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Evaluation of seaFAST, a Rapid Fluorescent In Situ Hybridization Test, for Detection of *Helicobacter pylori* and Resistance to Clarithromycin in Paraffin-Embedded Biopsy Sections

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A commercially available rapid fluorescent in situ hybridization (FISH) test, (seaFAST *H. pylori* Combi-Kit; SeaPro Theranostics International, Lelystad, The Netherlands) was used to simultaneously detect the presence of *Helicobacter pylori* and determine clarithromycin susceptibility in paraffin-embedded biopsy sections. The FISH method was found to be 97% sensitive, 94% specific for the detection of *H. pylori* and comparable to agar dilution for the detection of resistance to clarithromycin.

Helicobacter pylori is a pathogen of the gastric mucosa and a major cause of peptic ulcer disease and chronic gastritis (6). The usual treatment regimen for *H. pylori* infection consists of two antimicrobials in combination with an agent to lower gastric acid with or without bismuth subsalicylate. Clarithromycin used together with either amoxicillin or metronidazole is a common combination. A high eradication rate occurs when the isolate is susceptible to clarithromycin, but infection with clarithromycin-resistant organisms is often predictive of treatment failure (8, 10).

H. pylori resistance to clarithromycin has been increasing worldwide (3, 8, 10). The method approved by the National Committee for Clinical Laboratory Standards (NCCLS) to test for antimicrobial susceptibility of *H. pylori* is agar dilution (9). Due to the complexities of this methodology, it is usually performed only in specialized laboratories, making it generally unavailable for routine clinical use. An alternative method offered by some commercial laboratories is the EpsilonMeter (E test) test method (AB Biodisk, Solna, Sweden); however, this method has not been approved by the NCCLS for susceptibility testing of *H. pylori* (1, 9). Both agar dilution and the E test require culturing of the organism, which is by nature fastidious and slow growing. Therefore, results are often not available for 2 weeks or more. Consequently, empirical therapy for *H. pylori* infection is commonly employed. A more expeditious methodology for susceptibility testing of *H. pylori* could be an effective tool to help guide therapy of *H. pylori* infections. The purpose of this study was to evaluate a rapid fluorescent in situ hybridization (FISH) test that simultaneously detects *H. pylori* and clarithromycin resistance in formalin-fixed, paraffin-embedded biopsy sections.

Gastric biopsy specimens from both the antrum and the fundus were collected on all patients enrolled in an ongoing reinfection study at a tertiary care hospital in Anchorage,

Alaska (8). We randomly selected 35 isolates from 176 available biopsy specimens that tested *H. pylori* culture negative. From 286 specimens that tested *H. pylori* culture positive, we randomly selected 35 clarithromycin-sensitive *H. pylori* strains and 33 clarithromycin-resistant *H. pylori* strains, as determined by agar dilution, and tested them using FISH.

Cultures were performed by placing homogenized biopsy tissue on two types of solid media: blood agar (tryptic soy agar with 5% sheep blood) and brucella agar containing 10% horse blood, trimethoprim, vancomycin, and polymyxin B (Remel, Lenexa, Kans.). The inoculated media were incubated at 37°C, 12% CO₂ and 98% humidity for up to 10 days. Isolates were identified as *H. pylori* on the basis of positive catalase, oxidase, and urease reactions, typical colony morphology, and curved gram-negative bacilli on gram-stained smears.

The clarithromycin MIC was determined by the agar dilution method using the guidelines set by the NCCLS (9). The recommended MIC levels of this method for determining antimicrobial susceptibility of *H. pylori* to clarithromycin are as follows: sensitive, ≤ 0.25 $\mu\text{g/ml}$; intermediate, 0.5 $\mu\text{g/ml}$; resistant, ≥ 1.0 $\mu\text{g/ml}$. We defined a MIC of >0.25 $\mu\text{g/ml}$ as resistance to clarithromycin.

FISH was performed on deparaffinized tissue sections from the randomly selected specimens, using the following fluorescence-labeled oligonucleotide probes: A2143G (ClaR1), A2144G (ClaR2), and A2143C (ClaR3), targeting the most prevalent point mutations for clarithromycin resistance, and Hpy-1 for the specific identification of *H. pylori* (7, 13, 15). The probes for resistance were labeled with the fluorochrome Cy3 (red signal), and the probe for *H. pylori* identification was labeled with fluorescein (green signal). After hybridization was complete, the specimens were analyzed with a fluorescent microscope equipped with filters for green and red fluorescence.

Of the 68 culture-positive specimens, 66 (97%) were positive for *H. pylori* by FISH and 2 were negative. Of the 35 culture-negative biopsies, 33 (94.2%) were negative by FISH and 2 were positive. The sensitivity and specificity of FISH for the detection of *H. pylori* in biopsy specimens was 97% (95%

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TABLE 1. Discrepant results testing for *H. pylori* and resistance to clarithromycin between agar dilution and FISH among 103 specimens tested by both methods

No. of specimens	Culture result			FISH result	
	<i>H. pylori</i> result	MIC	Clarithromycin result	<i>H. pylori</i> result	Clarithromycin result
2 ^a	Positive	0.12	Susceptible	Positive	Resistant
1	Positive	0.25	Susceptible	Positive	Resistant
1	Positive	0.06	Susceptible	Positive	Resistant
1	Positive	8.0	Resistant	Positive	Susceptible
1	Positive	0.06	Susceptible	Negative	NA
1	Positive	≥16.0	Resistant	Negative	NA
2	Negative	NA ^b	NA	Positive	Resistant

^a Both clarithromycin-sensitive and resistant organisms seen with FISH.

