Detection and Clarithromycin Susceptibility Testing of *Helicobacter pylori* in Stool Specimens by Real-Time PCR: How To Get Accurate Test Results

Lottspeich et al. evaluated the novel *Helicobacter pylori* ClariRes assay (Ingenetix, Vienna, Austria) using feces of symptomatic children (2). This test, being a modified version of a bi-probe 23S rRNA gene real-time PCR assay published by Schabereiter-Gurtner et al. (5), allows the detection and clarithromycin susceptibility testing of *Helicobacter pylori* in gastric biopsy and stool specimens. In the study of Lottspeich et al., the sensitivity of the ClariRes assay was found to be only 63%, differing considerably from that shown in the previous publication. The authors suggested that this could be due to the use of frozen instead of fresh samples and also to differences between the gastrointestinal tract of children and that of adults. However, in the study of Schabereiter-Gurtner et al. as well as in another recent study (4), stool specimens were also frozen until they were used for DNA extraction. This latter study, also performed using stool specimens from pediatric patients, showed a high degree of concordance between the ClariRes assay and the monoclonal stool antigen test; using the urea breath test (UBT) and serology as the reference methods, real-time PCR was shown to be at least as sensitive as the antigen test but more specific by far.

It is well known that preanalytic handling, and thus the quality of the sample, is of crucial importance with regard to sensitivity for any test aimed at the detection of microbial DNA in clinical samples. Thus, prolonged storage or transportation of feces at room temperature and repeated thawing of the sample or the DNA extract may cause a loss of sensitivity of the ClariRes assay. Unfortunately, this kind of information is not provided at all in the manuscript of Lottspeich et al. For example, it would be of interest to know how many times these stool specimens had been unfrozen in the past prior to examination by real-time PCR. Was the DNA used for PCR directly after extraction or had it been stored prior to the reaction? Was the stool antigen test performed at the same time as the real-time PCR or some time before? Were the samples used for each of the tests equivalent in quality?

Throughout the manuscript, Lottspeich et al. repeatedly refer to the study of Schabereiter-Gurtner et al., where samples were run in duplicate in the PCR. In 7% of the cases with positive *H. pylori* status, only one of the two reactions was positive when stool samples were analyzed. For pediatric patients, however, this percentage was shown to be considerably higher (22.5%). If the authors did not run samples in duplicate (this information is not provided in the manuscript), this would definitely be one of the main reasons for the poor sensitivity of the ClariRes assay.

Thus, as for many other tests, including nonmolecular ones (e.g., *H. pylori* culture tests, the sensitivity of which has been shown to range between 55.9% and 100% in recent literature) (1, 3), appropriate preanalytic and laboratory practice is of crucial importance for the accurate performance of the *H. pylori* ClariRes assay with regard to sensitivity.

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Authors' Reply

We thank Dr. Makristathis and Dr. Hirschl for their critical comments with respect to our publication.

When we first read the paper by Schabereiter-Gurtner et al. (2) in 2004, we were delighted about the new method for the detection of *H. pylori* and clarithromycin susceptibility testing in stool specimens. Because we are especially interested in *Helicobacter* infections of children, we planned to evaluate this new real-time PCR protocol with stool samples from pediatric patients. We took advantage of the commercially available *H. pylori* ClariRes assay for our study. The Max von Pettenkofer-Institute for Hygiene and Medical Microbiology was interested in implementing the *H. pylori* ClariRes assay as a routine diagnostic method. Therefore, this test was bought directly from Ingenetix, Vienna (~\$650 U.S. for 50 reactions). The assay was performed according to the manufacturer's recommendations (unfortunately, these do not point out that samples have to be run in duplicate to increase sensitivity). We emphasize that running duplicates is an expensive procedure and that if a second stool specimen preparation is recommended, there is need for an additional QIAamp DNA stool minikit column. Altogether, a single run (without positive and negative controls) comes to approximately \$25 U.S. One should also keep in mind that the preparation of the stool is a relatively time-consuming multistep procedure and that a LightCycler per se is a pricey high-tech machine.

In contrast to the study published as a conference abstract by Puz et al. (1), our peer-reviewed paper used all gold standard methods (histology, culture, the UBT, and stool enzyme immunoassay) to define a positive or negative *H. pylori* status. Puz et al. used only the UBT and serology as reference methods. The latter method, especially used for samples from young children, does not help to accurately detect *H. pylori* colonization/infection.

We agree with Dr. Makristathis and Dr. Hirschl that pre-analytic handling of the stool sample is of crucial importance. In our study, we could not exclude prolonged storage or transportation at room temperature, because in many cases the samples were sent in by surface mail. Repeated thawing was applied to about one-third of the stool samples. However, the accuracy of the *H. pylori* ClariRes assay for repeatedly thawed samples was not worse than that for the remaining samples. Most importantly, the stool antigen test was performed at the same time as the *H. pylori* ClariRes assay. Thus, a direct and fair comparison of both methods was conducted. The facts that the cost for a single run of the stool antigen test is approximately \$8 U.S. and that this test is faster and less labor intensive and does not need expensive diagnostic machines represent clear advantages. However, the advantage of the *H. pylori* ClariRes assay is the simultaneous clarithromycin susceptibility testing that can be performed. As mentioned by Dr. Makristathis and Dr. Hirschl, running duplicate samples seems to be important to obtain a high sensitivity with the *H. pylori* ClariRes assay. It is desirable that protocols for isolation of DNA from stool samples and/or the assay itself be improved to obtain accurate results after single testing.

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