Good diagnostic accuracy of a chemiluminescent immunoassay in stool samples for diagnosis of *Helicobacter pylori* infection in patients with dyspepsia

María José Ramírez-Lázaro,[1,2,3] Josep Lite,[4] Sergio Lario,[1,2,3] Pepa Pérez-Jové,[4] Antònia Montserrat,[1,2] María Elisa Quílez,[1,3] Eva Martínez-Bauer,[1,2] Xavier Calvet[1,2,5]

[1]Digestive Diseases Service, Hospital de Sabadell, Corporació Sanitària Parc Taulí, Institut Universitari Parc Taulí-UAB, Sabadell, Spain
[2]Centro de Investigación Biomédica en Red de Enfermedades Hepáticas y Digestivas (CIBERehd), Instituto de Salud Carlos III, Madrid, Spain
[3]Fundació Parc Taulí, Corporació Sanitària Parc Taulí, Institut Universitari Parc Taulí-UAB, Sabadell, Spain
[4]Service of Microbiology, CatLab, Viladecavalls, Barcelona, Spain
[5]Departament de Medicina. Facultat de Medicina, Universitat Autònoma de Barcelona, Barcelona, Spain

Correspondence to
Sergio Lario, Servei de Malalties Digestives, Hospital de Sabadell, Institut Universitari Parc Taulí, Universitat Autònoma de Barcelona, CIBEREHD—Instituto de Salud Carlos III, Parc Taulí, s/n, Sabadell 08208, (Barcelona), Spain; slario@tauli.cat

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ABSTRACT

Laboratory-based chemiluminescence immunoassays (CLIA) are widely used in clinical laboratories. Some years ago, a CLIA test was developed for the detection of *Helicobacter pylori* in stool samples, known as LIAISON *H. pylori* SA, but little information on its use has been reported. To evaluate the accuracy of the LIAISON *H. pylori* SA assay for diagnosing *H. pylori* infection prior to eradication treatment. Diagnostic reliability was evaluated in 252 untreated consecutive patients with dyspepsia. The gold standard for diagnosing *H. pylori* infection was defined as the concordance of the rapid urease test (RUT), histopathology and urea breath test (UBT). The CLIA assay was performed according to the manufacturer's instructions. Sensitivity, specificity, positive and negative predictive values, and 95% CIs were calculated. According to the gold standard selected, 121 patients were positive for *H. pylori* infection and 131 negative. LIAISON *H. pylori* SA had a sensitivity of 90.1% and a specificity of 92.4%, with positive and negative predictive values of 91.6% and 90.1%, respectively. The accuracy of the LIAISON *H. pylori* SA chemiluminescent diagnostic assay seems comparable to that of ELISA or the best-performing LFIAs. Its sensitivity and specificity, however, seem slightly lower than those of histology, RUT or UBT. The advantages of the assay are that it is cheap, automated, and minimally labor-intensive.

Significance of this study

What is already known on this subject?

- The LIAISON *Helicobacter pylori* SA assay —a laboratory chemiluminescent test for the detection of *H. pylori* in stool samples —was developed some years ago. Few data about this assay have been reported, and none that comply with the STAndards for the Reporting of Diagnostic Accuracy Studies (STARD) recommendations.

What are the new findings?

- LIAISON *H. pylori* SA had a sensitivity of 90.1% and a specificity of 92.4%.
- The diagnostic reliability of LIAISON *H. pylori* SA is equivalent to that reported for ELISA.
- Its sensitivity and specificity are slightly lower than those of urea breath test, histology or rapid urease test.

How might it impact on clinical practice in the foreseeable future?

- LIAISON *H. pylori* SA may be acceptable for clinical practice. Its diagnostic accuracy is similar to those reported for ELISA and the best-performing lateral flow immunoassays.

INTRODUCTION

Diagnostic methods for *Helicobacter pylori* detection are classified into invasive and non-invasive. Histology, rapid urease test (RUT), culture and molecular diagnostic methods are invasive methods which require gastroscopy. Non-invasive tests include urea breath test (UBT) and stool antigen tests (SAT). Fecal tests are gaining acceptance because they are inexpensive and the samples are easy to collect; they are also especially convenient for pediatric diagnosis since they do not require the child's active collaboration. Stool tests can be performed as rapid in-office lateral-flow immunochromatographic assays (LFIA) or as laboratory-based ELISA. LFIA are less accurate than ELISA.[1] In fact, only Amplified IDEIA Hp StAR (Oxoid Ltd, UK) ELISA has generally shown high sensitivity and specificity in multiple settings, making it suitable for use in clinical practice.[2–11]

Laboratory-based chemiluminescence immunoassays (CLIA) are widely used in clinical laboratories due to their wide dynamic range, sensitivity, and automation. They may represent a convenient alternative to ELISA tests. The main advantage of chemiluminescence tests is that, by using a photomultiplier, the luminescence signal can be measured down to a few

photons. This allows the detection of low numbers of molecules, in the 10^{-18} to 10^{-21} mole range, and thus provides a similar technical sensitivity to radioisotopes.[12]

Some years ago, a new laboratory chemiluminescent test for the detection of *H. pylori* in stool samples was developed, the LIAISON *H. pylori* SA assay.[13] The information on this assay is limited, and none of the data available comply with the STAndards for the Reporting of Diagnostic Accuracy Studies (STARD) recommendations.[14] Therefore, the present study aimed to evaluate the accuracy of a monoclonal CLIA test for the diagnosis of *H. pylori* in a large series of consecutive untreated patients with dyspepsia.

