Immunoperoxidase in the Interpretation of Discordant Histologic and Urease Findings for *Helicobacter pylori*

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Abstract: Azure A and methylene blue (“Diff-Quik,” DQ) and tissue urease (U) tests are popular methods to diagnose *Helicobacter pylori*. These tests usually correlate well but sometimes produce discordant results. This study evaluates the DQ and U tests by comparing them with the immunoperoxidase reference method to resolve discordant results. DQ and U tests were performed on gastric biopsies. Results were tabulated as DQ(+) /U(+), DQ(+) /U(-), DQ(-)/U(+), and DQ(-)/U(-). Cases that were DQ(+) /U(+) were recorded as positive and not tested with immunoperoxidase. Cases that had discordant DQ/U results were tested by immunoperoxidase to resolve the discordance. Cases which were negative for both DQ/U were evaluated by immunoperoxidase to confirm the validity of DQ(-)/U(-). The groups were compared with concordant results (DQ(-)/U(-) group) and immunoperoxidase versus discordant DQ/U results and immunoperoxidase. There were 56 gastric biopsy specimens. Among all cases, 6 were DQ(+)/U(+). Of the remaining 50 cases, 38 were concordant DQ(-)/U(-), whereas 12 showed discordant DQ/U results. All 38 concordant DQ(-)/U(-) specimens were confirmed negative, 11 discordant DQ/U cases were confirmed negative, and 1 DQ(+)/U(-) specimen was confirmed positive by immunoperoxidase. Comparison of concordant versus discordant results was not statistically significant (P = 0.10). Among all discordant DQ and U, 11/12 (92%) were confirmed negative by immunoperoxidase. Thus, both concordant negative results and discordant results can be considered negative. Such interpretation of discordant results might prevent unnecessary additional procedures or treatment.

Key Words: *Helicobacter pylori*, Diff-Quik, urease, discordant tests, immunoperoxidase


*Helicobacter pylori* has been shown to cause gastritis and duodenal ulcers.1 Research suggests a correlation between *H. pylori* and gastric malignancies, especially lymphoma and adenocarcinoma.2 Infection with *H. pylori* may be asymptomatic or may produce signs and symptoms such as nonspecific abdominal pain, cramping, gastrointestinal bleeding, and ulcers. *H. pylori* does not thrive in the usual acidity of the stomach (pH 7.35 to 7.45). Therefore, the organism metabolizes urea, producing ammonia and raising the pH of the antrum to an alkaline level. The organism then colonizes the gastric mucosa, which can lead to inflammation and symptoms. This clinical development often correlates with an abnormal endoscopic appearance and concurrent histologic reaction in the gastric mucosa. The usual treatment of *H. pylori* is a combination of antibiotics to eliminate the bacteria, and medication to lower the gastric pH.

There are a variety of ways to diagnose *H. pylori*, each with different strengths and weaknesses. Serologic tests look for antibodies to *H. pylori*.3 However, the presence of antibodies to *H. pylori* in the disease process is time sensitive.4 Antibodies may not be present at the beginning of infection and may persist after successful treatment; thus, serologic tests may not detect early or recurrent disease,5 or reflect the effects of therapy.6,7 Serologic tests may be well suited for screening or epidemiologic purposes, however.8 Another antibody-based method relies on finding antibodies to *H. pylori* in stool.9,10 Dyspepsia urea breath tests for *H. pylori* use an assay for the characteristic enzyme of *H. pylori*, urease. The patient ingests a standard dose of radiolabeled urea, and the exhaled breakdown product of carbon dioxide with carbon-13 or carbon-14 is measured. However, this test is expensive and not widely available. In another method, the stomach is visualized by endoscopy and biopsied. This method is popular because it combines a real-time, clinical observation of the stomach with a histologic correlation. The tissue that is obtained is stained with routine hematoxylin and eosin and with a special stain for *H. pylori*. There are many such differential stains, including Genta, Warthin-Starry, modified Steiner, acridine orange, or the one used in our institution, azure A and methylene blue stain (Diff-Quik, DQ).11–13 Another option for diagnosing *H. pylori* infection using tissue is a metabolic test for urease activity (CLOtest). This test is a useful adjunct to routine histology and DQ stain, as it can be performed with fresh tissue obtained at the time of endoscopy.
The latter 2 methods, histochemical stain (in our case, DQ) and urease test on tissue, are practical methods for detecting *H. pylori* because they are performed using specimens from a single endoscopy procedure. In our community, we use them routinely. These methods are rapid, easy to use, and cost effective. These methods are not infallible, however. For example, there is a coccoid, nonhelical variant of *H. pylori*, which seems to develop in response to a therapeutic environment of lowered pH or antibiotic treatment. In this situation, the urease test could produce a false negative result. In addition, the coccoid variant can be difficult to identify morphologically on the DQ stain. Moreover, DQ may not differentiate between different organisms that resemble *H. pylori*, such as other species of *Helicobacter*, *Wolinella*, and *Campylobacter*.12

Given these limitations of the histochemical and urease methods, it is possible to obtain conflicting or discordant results from the 2 tests when run concurrently, in which 1 method generates a positive finding of *H. pylori* although the other method produces a negative result. As yet, there is no guidance regarding how to interpret these discordant results properly.

