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Diagnostic accuracy of three monoclonal stool tests in a large series of untreated *Helicobacter pylori* infected patients

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ABSTRACT

Objectives: Immunochromatographic tests need to be improved in order to enhance their reliability. Recently, several new kits have appeared on the market. The objective was to evaluate the diagnostic accuracy of three monoclonal rapid stool tests – the new Uni-Gold™ *H. pylori* Antigen (Trinity Biotech, Ireland), the *RAPID* Hp StAR (Oxoid Ltd., UK) and the ImmunoCard STAT! HpSA (Meridian Diagnostics, USA) – for detecting *H. pylori* infection prior to eradication treatment.

Design and methods: Diagnostic accuracy (sensitivity and specificity) and reliability (concordance between observers) were evaluated in 250 untreated consecutive dyspeptic patients. The gold standard for diagnosing *H. pylori* infection was defined as the concordance of two or more of rapid urease test (RUT), histopathology and urease breath test (UBT) or positive culture in isolation. Readings of immunochromatographic tests were performed by two different observers. Sensitivity, specificity, positive and negative predictive values and 95% confidence intervals were calculated. Sensitivity and specificity were compared using the McNemar test.

Results: The three tests showed a good correlation, with Kappa values > 0.9. *RAPID* Hp StAR had a sensitivity of 91%–92% and a specificity ranging from 77% to 85%. Its sensitivity was higher than that of Uni-Gold™ *H. pylori* Antigen and ImmunoCard STAT! HpSA ($p < 0.01$). Uni-Gold™ *H. pylori* Antigen kit showed a sensitivity of 83%, similar to ImmunoCard STAT! HpSA. Specificity of Uni-Gold™ *H. pylori* Antigen approached 90% (87–89%) and was superior to that of *RAPID* Hp StAR ($p < 0.01$).

Conclusions: Uni-Gold™ *H. pylori* Antigen and ImmunoCard STAT! HpSA present similar levels of diagnostic accuracy. *RAPID* Hp StAR was the most sensitive but less reliable of the three immunochromatographic stool tests. None are as accurate and reliable as UBT, RUT and histology.

1. Introduction

Various detection methods are used to diagnose *Helicobacter pylori*. Invasive methods requiring gastric endoscopy include rapid urease test (RUT), culture, histology, and molecular diagnostics. Noninvasive tests include urea breath test (UBT) and stool antigen tests (SAT). During recent years, noninvasive methods, like the stool antigen tests, have gained in importance and recognition [1]. Due to the ready availability and non-invasive nature of fecal samples, they are a convenient sample especially in the case of pediatric diagnosis, since children can be tested without needing their active collaboration, unlike tests such as UBT [2]. However, stool samples are challenging to analyze because they are heterogeneous and contain high quantities of microorganisms (up to 60% of fecal solids) [3], bile salts, polysaccharides, fiber, mucus, and other products of the gastro intestinal tract [4].

Several monoclonal SATs for *H. pylori* are available for diagnosing *H. pylori* infection. Fecal immunoassays for *H. pylori* may perform differently to each other and to other methods for two main reasons: Firstly, SAT assays may detect different antigens. In different geographical areas there may exist variations in the antigenic composition of the strains which may affect the tests' accuracy. Secondly, due to the technical

Abbreviations: RUT, rapid urease test; UBT, urea breath test; SAT, stool antigen test; LFIA, lateral flow immunochromatographic assay.

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and procedural characteristics of the tests, enzyme-linked immunosorbent assays (ELISA) have demonstrated greater accuracy than lateral flow immunochromatographic assays (LFIA) [5,6].

As the various kits may show marked differences in performance, new assays must undergo adequate evaluation before they can be extensively used in clinical practice. The Uni-Gold™ *H. pylori* Antigen kit (Trinity Biotech, Ireland) is a recently developed monoclonal LFIA. The current study aimed to evaluate the test's diagnostic accuracy (sensitivity and specificity) and reliability (concordance between observers) and to compare it with two other LFIA assays — *RAPID* Hp StAR (Oxoid Ltd., UK) and ImmunoCard STAT! HpSA (Meridian Diagnostics, USA), in testing a large series of consecutive dyspeptic patients.