PATIENTS AND METHODS

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 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 (Barcelona, Spain) for evaluation of dyspeptic symptoms from January 2009 to July 2014 were recruited for the study. Consecutive patients were contacted prior to the endoscopy and were asked to participate. Those who agreed were instructed to avoid antisecretory drugs in the 2 weeks before the test. Patients unable to stop antisecretory drugs, those who had received antibiotics in the 4 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 13C-UBT (UBiTest 100 mg, Otsuka Pharmaceutical Europe Ltd, UK) was administered. During endoscopy, two antral biopsies for histology and one for the 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. Thirty-eight were excluded because of the unavailability of UBT, RUT, and histology, for a variety of technical reasons, or because the fecal sample was insufficient to perform the three tests. The remaining 252 patients were analyzed. 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.[15] Patients who were positive for two or more of these tests or patients who were 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.

Table 1 Patients' characteristics

Gender (male/female) (N)	103/149
Age (years, mean±SD)	48.8±12.9
Endoscopy main indication	**N (%)**
Uninvestigated dyspepsia	202 (80.2)
Heartburn	27 (10.6)
Anemia	14 (5.6)
Other	9 (3.6)
Endoscopic diagnosis	**N (%)**
Peptic ulcer	12 (4.8)
Gastroduodenal erosions	60 (23.7)
Esophagitis	44 (17.5)
Normal or minor changes	136 (54.0)

CLIA stool test

The fecal test was performed according to the specifications of the manufacturer. Briefly, the LIAISON *H. pylori* SA assay (REF 318920, DiaSorin, Stillwater, Minnesota, USA) uses monoclonal antibodies against a *H. pylori* stool antigen in the form of a two-site sandwich assay. A 5 mm diameter stool sample was diluted in an 850 µL LIAISON *H. pylori* SA sample diluent, vortexed and processed using the LIAISON Stool Extraction Device. Two hundred microliter of the diluted sample was incubated with paramagnetic particles coated with capture antibodies. Isoluminol conjugated antibodies for *H. pylori* antigen were subsequently added and incubated and the unbound material was washed. Then the flash chemiluminescent reaction was initiated and chemiluminescent light measured by a photomultiplier. The process was performed automatically by the LIAISON analyzer (DiaSorin, Stillwater, Minnesota, USA). Relative light units (RLU) are recorded. RLU are proportional to the concentration of the *H. pylori* stool antigen present. Values <1.0 are considered negative, and values of ≥1.0 positive. The trained operator was unaware of the results of the reference tests.

Statistical methods

Sensitivity, specificity, positive predictive (PPV) and negative predictive (NPV) values, and their 95% CIs and positive and negative likelihood ratios were calculated by standard methods. Assuming a prevalence of *H. pylori* infection of 50% in the sample evaluated,[16 17] a sample size of 250 patients was required in order to obtain an estimation of sensitivity and specificity with a minimal CI of 0.1 and a confidence level of 0.95. All calculations were performed using SPSS V.21.0 for Windows (SPSS Inc., Chicago, Illinois, USA). The study was performed in compliance with the STARD recommendations.[14]

RESULTS

Sensitivity and specificity

According to the gold standard selected, 121 patients were positive for *H. pylori* infection (86 had three positive tests, 16 had two, and 19 had only one positive test but were

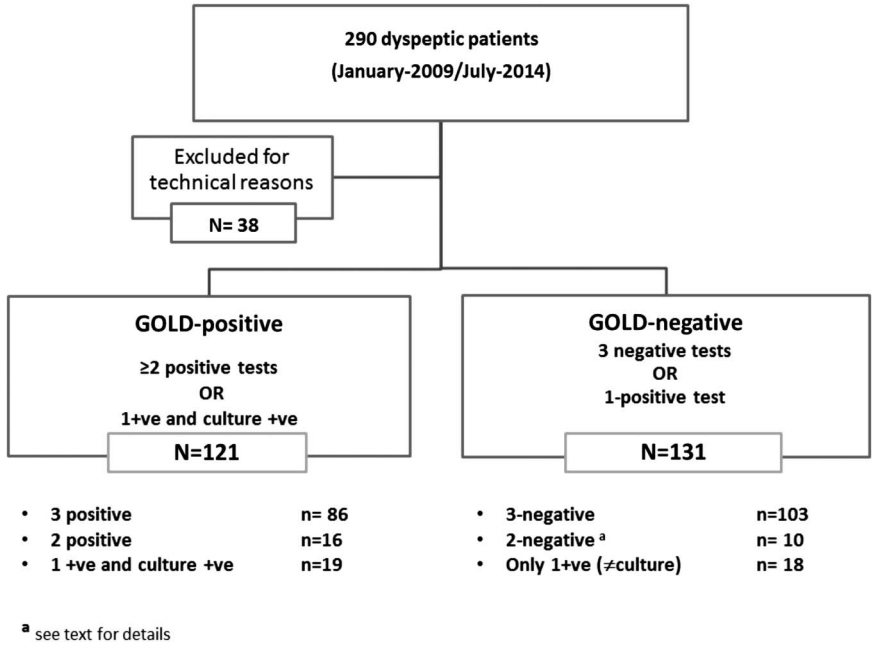

Figure 1 STAndards for the Reporting of Diagnostic Accuracy Studies (STARD) flow diagram of the study.

