterial colonies, bear no morphologic resemblance to *H. pylori*, nor do they elicit inflammatory mucosal changes typical of *Helicobacters*. *H. pylori* is morphologically distinct from *H. heilmannii*, and, in any event, distinguishing between the 2 is of little practical utility as both are similarly treated. Confirmatory immunohistochemical staining for *H. pylori* is indicated when organisms are not clearly recognized or have unusual morphologic features that prevent a definitive diagnosis. There is no incremental value to using special stains if *H. pylori* organisms are clearly visible with H&E-stained sections.

Is it Appropriate to Perform Ancillary Staining if There is a Chronic Active Gastritis but *H. pylori* Organisms Are NOT Visible by Routine (H&E) Staining?

The "chronic active" gastritis pattern of inflammation is present in approximately 30% of gastric biopsies and, when present, is associated with *H. pylori* infection in nearly 75% of cases (Fig. 1).[24] The positive predictive value of moderate lymphoplasmacytic and neutrophilic inflammation for *H. pylori* infection is $>90\%$. Bacteria may be scarce in patients who receive proton pump inhibitors or are hypochlorhydric for other reasons, and one may overlook the infection when very few organisms are present, involvement of the surface is patchy, bacteria are located within gastric epithelial cells, or bacteria appear as coccoid forms rather than curvilinear rods (Fig. 3).[10] Other causes of *H. pylori*-negative chronic active gastritis include idiopathic inflammatory bowel disease, Epstein-Barr virus infection, and less common etiologies such as poorly characterized immune-mediated disorders and other infections.[33] Of these, inflammatory bowel disease is the most practically relevant: 26% of pediatric patients with Crohn disease and 13% of ulcerative colitis patients have *H. pylori*-negative chronic active gastritis compared with just 2% of controls.[25] On the basis of these observations, we conclude that chronic active gastritis is a strong indication for using ancillary stains when H&E-stained sections fail to demonstrate *H. pylori*.

Is it Appropriate to Perform Ancillary Staining if There is a Chronic Inactive Gastritis and *H. pylori* Organisms Are Not Visible by Routine (H&E) Staining?

More than 95% of *H. pylori*-positive gastritis samples display moderate to severe chronic inflammation.[8] The vast majority of biopsies that show only chronic inactive gastritis, lack *H. pylori* organisms by any detection method, particularly when the inflammatory infiltrate is mild. Unfortunately, data regarding the utility of ancillary stains to detect *H. pylori* in patients with chronic inactive gastritis are limited because most studies have not evaluated this patient group as a separate category distinct from patients with more exuberant inflammatory activity.[28] Immunohistochemistry detects *H. pylori* organisms in $<10\%$ of patients who have a chronic inactive gastritis-type pattern of injury, even if they have proven *H. pylori* infection by other means.[24,34] However, clinicians are interested in detecting even mild infections and, thus, may pressure pathologists to "rule out" *H. pylori* despite its low prevalence in chronic inactive gastritis. Indeed, the chronic inactive gastritis cohort is the largest group of patients in which the use of ancillary stains for *H. pylori* detection should be considered.

Immunohistochemical staining directed against *H. pylori* is strongly advised whenever chronic inactive gastritis cases display well-formed lymphoid follicles with

TABLE 2. Utility of Key Findings and Stains in Detecting *H. pylori* in Gastric Biopsies

Study Findings	Hartman and Owens[8] (%)	Smith et al[10] (%)	Wang et al[24] (%)
Sensitivity of moderate to severe gastritis	97	94	93
Specificity of moderate to severe gastritis	98	88	91
Sensitivity of H&E	93	91	95-100
Specificity of H&E	100	100	100
Sensitivity of immunohistochemistry	97-100	100	100
Specificity of immunohistochemistry	100	100	100

germinal centers, as this finding is highly predictive of underlying *H. pylori* infection.[10] Gastric biopsies from patients with coexistent gastric or duodenal ulcers not clearly associated with chemical injury or reactive gastropathy should also be evaluated with additional stains, as failure to detect *H. pylori* may result in substantial patient harm. Finally, biopsies from patients with coexistent gastric lymphoma, particularly MALT-type lymphoma, or adenocarcinoma should be evaluated with immunohistochemistry for *H. pylori*, because eliminating the organism in these situations may modify the disease course or management to some degree. Biopsies of non-neoplastic mucosa are preferred in such cases, as staining of tumor samples is less likely to yield meaningful information. There is no need to perform staining analyses on samples of both neoplastic and non-neoplastic tissues.

