Evaluation of 3 different tests for the detection of stool antigens to confirm *Helicobacter pylori* eradication after treatment. A pilot study


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**ABSTRACT**

Recently, several new diagnostic methods aimed to detect *Helicobacter pylori* stool antigens have been developed. Our aim was to evaluate the accuracy of 3 different stool tests to confirm *H. pylori* eradication.

**Patients and Methods**: Twenty-six patients received *H. pylori* eradication treatment. Eradication was confirmed with 13C-Urea breath test 6-8 weeks later, when stool samples were analyzed by polyclonal (Premier-Platinum-HpSA™), monoclonal (Amplified-IDEIA™-HpS®), and rapid test (ImmunoCard-STAT-HpSA™).

**Results**: *H. pylori* was eradicated in 85% of the cases. Sensitivity, specificity, positive predictive value and negative predictive value with the polyclonal test were: 25%, 91%, 33% and 87%. Corresponding results with the monoclonal test, using the cut-off point recommended by the manufacturer, were 100%, 46%, 25% and 100%. However, the best cut-off point in our study had 100% sensitivity and 91% specificity. The area under ROC curve for the polyclonal and the monoclonal tests was 0.65 and 0.95. Diagnostic accuracy with the rapid test was 75%, 90%, 60% and 95%.

**Conclusion**: Neither the polyclonal stool antigen test nor the rapid stool antigen test can be recommended to confirm *H. pylori* eradication after treatment. The monoclonal test has better diagnostic accuracy, although more studies are necessary to definitively recommend its use for the confirmation of *H. pylori* eradication success.
INTRODUCTION
Helicobacter pylori infection plays a fundamental role in the development of several gastroenterological diseases and, therefore, the diagnosis of the infection represents a clinically relevant chapter. The methods for the diagnosis of H. pylori infection are classically divided into invasive and non-invasive. The former are based on the demonstration of the organism from gastric biopsy samples, therefore an endoscopy is needed. On the other hand, non-invasive methods, which require no endoscopic examination, are also available. Among non-invasive techniques, serology and urea breath test are the classically considered and the most widely used.

Most recently, a new non-invasive diagnostic test based on the detection of H. pylori stool antigens has been developed, and it has shown to be an accurate method for the detection of infection in non-treated patients. However, the experience in the post-treatment setting, to confirm H. pylori eradication, is much more limited, and discouraging results have been reported in some studies. After the first commercially available H. pylori stool antigen test, which used polyclonal antibodies to H. pylori, newer methods based on monoclonal antibodies have been developed, including an ELISA test and, even more recently, a rapid in-office test whose result is read after only 5 min. In summary, very limited experience exists with both the polyclonal ELISA and the monoclonal ELISA stool test in the confirmation of H. pylori eradication after treatment, and the rapid monoclonal immunochromatographic test has never been evaluated before in this setting.

Therefore, our aim was to assess the accuracy of these 3 tests for the detection of stool antigens to evaluate H. pylori eradication success or failure after treatment.

PATIENTS AND METHODS

Patients
Twenty-six H. pylori-positive patients (62% with peptic ulcer disease, and 38% with functional dyspepsia) were included in this pilot study. Exclusion criteria were: previous H. pylori eradication therapy administration, previous gastric surgery, and presence of associated conditions (hepatitis, cardiovascular or renal diseases, diabetes treated with insulin, malignancies or cigalopathy). Informed consent was obtained from all the patients. All patients received H. pylori eradication treatment for 7 days with proton pump inhibitors, metronidazole, tetracycline, and clarithromycin.

Reference methods for the diagnosis of H. pylori infection
H. pylori eradication was defined as a negative 13C-urea breath test (TAT-KIT®, Isommed S.L., Madrid, Spain) 8 weeks after completing treatment. The standard protocol was performed with 4.2 μg of citric acid (Citrat pylori®) and 100 mg of 13C-urea, as detailed in previous publications. Breath samples were analyzed by means of isotope ratio mass spectrometer (AIRC, PDG, Crew, Manchester, England). The result of 13C-UBT was expressed as delta over baseline, values higher than 5‰ being considered positive.

Stool tests
At the time of breath test performance, the patient provided a stool sample. The samples were stored at −20 °C until analysed by the 3 stool tests: a) polyclonal ELISA test (Premier Platinum HpSA™, Meridian, Cincinnati, OH, USA); b) monoclonal ELISA test (Amplified IDEIA™ HpSA® DAKO AS, Denmark); c) rapid monoclonal immunochromatographic test (ImmunoCard STAT® HpSA™, Meridian Bioscience, Santa, Italy). All tests were performed in accordance with the manufacturer’s protocol. The protocol for the first 2 tests has been detailed in previous publications. For the novel rapid test, detailed protocol information is provided in the next section. All stool tests were performed without knowledge of the other stool tests results or the urea breath test result.