A possible resolution of discordant DQ and urease tests is to perform an immunoperoxidase stain on tissue recovered from endoscopy. This method may be helpful for detecting *H. pylori* on a wide range of infections with different morphologies and environments. Although it is not 100% sensitive and specific, this method often overcomes the deficiencies of the other methods. The immunoperoxidase method is very specific for the antigens of *H. pylori* regardless of helical versus coccoid morphology, and the method does not rely on the metabolic activity of the bacteria as the urease test does. Unlike a histochemical stain, immunoperoxidase is also reliable at separating the different organisms that resemble *H. pylori*.12 Immunologic methods are also generally more sensitive and/or specific than other methods. For example, a study comparing immunofluorescent studies with urease and with acridine orange found the immunologic method to be positive more often compared with culture (98%) than the histochemical method (61%) or urease method (75%).12 A study comparing a histochemical stain (Genta) and immunoperoxidase found the latter to be 97% sensitive and 98% specific.13 Another study demonstrated improved overall performance of immunoperoxidase relative to 2 histochemical stains.13 Immunoperoxidase-stained biopsies may also be easier to interpret and produce better reproducibility between interpreters than histochemical-stained biopsies.13,14,17,18

It is noteworthy that other methods may in time replace immunoperoxidase for sensitivity and specificity. In situ hybridization for oligonucleotides characteristic of *H. pylori* has shown promise,14,19 as has polymerase chain reaction with Western blotting.12,14 However, these methods are not in wide commercial use as yet, and require dedicated personnel to perform and interpret them. Tissue culture is also an option, but it is not rapid and requires good sampling and a fastidious technique. At present, the immunoperoxidase method can be considered as the practical reference method of *H. pylori* testing.13,14

However, the immunoperoxidase method is still more expensive and more time consuming than the histochemical and enzymatic methods. Therefore, for the purpose of diagnosing *H. pylori* infection relatively inexpensively and rapidly, the DQ stain and urease test remain the most common methods used in our community. But, we do occasionally experience discordant results from the urease and DQ methods. We undertook in the present study to determine how to interpret discordant urease and DQ tests by comparing them with the immunoperoxidase method. We sought to learn what conflicting results should tell us about the diagnosis, and possibly subsequent treatment, of patients whose specimens produced discordant urease and DQ results, and it is hoped to reduce the need for additional tests or repeat studies.

**MATERIALS AND METHODS**

Cases were collected from a review of endoscopic gastric biopsy procedures performed over a 6-month period in 2002 at Mercy Medical Center in Cedar Rapids, IA. Original slides were obtained for review, results of urease tests were retrieved, and tissue blocks were recovered for additional sectioning and performance of immunoperoxidase stains. Cases were evaluated at the time of the procedure by a urease test using fresh tissue (CLOtest, Kimberly-Clark, Draper, UT) and the DQ stain (azure A and methylene blue, Dade-Bering Newark, DE). Hematoxylin and eosin slides that were made for diagnostic purposes at the time of the procedure were reviewed, although they were not used to assess the presence of *H. pylori*. The urease test was interpreted visually by laboratory technologists at the time of the procedure. DQ stains were interpreted by pathologists using light microscopy. *H. pylori* were identified by the presence of characteristic dark-blue staining, curved or helical bacilli which were localized to the apical cells and mucus of the gastric mucosa (Fig. 2). Appropriate histochemical and enzymatic method controls were performed for comparison at the time of the initial interpretations. Results from these studies were tabulated into 4 groups: DQ positive and urease positive (DQ(+)/U(+)); DQ positive and urease negative (DQ(+)/U(-)); DQ negative and urease positive (DQ(-)/U(+)); and DQ negative and urease negative (DQ(-)/U(-)).

Cases that were positive by both the urease and DQ methods (the DQ(+)/U(+) group) were recorded as positive and not further tested with immunoperoxidase, because in clinical practice, cases such as these would be reported as positive with no discordance between methods. Cases that had discordant results between DQ/U were further tested by an immunoperoxidase method to resolve the discordant results. Cases that were negative for both DQ/U were also evaluated by immunoperoxidase to confirm the validity.
of a DQ(−)/U(−) result regarding the presence of *H. pylori*. The immunoperoxidase test used an antibody to *H. pylori* in an avidin-hydrogen peroxidase conjugation reaction with diaminobenzidine as the precipitate (Ventana, Tucson, AZ). The immunoperoxidase slides for these cases were interpreted by the authors using light microscopy. Appropriate positive and negative controls were generated with each run. The 3 groups were then grouped by concordant results (DQ(−)/U(−) group) and immunoperoxidase versus discordant results (DQ(+)/U(−) plus DQ(−)/U(+) groups) and immunoperoxidase. These data were compared using a $\chi^2$ test. The customary $P$ value necessary to reject the null hypothesis, that there is no difference between concordant negative DQ/U results and discordant DQ/U results compared with immunoperoxidase results, was 0.05. Statistica software (StatSoft, Tulsa, OK) was used to perform the calculation.