^b NA, Not applicable.

confidence interval [CI], 91% to 99%) and 94% (95% CI, 81% to 99%), respectively.

When tested by agar dilution, *H. pylori* clarithromycin MICs were predominately either ≤0.25 µg/ml (35 isolates) or ≥16 µg/ml (31 isolates). Two isolates had a MIC of 8.0 µg/ml, and no isolates had intermediate MICs.

Of 33 specimens resistant to clarithromycin by agar dilution, 32 were *H. pylori* positive by FISH, and 31 of these were also resistant to clarithromycin by FISH. Of 35 specimens sensitive to clarithromycin by agar dilution, 34 were *H. pylori* positive by FISH, and 30 of these were also sensitive to clarithromycin by FISH, resulting in a sensitivity of 97% (95% CI, 84% to 100%) and a specificity of 88% (95% CI, 73% to 96%) for the detection of clarithromycin resistance for FISH compared with agar dilution.

Discrepant results between FISH and agar dilution were found in nine biopsy specimens; four isolates determined to be clarithromycin sensitive by agar dilution were found to be clarithromycin resistant by FISH. Of these, two were found to contain a mixed population of both sensitive and resistant *H. pylori* organisms by the FISH method. One specimen yielded an *H. pylori* isolate resistant by agar dilution (MIC, 8.0 µg/ml) but sensitive by FISH. Two specimens were found to be negative for *H. pylori* by the FISH method but by culture grew *H. pylori* isolates that had MICs of 0.06 µg/ml and >16.0 µg/ml by agar dilution, and two specimens that were *H. pylori* negative by culture were positive by the FISH method (Table 1).

Our study demonstrates that this rapid FISH test is a sensitive and specific method for the detection of *H. pylori* and is comparable to agar dilution for the detection of clarithromycin-resistant organisms. Our results are consistent with those in previous studies by Rüßmann et al., which found the same method to be comparable to E test, disk diffusion, and culture when using shock-frozen biopsies (13, 14), and the more recently published study by Jüttner et al., who found this FISH method to be comparable to culture and E test when using formalin-fixed, paraffin-embedded biopsy specimens (4).

The advantages of the FISH method are the rapid detection of *H. pylori* and its susceptibility to clarithromycin using paraffin-embedded biopsy sections and fluorescence microscopy. For example, once tissue sections are deparaffinized in the laboratory, results from FISH testing can be available in 3 h. In addition, the FISH method is able to detect mixed populations

of clarithromycin-sensitive and resistant organisms and may explain some of the treatment failures that occur in persons infected with clarithromycin-sensitive isolates as determined by culture and agar dilution. For instance, in a separate study (J. M. Morris, D. L. Bruden, and A. J. Parkinson, unpublished data), we identified 11 biopsy specimens containing isolates sensitive to clarithromycin by agar dilution from seven patients who failed clarithromycin therapy in spite of a 100% reported medication compliance; three specimens from two patients demonstrated the presence of a mixed population of clarithromycin-sensitive and resistant *H. pylori* by FISH. These discrepancies and those in our current study are most likely due to inadequate biopsy sampling coupled with the patchy growth distribution of the organism in the stomach (5, 7, 11). It is also possible that low numbers of clarithromycin-resistant organisms present within a large population of clarithromycin-susceptible organisms become suppressed and are not expressed by phenotypic methods, such as culture and agar dilution (3). Since the FISH method is not dependent on the viability of microorganisms, it is likely that it would detect *H. pylori* in biopsies that fail to yield *H. pylori* by culture. Also, FISH may be more successful than histology in detecting low numbers of *H. pylori* in gastric tissue, as may occur after recent antibiotic treatment.

One of the limitations of FISH is its inability to determine the susceptibility of *H. pylori* to antibiotics other than clarithromycin. For example, it has been found that tetracycline resistance is due to specific mutations in both copies of the 16S rRNA of *H. pylori* (2, 12). These changes are analogous to the specific point mutations found at the 23S rRNA when *H. pylori* is resistant to clarithromycin. Therefore, it is likely FISH could be adapted to determine tetracycline susceptibility; however, at present, resistance to tetracycline is rare (2).

In conclusion, we suggest that an additional slide could be prepared for FISH testing from formalin-fixed, paraffin-embedded biopsy sections at the same time a histology slide is prepared, in order to determine antimicrobial susceptibility to clarithromycin in instances where the provider is interested in treatment with a clarithromycin-based regimen. This could potentially result in a more judicious use of clarithromycin and a higher eradication rate than that with empirical therapy.

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