2. Patients and methods

2.1. Patients

All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. Informed consent was obtained from all individual participants included in the study. Outpatients referred to the Endoscopy Unit of the Hospital de Sabadell for evaluation of dyspeptic symptoms from January 2009 to July 2014 were recruited for the study. Patients were contacted prior to the endoscopy and were asked to participate. Those who agreed were instructed to avoid antisecretory drugs in the two weeks before the test. Patients unable to stop antisecretory drugs, those who had received antibiotics in the four weeks before the endoscopy and those with previous *H. pylori* treatment were excluded. Patients were asked to bring a fecal sample on the day the endoscopy was to be performed. Before the endoscopy, the patients signed informed consent and a ^{13}[C]-urea breath test (UBT) (UBiTest 100 mg, Otsuka Pharmaceutical Europe Ltd., UK) was administered. During endoscopy, two antral biopsies for histology and one for rapid urease test (RUT, JATROX HP test CHR Heim Arzneimittel GmbH, Germany) were obtained. Isolation, culture and identification of *H. pylori* were performed after a positive RUT test. The RUT biopsy was plated on Pylori Agar (Biomerieux, Spain) in microaerophilic conditions in microaerophilic jars (Jar Gassing System, Don Whitley Scientific Limited, UK). After a maximum of a week, *H. pylori* isolates were subcultured on Columbia plates (Biomerieux) and identified by colony morphology, Gram-negative staining and a positive result for urease, catalase, and oxidase tests. Aliquots of the feces were frozen and stored at −80 °C until analysis.

Two hundred and ninety consecutive patients were included in the study. Forty of them were excluded because of the unavailability of UBT, RUT, histology, for a variety of technical reasons, or because the fecal sample was insufficient to perform the three tests. The remaining 250 patients were available for analysis. Patients' clinical and demographic data are shown in Table 1.

The gold standard for diagnosing *H. pylori* infection was defined by the concordance of RUT, UBT and histopathology (Giemsa staining), in accordance with the recommendations of the European *Hp* Study Group [7]. Patients who were positive for two or more of these tests or patients positive for *H. pylori* culture, with or without a positive test with RUT, UBT or histopathology, were considered infected; the remaining patients were considered uninfected.

2.2. Lateral flow immunochromatographic assays

Stool samples were thawed at room temperature for 30 min, and processed in batches of 24 (12 per observer). After the stool samples were thawed at room temperature, all the tests were performed simultaneously. Positive and negative controls were included in each batch of samples. All three commercial tests were performed according to the specifications of their respective manufacturers. The three kits use a similar quantity of solid sample (~5 mm) that is dissolved in the buffers provided. Dilution buffers for the three kits analyzed were equivalent in volume. Liquid samples are processed using disposable Pasteur pipettes. For solid samples, ImmunoCard STAT! HpSA and Uni-Gold™ *H. pylori* Antigen provide a sample preparation vial with an integrated sample applicator stick in the cap, whereas for RAPID *Hp* StAR the sample is collected with a separated stick and dissolved in a vial. In the case of ImmunoCard STAT! HpSA and Uni-Gold™ *H. pylori* Antigen kits, four drops of the diluted sample are subsequently applied to the cassette device, through an integrated dispenser in the cap of the vial. For the RAPID *Hp* StAR the base of the test strip is immersed in the diluted sample. After applying the sample, the ImmunoCard STAT! HpSA is read after 5 min of incubation, whereas RAPID *Hp* StAR and Uni-Gold™ *H. pylori* Antigen are read after 15 min.

Two independent observers performed the readings of all three tests. They were unaware of: *i*) the results of the reference techniques, *ii*) the results of the other tests, and *iii*) the readings of the other evaluator. Results obtained with the three LFIAs were graded as follows:

0 No test line (control line only)
0.5 Trace test line
1 Clear pale test line
2 Clear test line slightly weaker than the average control line
3 Strong clear test line equal to or stronger than the average control line

Tests were considered positive when a score of 0.5 or more was assigned by the reader. Observers also annotated the number of failed or clogged devices.

2.3. Statistical methods

Results of the LFIAs were analyzed by the two different observers. No attempt was made to achieve consensus on the discordant results. Sensitivity, specificity, positive (PPV) and negative predictive values (NPV), their 95% confidence intervals, positive (+LR) and negative likelihood (−LR) ratios were calculated by standard methods. The McNemar test was used to compare the sensitivity and specificity of the different tests [8]. To correct for multiple comparisons, only p values

Table 1
Characteristics of the patients.

Age (years)	Mean ± S.D. 48.9 ± 13
Gender	N (%)
Male	102 (41%)
Female (n)	148 (59%)
Endoscopy main indication	N (%)
Dyspepsia	201 (78%)
Heartburn	21 (8%)
Anemia	8 (3%)
Other	27 (11%)
Endoscopic diagnosis	N (%)
Peptic ulcer	14 (6%)
Gastroduodenal erosions	58 (23%)
Esophagitis	40 (16%)
Normal or minor changes	138 (55%)
Helicobacter pylori status[a]	N (%)
Negative	131 (52%)
Positive	119 (48%)
Number of positive tests	N (%)
0	113 (45%)
1	18 (7%)
1 + positive culture	19 (8%)
2	16 (6%)
3	84 (34%)

[a] *H. pylori* infection was considered positive if patients had a positive culture or at least two of the remaining tests were positive. Negativity was defined as having one or no positive tests.