Use of immunohistochemistry to identify *H. pylori* organisms in patients with chronic inactive gastritis is also reasonable in other circumstances, although this practice yields a low rate of *H. pylori* detection. Biopsies that show moderate or severe mononuclear cell-rich inflammatory infiltrates in the superficial mucosa are more likely to be associated with *H. pylori* than those that show only minimal, or mild, chronic inflammation, so the use of ancillary stains is recommended when biopsies contain substantial chronic inflammation. Samples obtained from patients at high risk for *H. pylori* infection from an epidemiologic standpoint may be evaluated with immunohistochemistry when they show only mild inflammation, as the likelihood of detection is probably higher in this patient group. We also advocate use of ancillary stains among pediatric patients with chronic inactive gastritis, although there is a paucity of literature supporting this practice.

Is it Appropriate to Perform Ancillary Staining if the Stomach is Histologically Normal?

Although some expert pathologists report the rare occurrence of *H. pylori* in otherwise normal stomach biopsies, the literature suggests that this finding is extremely uncommon.[24] Two large studies evaluating the utility of reflexive immunostaining for *H. pylori* demonstrated the presence of at least mild inflammation in biopsies from all patients who had immunohistochemically detected organisms.[8,10] It is likely that the discrepancies between anecdotal reports and those established in the literature reflect a tendency to consider mild inflammatory changes in the gastric mucosa within the spectrum of normal findings. Indeed, criteria distinguishing between the upper limit of normal findings and the lower threshold of mild chronic gastritis are poorly defined and highly subjective. There are no data regarding the long-term morbidity and mortality related to exceptional cases of *H. pylori* infection unassociated with chronic gastritis. We advise against using ancillary stains on normal gastric biopsies.

Is it Appropriate to Perform Ancillary Staining if Biopsies Display a Classic Chemical (Reactive) Gastropathy?

Chemical (reactive) gastropathy is characterized by the presence of foveolar hyperplasia, reactive epithelial cell changes with mucin depletion and mild nuclear enlargement, and variable fibrosis of the lamina propria with prominent vascular channels. Chronic inflammation is minimal. Neutrophils may be detected, but they are usually identified in the context of an erosion or ulcer. The likelihood of detecting *H. pylori* by any method is essentially nil when a pure chemical gastropathy pattern is present.[24] However, the presence of chemical gastropathy does not preclude coexistent *H. pylori* gastritis. Thus, it is reasonable to perform *H. pylori* immunohistochemical analysis when patterns of chemical gastropathy and chronic active gastritis coexist and organisms are not detected in H&E-stained sections.

For What Types of Unusual Gastritis is it Appropriate to Perform Ancillary Staining if *H. pylori* Organisms Are Not Detected in H&E-stained Sections?

H. pylori has been implicated as a cause, or mimic, of lymphocytic gastritis, granulomatous gastritis, and eosinophilic gastritis, although the role of this organism in the development of these disorders is unclear. At least 50% of the world population is infected with *H. pylori*, and, thus, any reported association between the organism and these uncommon diseases may simply be coincidental. There are essentially no data demonstrating the added value of ancillary stains to enhance *H. pylori* detection in these situations.

Lymphocytic gastritis, as defined by >25 intraepithelial lymphocytes per 100 epithelial cells, may be seen in association with gluten sensitivity or as a cause of either "varioliform" gastritis or a hypertrophic gastropathy resembling Menetrier disease.[35] Although $<5\%$ of *H. pylori*-associated gastritis cases contain adequate numbers of intraepithelial lymphocytes to mimic lymphocytic gastritis, *H. pylori* gastritis is very common and, thus, accounts for 29% of patients who carry a diagnosis of "lymphocytic gastritis."[36] For this reason, the presence of intraepithelial lymphocytosis should be an indication for *H. pylori* immunohistochemistry when organisms are not detected upon examination of H&E-stained sections. Patients with detectable *H. pylori* should be treated for *H. pylori* and undergo repeated endoscopic evaluation

with biopsy in 6 months. Resolution of lymphocytosis would support a diagnosis of *H. pylori* gastritis with increased intraepithelial lymphocytes rather than lymphocytic gastritis.[37]

