Evaluation of a Monoclonal Antibody-Based Test for Detection of Helicobacter pylori-Specific Antigen in Stool Samples from Mice

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A test using monoclonal antibodies for detection of antigen in stool samples was compared with culture and histology for noninfected (n = 25), Helicobacter pylori-infected (n = 25), and Helicobacter felis-infected (n = 6) mice. Sensitivity and specificity were 96%. The monoclonal antibody-based test is therefore a noninvasive technique that is able to diagnose H. pylori infection in mice.

Mice infected with Helicobacter pylori are a convenient animal model for investigations (5, 6); however, noninvasive techniques to assess infection status are limited. The “gold standard” technique for indicating gastric colonization by H. pylori is considered to be a culture of gastric tissue. This requires the animal to be sacrificed, as does any histological analysis, therefore preventing the same animal to be monitored before, during, and after treatment. A noninvasive technique for detection of H. pylori is a test using monoclonal antibodies for detection of antigen in stool samples from mice. The aim of the present study, therefore, was to assess the sensitivity and specificity of the H. pylori-specific antigen test. All experiments were approved by the animal ethics committee of the Women’s and Children’s Hospital, North Adelaide, South Australia.

Female C57BL/6 mice (age, >7 weeks) were divided into three groups, noninfected (n = 25), Helicobacter pylori-infected (n = 25), and Helicobacter felis-infected (n = 6), and housed separately. Inoculations with H. pylori (Sydney strain 1) (6) and H. felis (5, 6) were performed via gavages of 100-μl suspensions (10^9 CFU/ml). Following an infection time of 4 weeks and by using age-matched noninfected controls, mouse fecal pellets were collected from each group. Mice were then sacrificed by CO2 asphyxiation and cervical dislocation, after which the stomachs were excised for histological examination and bacterial culture. Paraffin-embedded sections were stained with hematoxylin and cosin for histology and with a modified May-Grünwald-Giemsa stain to assess bacterial colonization (4). Gastritis was assessed in the body and the antrum by using a modified Sydney grading system for gastritis (6). The severity of gastritis and bacterial colonization density were assessed blindly by an impartial observer.

The remaining tissue was homogenized, and serial 10-fold dilutions were performed, with 200 μl of each dilution for all mice plated out in duplicate on Helicobacter-selective agar. In addition, 200 μl of each dilution of the homogenized stomachs of the H. felis-infected group were plated out in duplicate on Campylobacter-selective agar. Plates were incubated in a 10% CO2 incubator set at 95% humidity at 37°C for 5 to 7 days.

Fecal samples were stored at −20°C until analyzed. A monoclonal antibody-based enzyme-linked immunosorbent assay (FemtoLab H. pylori Cax; Connex, Martinsried, Germany) was used to detect H. pylori-specific antigen in stool samples by a modified method. Briefly, the size of the stool sample was reduced (mean weight, 0.047 g; range, 0.015 to 0.200 g), and the stop solution for the reaction was added at 30 min. The results were read by spectrophotometry (450 and 630 nm, double wavelength). According to manufacturer’s guidelines, an optical density (OD) of <0.150 was considered a positive test result.

No viable bacteria were recovered when the stomach homogenate was cultured from the noninfected control mice, nor was there any gastric inflammation as determined by histology.

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but one stool sample gave a false-positive result (Fig. 1). \textit{H. pylori} was present in the stomach homogenate of all \textit{H. pylori}-infected mice, with both culture and histology, and no \textit{H. pylori} was present in the gastric tissue of the \textit{H. felis}-infected mice. A mild severity of gastritis was detected in \textit{H. pylori}-infected mice, and moderate gastritis was present in the \textit{H. felis}-infected mice. The stool test was positive in 24 out of 25 \textit{H. pylori}-infected mice, with one stool sample giving a false-negative result (Fig. 1). The stool test was negative in all of the \textit{H. felis}-infected mice (Fig. 1).

No significant correlations existed between the OD value of the \textit{H. pylori}-specific antigen test and the bacterial colonization density as measured by culture and histology (Fig. 2). There was also no significant correlation between gastritis severity and the stool test results \((r = -0.096, P > 0.05)\). The overall sensitivity and specificity were high (both 96%), as were the positive predictive and negative predictive values (both 96%).

The present study was designed to evaluate for the first time the novel monoclonal antibody-based test for detection of \textit{H. pylori}-specific antigen in stool samples from mice and compare it to commonly used invasive methods. Monoclonal antibody-based tests are widely accepted for clinical practice with humans since they are sensitive, specific, noninvasive, and easy to perform (1, 7, 8). Our results show a high sensitivity and specificity of the test for stool samples from mice, which is in agreement with the values given for humans by the manufacturer. The \textit{H. pylori}-specific antigen test discriminates well between \textit{H. pylori}-infected and uninfected mice, is easy to perform, and does not have limitations like the \textsuperscript{13}C urea breath test, where dietary uptake and coprophagy adversely affect the validity of the test (2). Cross-reactivity of the \textit{H. pylori}-specific antigen test with other types of \textit{Helicobacter} infection was also not a concern, as all mice infected with \textit{H. felis} had negative test results, showing that the monoclonal antibodies utilized by this test are specific for \textit{H. pylori} antigen.

The \textit{H. pylori}-specific antigen test for stool samples from mice resulted in one false-negative and one false-positive result for \textit{H. pylori} infection. The false-negative result may have been due to the level of antigen in the assay being below the limit of quantitation for the test. This could have been due to the freezing and thawing of the fecal sample, as this has recently been shown to decrease the sensitivity of the test (3). The false-positive result could have been due to cross-reaction with another bacterial species or, more likely, from cross-contamination from a positive sample in a nearby well. To eliminate false-positive results due to operator error, the samples should be tested in duplicate, but this greatly increases the cost of the test.

In conclusion, this study shows that the monoclonal antibody-based test for detection of antigen in stool samples is a reliable and rapid diagnostic tool for assessment of \textit{H. pylori} infection in mice. It has the potential to be utilized in mouse studies that evaluate novel treatments over a period of time. Future investigations should determine the usefulness of this test in \textit{H. pylori}-infected mice over a longer period of time and before, during, and after eradication therapy.

**REFERENCES**