Rapid monoclonal immunochromatographic test
The ImmunoCard STAT® HpSA immunochromatographic test was a rapid in vitro qualitative procedure based on a lateral flow chromatography technique, for the detection of H. pylori antigens in human stool. The test utilizes a monoclonal anti-H. pylori antibody. Diluted patient samples are dispensed to the sample port of the test cassette and the appearance of a pink-red line in the reading window indicates a positive result after 5 min of incubation at room temperature. The stool specimen should be stored at 2–4 °C until tested. The specimen should be tested as soon as possible, but may be held up to 24 h at 2–8 °C prior to testing. Using the applicator stick of the diluent vial, a small portion (5-6 mm diameter) is transferred to thoroughly mixed stool into the sample dilution. The specimen is then emulsified using the applicator stick. The sample diluted vial containing the specimen should be held and the tip broken off. Four drops should be dispensed into the round window indicated by an arrow. Finally, the result should be read after exactly 5 min. The interpretation of results is as follows:

- Negative test result. Only one blue colored band (control line) appears across the central window of the device close to the letter T.
- Positive test result. In addition to the blue band (control line), a distinguishable pink-red band (test line) also appears across the central window of the device close to the letter T. Any pink-red line, even very weak, must be considered as a positive result.
- Any line or colour appearing after 5 min has no diagnostic value (reagents, not re-tested the result after 30 min, with the aim to evaluate concordance between the 5 and 30 min lecture).
- Invalid test result. The blue band (control line) is absent, with or without a visually detectable pink-red band (test line).

Results of the rapid stool test were independently evaluated by 3 observers, who classified the results as positive, negative or indeterminate.

Statistical analysis
Sensitivity, specificity, positive and negative predictive values, and positive and negative likelihood ratios of the stool antigen tests were calculated, and the 95% confidence interval was provided. Comparisons between independent proportions were carried out by chi-square test (χ2 test). For homogeneity test regarding stool antigen methods in the same patients, McNemar statistic was used. Concordance for the rapid stool test interpretation between the 2 observers was evaluated by the kappa statistic. Concordance for the polyclonal ELISA test interpretation of the 2 observers was calculated using the area under the ROC (receiver operating characteristic) curve. The operating characteristics were determined from the means of the sensitivity analysis (using contingency tables) and likelihood ratio.

RESULTS
Twenty-six infected patients received H. pylori eradication therapy. Mean age (± standard deviation) was 55 ± 14 years. 42% were males, and 23% were smokers. H. pylori eradication was achieved in 85% of the cases and, therefore, prevalence of the infection post-treatment was of 15% (4 patients). Delta over baseline values of the 13C-urea breath test in H. pylori-positive patients were: 93, 26, 22, and 57‰.

Sensitivity, specificity, positive predictive value and negative predictive value with the polyclonal ELISA test was: 1/4 (25%); 95% confidence interval [85% CI, 0.62–0.88], 2/0 (25%); 95% CI, 72–97%); 1/3 (33%); 95% CI,
The first commercially available monoclonal ELISA stool test has better diagnostic accuracy of antigens than the polyclonal ELISA test. The results of the present study show that the polyclonal ELISA test, using the cut-off point recommended by the manufacturer (optical density of 0.15), were: 4/4 (100%; 95% CI, 66-100%), 10/22 (46%; 95% CI, 24-68%), 4/16 (25%; 95% CI, 7-52%) and 10/10 (100%; 95% CI, 69-100%). Positive and negative likelihood ratios were 11.1 and 0, respectively. Corresponding results of sensitivity, specificity, positive predictive value and negative predictive value with the monoclonal ELISA test, using the cut-off point recommended by the manufacturer were: sensitivity 3/4 (75%; 95% CI, 40-100%), 20/22 (91%; 95% CI, 72-97%), positive predictive value 2/4 (50%; 95% CI, 7-93%), and negative predictive value 20/22 (91%; 95% CI, 72-97%). Positive and negative likelihood ratios were 5.5 and 0.55, respectively.

DISCUSSION

The results of the present study show that the polyclonal ELISA stool test and the rapid monoclonal immunochromatographic test are insufficiently accurate for the confirmation of H. pylori eradication after therapy. However, monoclonal ELISA stool test has better diagnostic accuracy. The first commercially available H. pylori stool antigen test, Premier Platinum HpSA™ (Meridian Diagnostics), used polyclonal antibodies to H. pylori as capture antibodies, absorbed to microwells. More recently, a new stool antigen test, a quantitative enzyme immunoassay based on monoclonal – instead of polyclonal – antibodies, has been commercialized. The «old» Premier Platinum HpSA (Meridian) uses polyclonal antibodies obtained from intraperitoneal injection of H. pylori antigens to rabbit. This method obtains a profile of antibodies which is different in each animal, and this could generate, in theory, differences among diagnostic kits. In fact, considerable variability has been reported when several stool determinations with the polyclonal method have been performed in the same patients; this, in turn, could explain remarkably differences among the different studies from the literature.