also positive for culture) and 131 negative (103 were negative for all reference tests and 18 had one positive test—12 UBT, five histology and one RUT). For technical reasons, only two gold standard tests were performed in 10 cases: all 10 were negative for both tests and were considered negative. The STARD flow diagram of the study is shown in figure 1.

Sensitivity, specificity as well as PPV and NPV of the LIAISON *H. pylori* SA assay are shown in table 2. LIAISON *H. pylori* SA had a sensitivity of 90.1% and a specificity of 92.4%. Positive predictive value and NPV were 91.6% and 90.1%, respectively. The positive (LR+) and negative likelihood ratios (LR−) were 11.9 and 0.10, respectively.

The sensitivity of the LIAISON *H. pylori* SA test was similar to that of the UBT and lower than those of the invasive tests RUT and histology. However, this finding should be treated with caution because, in contrast to the fecal test, UBT, RUT and histology were used to build the gold standard.

DISCUSSION

This study evaluates the diagnostic accuracy of a chemiluminescence test for identifying *H. pylori* infection in a large series of consecutive untreated patients with dyspepsia. The assay offered sensitivity and specificity values of 90.1% and 92.4%, respectively. The reliability of this specific test seems comparable to that of ELISA or the best-performing LFIAs.[1–11]

The currently existing SAT for *H. pylori* diagnosis have practical and economic advantages over other non-invasive and invasive tests. However, the diagnostic accuracy of many of these tests seems slightly lower than those of UBT or invasive tests, and improvements in their sensitivity and specificity seem necessary. CLIA may represent a technical advance. To the best of our knowledge, this is the first well-devised report of the diagnostic value of the chemiluminescent LIAISON *H. pylori* SA assay. Data on this test's accuracy are very scarce. The product data insert reports the concordance of LIAISON *H. pylori* SA results with an unspecified ELISA stool test; the correlation was performed in 201 symptomatic patients from Italy, in which the agreement between the stool tests was 97%,[13] but no data were reported on how patients were recruited or diagnosed, nor on their current status. Another evaluation of the test in 103 patients with dyspepsia was performed in Spain but was reported only in abstract form.[18] In that study, the gold standard was only the UBT: this may help explain the low sensitivity reported (72.0%), much lower than in this study (90.1%). The specificity was, however, excellent (96.2% vs 92.4%).

Table 2 Sensitivity, specificity, positive and NPVs of the reference tests

	Test	Sensitivity (95% CI)	Specificity (95% CI)	PPV (95% CI)	NPV (95% CI)
Non-invasive tests	LIAISON *Helicobacter pylori* SA	90.1 (85 to 93)	92.4 (88 to 95)	91.6 (87 to 95)	90.1 (86 to 93)
	UBT	90.9 (86 to 94)	99.2 (97 to 100)	99.2 (97 to 100)	90.8 (86 to 94)
Invasive tests	RUT	99.0 (96 to 100)	92.4 (88 to 95)	90.5 (86 to 94)	99.2 (97 to 100)
	Histology	95.5 (92 to 98)	95.4 (92 to 98)	94.6 (91 to 97)	96.2 (93 to 98)

NPV, negative predictive value; PPV, positive predictive value; RUT, rapid urease test; UBT, urea breath test.
Values are given as percentages and 95% CIs.

The strengths of this study are the inclusion of prospectively evaluated patients, its sample size, and the fact that the gold standard and the analysis were performed in strict observance of current recommendations.[14 15] Its limitations are the lack of a direct comparison with equivalent assays in other formats (LFIA, ELISA) and that—as is habitual for SATs—its results cannot be extrapolated to other populations because the antigenic composition of *H. pylori* may present geographical variations.[19]

In conclusion, the accuracy of the LIAISON *H. pylori* SA chemiluminescent diagnostic assay seems comparable to that of ELISA or the best-performing LFIAs. Its sensitivity and specificity, however, seem slightly lower than those of histology, RUT or UBT. The advantages of the assay are that it is cheap, automated, and minimally labor-intensive.

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Contributors MJR-L and MEQ performed the breath tests and microbiological analysis. JL and PP-J performed the CLIA test and reviewed the manuscript. SL wrote the manuscript. AM enrolled patients and recorded clinical data. EM-B and XC performed gastroscopies. XC designed the experiments, analyzed the data and reviewed the manuscript.

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Competing interests None declared.

Patient consent Obtained.

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