Granulomatous gastritis can be secondary to systemic conditions (eg, sarcoidosis, Crohn disease, mycobacterial or fungal infections) or localized to the stomach, in which case it is classified as "isolated granulomatous gastritis." Rare cases of granulomatous gastritis have been linked to *H. pylori*, in which case the granulomas are generally accompanied by chronic gastritis.[38] We suggest that granulomatous gastritis is an indication for *H. pylori* immunohistochemistry, but single, or rare, granulomata in otherwise normal gastric mucosae are unlikely to reflect infection.

"Histologic eosinophilic gastritis" is a term suggested to describe the finding of >30 eosinophils per high-power field in at least 5 examined fields (normal range <9/HPF).[39] The link between *H. pylori* and eosinophilic gastritis is tenuous at best. Very rare reports describe a possible association between *H. pylori* and eosinophilic gastritis, and a large US study failed to identify any patients with *H. pylori* infection by immunohistochemistry.[40,41] One Chinese study found that only 15% of patients with eosinophilic gastroenteritis were infected with *H. pylori* compared with 58% of controls.[42] These data do not lend much evidence supporting a relationship between *H. pylori* and eosinophilic gastritis. However, the rare nature of "histologic eosinophilic gastritis" probably permits use of *H. pylori* immunohistochemistry in these cases.

Is it Appropriate to Perform "Up Front" Ancillary Staining Studies for *H. pylori* in Every Gastric Biopsy?

Some pathologists and laboratories perform "up front" special staining on all gastric biopsies for the sake of expediency and/or convenience. However, it is our position that *H. pylori* infection is not an imminently life-threatening condition, and a slightly delayed (ie, <24 h) diagnosis is unlikely to cause patient harm. Thus, routine application of ancillary stains to all gastric biopsies is not justified by a perceived need to expedite the few cases in which staining might be necessary. If a laboratory performs "up front" testing to facilitate workflow, then it should absorb expenses due to technician time, slides, and reagents when staining proves unnecessary, rather than transmit those costs to patients or third-party payors. It is also important to ensure that selected ancillary stains used in this manner adequately detect *H. pylori* when they are needed (ie, organisms are present, but are not readily identified in H&E-stained sections). Histochemical stains are generally less sensitive than immunohistochemical stains, so "up front" histochemical stains do not necessarily prevent a third line of staining (immunohistochemistry).

Is it Appropriate to Perform Ancillary Staining in Biopsies of Gastric Polyps?

Gastric polyps are commonly encountered among patients undergoing upper endoscopic evaluation. Most represent either fundic gland polyps or hyperplastic/regenerative polyps. Fundic gland polyps are incidentally detected in up to 2% of endoscopic procedures, whereas hyperplastic/regenerative polyps frequently occur in association with chemical gastropathy or chronic gastritis, including *H. pylori* infection and autoimmune gastritis. There is a negative association between the presence of fundic gland polyps and *H. pylori* infection, and no relationship exists between these lesions and gastric cancer risk.[43,44] Approximately 25% of hyperplastic/regenerative polyps of the distal stomach occur in association with *H. pylori* infection, whereas those near the gastroesophageal junction are virtually never associated with *H. pylori* infection.[45,46] Importantly, *H. pylori* organisms are not usually present in hyperplastic/regenerative polyps, and, if detected on the polyp surface, they are also present in the background mucosa. We suggest that application of ancillary stains to gastric polyps be limited to certain circumstances. Ancillary staining studies for *H. pylori* may provide useful information when applied to chronically inflamed hyperplastic/regenerative polyps but are, otherwise, unhelpful. There is essentially no literature support for use of *H. pylori* immunohistochemistry in the evaluation of fundic gland polyps. Ancillary staining patterns should be evaluated in biopsies of background mucosa, rather than the polyp, when samples of both are submitted.

Is it Appropriate to Perform Ancillary Staining on an Inflamed Biopsy of the Cardia?