On the other hand, the test based on monoclonal antibodies may have greater reproducibility of test results. In this respect, some studies have reported excellent results despite the fact that the test was performed in 3 different laboratories using 2 different production lots. In a recent systematic review of the stool antigen test, overall results of the studies evaluating (pre-treatment) the monoclonal technique were better than those obtained with the polyclonal method. Of special interest are those studies that compare the monoclonal and polyclonal method in the same protocol. Thus, although some of these studies have obtained similar results with both techniques, others have reported a tendency for better results or a clear advantage with the monoclonal method. In the post-treatment setting, relatively low accuracy was reported in some studies with the polyclonal ELISA stool antigen test; thus, mean sensitivity calculated from 33 studies evaluating polyclonal stool antigen test 4-8 weeks after finishing therapy was of only 84%, and even worse results were achieved in our study. When the results of the polyclonal ELISA test were interpreted by visual reading instead of by spectrophotometry, results were relatively similar, which coincides with other studies where the concordance of H. pylori stool antigen test interpreted by these 2 methods has been very high. However, better results were achieved with the monoclonal ELISA test in our experience, specially when the best cut-off point calculated by statistical methods, and it needs to be clarified which factors influence it (for example, pre-treatment setting). It should be stressed, however, that the use of the cut-off point arising from a study as a reflection of the performance accuracy of the test in the general population may flaw the results, and therefore it will be necessary to confirm the encouraging results of this cut-off point in a new sample of patients. Nevertheless, the area under the ROC curve, which globally evaluates the yield of all the cut-off points of the monoclonal ELISA test, was 0.95, which indicates an excellent diagnostic performance (better than the 0.65 obtained for the polyclonal test). These excellent results are in agreement with those reported in the few studies from the literature evaluating the monoclonal ELISA technique 4-8 weeks post-treatment, which achieved mean 95% sensitivity and mean 97% specificity. Furthermore, the differences between positive and negative results obtained with the monoclonal ELISA test are generally greater in comparison to the polyclonal ELISA test and therefore the monoclonal test allows a better distinction between infected and non-infected patients. Thus, in contrast with the polyclonal test, no grey zone seems to be necessary when the monoclonal test is used.
The rapid monoclonal immunochromatographic stool test is currently incorporated in a device which integrates sampling, processing and analysis in one test unit allowing for simple and hygienic handling. Although limited experience exists on this new method, encouraging results have been reported in patients without prior treatment of H. pylori16,17. As the results with this novel office-test are available in approximately 5 min, it has the advantage of being suitable for the use at the doctor’s office (making possible the prescription of H. pylori eradication therapy, if necessary, during the same visit). Although it has been suggested that a methodological limitation is the sometimes very low intensity of the bands on the test device, resulting in difficulties to interpret the results, concordance between the 2 observers in our study was perfect (kappa = 1). However, the disappointing results obtained in our study (with a sensitivity of only 75%, and a positive predictive value of only 60%), which represents the first experience evaluating this rapid monoclonal immunochromatographic stool test in the post-treatment setting, suggests that this test may not be accurate to confirm H. pylori eradication. Finally, several advantages of the stool antigen determination should be underlined: The H. pylori stool antigen test does not need an endoscopy, is easy and simple to perform, rapid (the test provides results in approximately 2 h with the ELISA stool test, and in only 5 min with the immunochromatographic test), only one stool specimen is required (instead of 2 breath samples with urea breath test), and it does not require a technician or nurse. The stool sample can be stored at 2 °C -8 °C for up to 3 days, or indefinitely at –20 °C before the test; this makes it possible to collect multiple samples over several days or weeks, which is valuable in a small hospital with a low number of patients to be tested in one session, thus reducing the cost. Lastly, preliminary studies using decision analysis suggest that stool antigen test is associated with a high cost-effectiveness ratio for the diagnosis of H. pylori infection18. On the other hand, as any diagnostic method, stool antigen test has also some disadvantages: In particular, collection of stools may be a disagreeable task for some patients, and it remains to be seen whether patients will be more or less willing to provide a stool or breath sample for H. pylori testing; we must not forget that ease of specimen acquisition is likely to affect the patient’s compliance.

In summary, the results of the present pilot study, although including a low number of patients, suggest that neither the polyclonal ELISA stool antigen test nor the rapid monoclonal immunochromatographic stool antigen test can be recommended to confirm H. pylori eradication. The monoclonal ELISA test has better diagnostic accuracy, although more studies are necessary to definitively recommend its use for the confirmation of H. pylori eradication. Therefore, future studies will need to demonstrate whether stool antigen tests are equally reliable than 13C-urea breath test before the generalized use of these tests are recommended in the post-treatment setting.

ACKNOWLEDGEMENTS

We are indebted to Brenda Ashley for her English technical assistance.

REFERENCES