

oped to detect infection by *H. pylori*. Detection of *H. pylori* in gastric biopsy specimens remains the gold standard for diagnosis of *H. pylori* infection.² *H. pylori* is fastidious and slow growing, so the culture of this organism from gastric mucosal biopsy specimens is both difficult and time consuming, requiring 3 to 7 days of incubation.^{3,4} Therefore, histological detection of *H. pylori* in gastric biopsy specimens is another important diagnostic method.^{2,5,6} Histological evaluation of *H. pylori* status solely with hematoxylin and eosin (H&E)-stained slides has always produced inconsistent results,^{3,4,7} so stains with more sensitivity and more specificity are needed. It is desirable to have a rapid, easy stain to demonstrate the organism. The most widely used special stains for histological diagnosis of *H. pylori* include Giemsa, Warthin-Starry silver, and Genta stains.^{2,5,8} However, most of these special stains are technically difficult, expensive, and time consuming. Diff-Quik stain was reported to be a rapid, easy, and reproducible special staining method for the detection of *H. pylori*.⁹⁻¹²

The aim of this study was to evaluate the usefulness of a combination of stains in the diagnosis of *H. pylori*, using H&E stain for gastric morphologic features, plus Diff-Quik stain for *H. pylori* identification.

PATIENTS AND METHODS

Ninety-five consecutive patients with dyspeptic symptoms referred to our division for endoscopic examination were enrolled in this study. All patients had previously received a ¹³C-Urea Breath Test (¹³C-UBT) before the endoscopic examination. Our series was made up of 58 men and 37 women with a mean age of 46 years (range 20-72 years). Informed consent was obtained from all patients.

Endoscopy was performed with an Olympus GIF-XQ230 gastroduodenoscope. Biopsy forceps were sterilized, and endoscopes were fully disinfected using an automatic washer (Olympus Endoscope Washer EW-30, Tokyo, Japan) before and after each examination. During endoscopy, 4 biopsies were taken from the gastric antrum and corpus and subjected to a rapid urease test (CLO test, Tri-Med Specialties, West-

ern Australia) and histological examination.

Infection by *H. pylori* was defined as both ¹³C-UBT and CLO test being positive or by the organisms being identified histologically. However, a single test being positive and the other 2 tests being negative was considered a false positive result.

Two gastric biopsy specimens for histological examination were preserved in formalin, processed routinely, embedded in paraffin, and stained with H&E to show the bacteria histologically. All gastric specimens were interpreted by the same pathologist, and results of other tests on the same subject were not known by the pathologist. If *H. pylori* was not found in the H&E-stained sections, the pathologist prepared additional slides stained with Diff-Quik to identify *H. pylori*.

The Diff-Quik staining set is composed of 2 solutions. Solution I is a buffered solution of eosin Y. Solution II is a buffered solution of thiazine dyes consisting of methylene blue and azure A. The former is an anionic dye and acidophilic, and the latter is a cationic dye with a basophilic character. We prepared the slides stained with Diff-Quik according to the protocol described by Potvin.¹¹ The Diff-Quik-stained slides were re-examined by the same pathologist.

Samples for rapid urease test were immediately inoculated into the CLO test gel after collection, and readings were taken for up to 24 h.

RESULTS

Among the 95 patients, 56 patients were negative by all demonstrated methods (Table 1). ¹³C-UBT was positive in 37 patients, 27 of whom also had a positive CLO test, and *H. pylori* was identified in H&E-stained slides. These 37 patients were considered to have *H. pylori* infection. There were 2 patients whose gastric biopsy specimens showed *H. pylori* on H&E-stained slides, but these patients had negative results in both the CLO test and ¹³C-UBT. Therefore, these 2 patients were considered to have a false-positive histological examination on H&E-stained slides.

Seven patients had positive results in both ¹³C-UBT and the CLO test, but *H. pylori* was not found