Inflammation of the gastric cardia may reflect *H. pylori* infection, GERD, or a mild degree of acid injury in asymptomatic patients. *H. pylori* gastritis can affect the entire stomach (pangastritis) and extend into the cardia to produce chronic active gastritis in that region, but *H. pylori*-related inflammation localized to the gastric cardia with sparing of the body and antrum has not been described.[47] Inflammation limited to the cardia most commonly reflects GERD, which tends to elicit a less dense chronic inflammatory infiltrate with fewer plasma cells, lymphoid aggregates, and neutrophils than *H. pylori* pangastritis involving the cardia.[48]

It is difficult to justify use of special stains for *H. pylori* on inflamed cardia biopsies when reasonable sampling of the distal stomach shows uninflamed mucosa without *H. pylori* or evidence of prior infection (ie, intestinal metaplasia). In contrast, ancillary staining may be indicated when chronic active inflammation is detected in the cardia but biopsies of the distal stomach have not been obtained. This is particularly true if the patient is at risk for *H. pylori* infection and has either a positive or unknown *H. pylori* serology result. Ancillary stains for *H. pylori* have essentially no utility in cases of mild chronic inactive "carditis."

Is it Appropriate to Perform Ancillary Staining for *H. pylori* in Esophageal Samples?

Some laboratories use ancillary stains for *H. pylori* on every esophageal biopsy that contains columnar mucosa. We can find no sound medical reason or supportive

literature to justify use of special stains to detect *H. pylori* in cases of Barrett esophagus.

Is it Appropriate to Perform Ancillary Staining for *H. pylori* in Duodenal Samples?

H. pylori do not normally inhabit intestinal mucosa, so "up front" ancillary staining of duodenal biopsies are not indicated. However, patients with peptic duodenitis who develop foveolar metaplasia in the duodenum may harbor *H. pylori* organisms in metaplastic epithelium.[49] In this situation, duodenal *H. pylori* infection only occurs in combination with gastric involvement. Thus, ancillary staining of duodenal samples are reasonable only in a very narrow set of circumstances, namely when foveolar metaplasia is present in a patient with no available gastric biopsies and a positive or unknown *H. pylori* serology status.

SUMMARY AND CONCLUSIONS

Approximately 5% to 20% of gastric biopsy cases in North America harbor *H. pylori*. Infection rates show regional variation and are higher in lower socioeconomic areas. Optimal sampling to characterize gastritis and detect *H. pylori* includes biopsies of the antrum (preferably 2 sites), body (preferably 2 sites), and incisura angularis. Most cases of *H. pylori* infection can be diagnosed, or suspected, on the basis of H&E evaluation alone because biopsies from infected patients are essentially never normal; they display some degree of chronic inflammation and frequently show neutrophils. We recommend use of immunohistochemistry as the diagnostic tool of choice when special staining for *H. pylori* is indicated. This methodology shows superior sensitivity compared with histochemical staining in those situations that require ancillary staining (ie, cases with histologic features of *H. pylori* infection that lack detectable organisms in H&E-stained sections). If immunostains are not available or affordable, use of histochemical stains may be considered, but they likely add little value over a well-performed and carefully reviewed H&E-stained slide. Ancillary staining for *H. pylori* is not indicated when organisms are detected in H&E-stained sections of any gastric biopsy. The literature does not show that these stains are useful when applied to normal gastric biopsies or those with chemical (reactive) gastropathy alone, and they are unlikely to reveal organisms in uninflamed gastric polyps. *H. pylori*-negative H&E stains may be supplemented with immunohistochemistry when chronic inflammation is present in gastric biopsies, including the cardia, although the yield of these stains is generally low. Ancillary staining should not be performed simply because pathologists are requested to "rule out" *H. pylori* unless the patient has been previously treated for infection.

Our review emphasizes the critical role of pathologists in providing accurate and efficient information to clinicians. "Up front" staining were initially introduced to reduce turnaround time of diagnoses but are increasingly used for a variety of other reasons. In fact, data from our GIPS member survey indicate that nearly half of respondents reflexively perform *H. pylori* staining on all gastric biopsies. We do not find substantive support in the literature for this practice. In our own experience, pathologists are highly skilled at identifying situations in which ancillary stains are needed, and, thus, laboratories choosing to utilize "up front" staining should report and bill only those stains that are justified on the basis of the H&E findings. We believe that a slight delay in diagnosis of *H. pylori* infection, which is not an immediately life-threatening condition, is an acceptable alternative to practices that do not promote accuracy while unnecessarily contributing to health care costs.

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