Usefulness of Leifson Staining Method in Diagnosis of *Helicobacter pylori* Infection

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NOTES

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The Leifson staining method was used to diagnose Helicobacter pylori infection and was compared to histology, culture, and the rapid urease test (RUT). Histology gave the best sensitivity (98%), compared to Leifson staining (97%), culture (92%), and RUT (85%) (P < 0.005). Leifson staining is a sensitive, rapid, economical method for diagnosis of H. pylori infection in dyspeptic patients.

Since the initial identification and successful culture of Helicobacter pylori from human gastric biopsy specimens by Marshall and Warren (14, 15), H. pylori has been accepted as having an etiologic role in such gastroduodenal diseases as chronic active gastritis, peptic ulcer, and gastric carcinoma (6, 8, 11, 17, 18). A large number of invasive and noninvasive methods have been used to diagnose H. pylori infection in humans (1, 16, 23).

At present, a universally accepted “gold standard” for the diagnosis of H. pylori infection is not available, and the choice of test depends on the specific clinical situation and the questions that need to be answered. Smear examination of a gastric biopsy specimen can be useful in the rapid diagnosis of H. pylori infection (3–5, 7). H. pylori, like most motile bacteria, has flagella, whose aspect (sheathed), number (three to seven), and arrangement on the cell (polar) are important differentiating characteristics in identification, especially when biochemical reactions are uncertain or weak.

In this study, using the Leifson tannic acid-fuchsin method (13), we performed touch cytology on smears obtained from gastric biopsies to demonstrate H. pylori flagella. The primary goal of this study was to determine the accuracy of Leifson flagellum staining for the diagnosis of H. pylori infection compared to those of histology, the rapid urease test, and culture.

(B)acteriologial recovery. The study population consisted of 240 consecutive patients undergoing endoscopy because of clinical symptoms attributable to the upper gastrointestinal tract. At endoscopy, five antral biopsy specimens were taken from each patient. The first biopsy specimen was used for the rapid urease test (CP test; Yamanouchi Pharma S.p.A., Milan, Italy). The second and third biopsy specimens were placed in brucella broth (Oxoid S.p.A.; Garbagnate Milanese, Milan, Italy) and transferred to the microbiology laboratory within 3 h for culture. The tissue was streaked in parallel onto egg yolk emulsion agar and modified chocolate agar (20) and incubated in a closed jar in a microaerophilic gas mixture composed of 10% CO2–5% O2–85% N2 (Campy-Pak; Oxoid S.p.A.) at 37°C for up to 7 days. Colonies of H. pylori were identified by Gram staining and oxidase, catalase, and urease reactions. The fourth biopsy specimen was processed in formalin and sent for histology (Giemsa). The fifth biopsy specimen was used to perform Leifson staining. The infection was confirmed when (i) culture was positive for H. pylori or, in the case of negative culture, (ii) at least two tests (histology, the rapid urease test, and/or Leifson staining) were positive for H. pylori.

Staining solution and procedures. The staining solution was obtained by mixing equal volumes of 1.5% sodium chloride in distilled water, 3% tannic acid in distilled water, and 1.2% basic fuchsin in 95% ethanol. This mixture remains stable for 1 month at 4°C and for at least 1 year at −20°C. The Leifson technique was accomplished in four steps for a total of 15 min turnaround time: (i) the biopsy specimen was rolled on a clean glass slide to obtain an imprint of all sides of the specimen and was allowed to air dry (the slides were cleaned by soaking them in 3% concentrated hydrochloric acid in 95% ethanol for 4 to 5 days and were flamed in a gas burner just before use); (ii) 1 ml of the staining solution was placed on the slide and left until a fine, metallic red precipitate, as well as a golden film, was formed (about 8 min); (iii) the slide was rinsed gently in tap water and allowed to air dry; (iv) the slide was examined by light microscopy under oil immersion (magnification, ×1,000), and bacterial bodies and flagella appeared dark red or blue-black (2).

The results of each diagnostic assay for the 240 patient specimens are presented in Table 1. H. pylori infection was confirmed in 130 (54%) of them. The overall specificity of all methods was 91%. Figure 1 shows a photomicrograph of flagellated H. pylori to illustrate the quality of the results.

Various techniques have been proposed for the diagnosis of H. pylori infection, suggesting that none of them proves perfect for all situations. Even though not generally required, flagellum staining may be useful for identification of nonfermenting,
motile bacteria, particularly when their biochemical reactions are weak or uncertain. A number of methods for staining bacterial flagella have been described (9, 12, 22, 24), one of the most commonly used being the original Leifson tannic acid–fuchsin method (13).

The accuracy of Leifson staining in the diagnosis of H. pylori infection was evaluated in our study for the first time. Our data showed that Leifson flagellum staining alone proved useful for detection of H. pylori in gastric biopsy specimens. The sensitivity and specificity of the method showed satisfactory levels compared to those of other invasive tests, such as culture, the rapid urease test, and histology, and these results agree with previous data on touch cytology as a useful diagnostic tool for diagnosis of H. pylori infection (3–5, 10).

There are problems associated with histology (interobserver variability and difficulty in differentiating H. pylori coccoidal forms from cocci and spores that may colonize the human stomach), the rapid urease test (false-positive results), and culture (possible uncertain or weak biochemical reactions), while the flagellated phenotype of H. pylori (three to seven sheathed polar flagella) permits differentiation of the bacterium from Proteus spp. (peritrichous flagella), Pseudomonas aeruginosa (polar monotrichous flagella), Streptococcus spp. (no flagella), and Candida spp. (no flagella), well-known contaminants of biopsy specimens (21). Unlike culture and the rapid urease test, Leifson staining allows a retrospective analysis if the slides have been properly stored at room temperature. Debongnie et al. (4) demonstrated that the biopsy sample used for touch cytology is not altered or depleted in bacteria, allowing further histologic examination or culture, eliminating sampling bias, and preventing contamination. Leifson staining can be performed in most laboratories and easily adapted to their workflow. It can be used, in the economy of the laboratory, as a substitute for the rapid urease test because of its speed, sensitivity, inexpensiveness, and availability. In our experience, the critical factors affecting the quality and reproducibility of the Leifson staining results are (i) special cleaning of the slides; (ii) drying of the smear at room temperature before proceeding to staining to prevent the flagella and bacteria from being distorted, dislodged, or both; (iii) thorough washing of both sides of the slide, to prevent the formation of artifacts; (iv) appropriate determination of the best time for application of the stain to the dry slide; and (v) the bacterial load of the biopsy specimen. Leifson staining can be performed in about 15 min, and the results are available before the patient leaves the endoscopy room. This represents a practical advantage for the gastroenterologist, who requires a rapid and specific technique for detection of H. pylori in gastric biopsy specimens from patients with dyspepsia.

In conclusion, we think that visualization of characteristic flagellated H. pylori in cytologic smears adds important information and in some circumstances is crucial for a timely diagnosis of infection. In this sense, Leifson flagellum staining fulfills numerous criteria considered in evaluating a new diagnostic test (e.g., sensitivity and specificity, early availability of results, possible retrospective analysis, and cost-effectiveness). For these reasons, the Leifson tannic acid–fuchsin method can be considered a useful invasive test for the diagnosis of H. pylori infection.

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