RESULTS

There were 56 patients who produced gastric biopsy specimens for the evaluation of possible *H. pylori* infection. When present, gastritis was demonstrated by acute inflammatory cells in the mucosa and lamina propria of the gastric biopsy specimen (Fig. 1). Among all cases, 6 showed such histology, and were DQ(+/U(+) for *H. pylori*. DQ stains showed *H. pylori* by the presence of characteristic dark-blue, curved or helical bacilli (Fig. 2). Commercial controls were appropriate on these enzymatic and histochemical tests. These 6 DQ(+)/U(+) cases presented no discordance between the 2 routine methods and posed no diagnostic ambiguity regarding the presence of *H. pylori*, and thus were not further worked up by immunoperoxidase. Of the remaining 50 cases, 38 were concordant DQ(−)/U(−), whereas 12 showed discordant DQ and urease results. These 50 negative and discordant cases were stained with antibody to *H. pylori*. The comparison of these chemical tests with immunoperoxidase is shown in Table 1. Of 50 immunoperoxidase-stained tissue samples, 1 was positive for *H. pylori* (Fig. 3). All 38 concordant DQ(−)/U(−) specimens were confirmed negative, although 1 DQ(+)/U(−) specimen was confirmed positive by immunoperoxidase. The remaining immunoperoxidase-stained tissues were negative. Appropriate positive and negative controls were performed and reported with each run. Comparison of concordant DQ(−)/U(−) and immunoperoxidase results versus discordant DQ(+)/U(−) plus DQ(−)/U(+) results and immunoperoxidase was not statistically significant ($P = 0.10$).

DISCUSSION

The absence of *H. pylori* in cases that were DQ(−)/U(−) was confirmed by immunoperoxidase in 100% (38/38) of biopsies. Among cases with discordant DQ/U results, 1 case was found to be positive for *H. pylori* by immunoperoxidase. This case was DQ(+)/U(−), and thus represented a false negative by urease. All the other cases with discordant DQ(−)/U(+) results were found to be negative for *H. pylori* on immunoperoxidase, and thus represented false positives by urease method. Thus, among all discordant DQ and urease results in which at

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<th>DQ(+/U(+)</th>
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<tr>
<td>Immunoperoxidase (+)</td>
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<td>Immunoperoxidase (−)</td>
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Statistical difference between discordant (DQ(+)/U(−) plus DQ(−)/U(+) and immunoperoxidase staining for *H. pylori* versus concordant (DQ(−)/U(−) and immunoperoxidase staining for *H. pylori* not significant ($P = 0.10$).
least 1 method was positive, 11/12 (92%) were confirmed negative by immunoperoxidase. Because the difference between discordant DQ/U results with immunoperoxidase versus concordant DQ(−)/U(−) results with immunoperoxidase was not statistically significant, it can be proposed that there is no difference between the comparison of concordant and discordant results regarding the results of the reference method, and that both discordant negative results and discordant results can therefore be considered negative.

Another potential source of error in pairing DQ and urease results for this study is that separate biopsies are submitted for the DQ and urease tests. These separate biopsy sites could represent different areas of the stomach, which are not uniformly colonized by H. pylori. Thus, sampling error could be a source for discordant results between the different test methods. However, this issue has not been found to be a significant source of error in previous studies. It is also noteworthy that our study populations were small, and further work might be necessary to evaluate accuracy and reproducibility of this study.

In summary, the routine histochemical and biochemical tests of DQ and urease, respectively, seem to be useful for determining the presence or absence of H. pylori when both methods produce positive results, and when both methods produce negative results. However, occasionally, the 2 methods produce discordant results. In these cases, most are likely to be negative when compared with immunoperoxidase. Therefore, this study suggests that discordant DQ and urease tests could be considered negative for H. pylori.

It has been suggested that serologic tests may be a practical method to confirm negative urease results. However, the present study suggests that additional tests beyond urease and DQ are not necessary. The findings of this study may assist in interpreting discordant results from these common and routinely available tests. Using these findings, interpretations of discordant tests may preclude the need for further testing by more labor-intensive and expensive adjuvant methods. In addition, such interpretation with other clinical evaluation and judgment might prevent unnecessary treatment based on discordant results.

REFERENCES