

RAPID COMMUNICATION

## Diagnostic accuracy of a rapid fecal test to confirm *H pylori* eradication after therapy: Prospective comparison with a laboratory stool test

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### Abstract

**AIM:** To investigate the clinical performances of rapid stool test (ImmunoCard STAT HpSA, Meridian Diagnostic Inc.) in the evaluation of eradication therapy of *H pylori* and to compare it with a well-known and validated laboratory stool test (Amplified IDEA Hp StAR, Dako).

**METHODS:** Stool samples of 122 patients were evaluated after eradication therapy of *H pylori*. *H pylori* status was assessed by 13C-urea breath test (UBT). Stool specimens were tested using either the rapid immunoassay kit or the laboratory immunoassay kit.

**RESULTS:** Forty-three patients were infected and 79 non-infected. Sensitivity and specificity of ImmunoCard STAT and Hp StAR were 58.14% and 76.4%, and 97.47% and 98.73%, respectively ( $P > 0.05$ ). Overall agreement between the two tests was 92.6% (113 of 122 cases).

**CONCLUSION:** ImmunoCard STAT seems to have rather low performances, and it cannot be regarded as a reliable tool in the post-treatment setting. Also Hp StAR cannot be recommended to confirm *H pylori* eradication after treatment.

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**Key words:** *H pylori*; Diagnosis; Feces

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### INTRODUCTION

Nowadays, there is an increasing interest in non-invasive methods to diagnose *H pylori* infection. Indeed, they can profitably replace endoscopy in predicting the diagnosis and determining the management of some types of patients, according to a "test and treat" strategy<sup>[1]</sup>. Post-therapy testing to confirm eradication is also growing in importance, as resistant strains of *H pylori* are now widely prevalent in both USA and Europe, with a failure rate of current eradication regimens ranging from 10% to 20%<sup>[2,3]</sup>. Furthermore, in patients with bleeding peptic ulcer, the risk for rebleeding is greatly increased if *H pylori* infection persists<sup>[4]</sup>.

Until some years ago, the only non-invasive test that reliably demonstrated whether eradication was successful was the urea breath test (UBT)<sup>[5]</sup>. It is easy to perform and does not need special transport conditions, but requires expensive and specific instruments. In the last years, several stool antigen tests have been put on the market and approved by the U.S. Food and Drug Administration for detection of *H pylori* before and after therapy. They are considered reliable in either pre-treatment or post-treatment settings<sup>[1,6,7]</sup>, even though some controversial results have been reported after eradication therapy<sup>[8-12]</sup>. These tests are based on an enzyme immunoassay carried out in laboratory, and this limit delays the diagnostic report. Moreover, if stool samples are not frozen immediately after receipt and stored frozen until titration at a temperature lesser than -20°C, sensitivity of the test can drop<sup>[13]</sup>. More recently, some rapid stool tests not requiring laboratory assay have been put on the market. These near-patient tests are cheap, easy and quickly performed, and have good diagnostic accuracy in the pre-treatment setting<sup>[14-19]</sup>. For these reasons, they could represent a valid alternative to both UBT and traditional stool tests. However, at present there are few data about their clinical usefulness in the post-treatment setting.

In this prospective pilot study, we investigated the clinical performances of a rapid stool test in the evaluation

of eradication therapy and compared it with a well-known and validated *H pylori* stool test requiring laboratory assay.

## MATERIALS AND METHODS

### Patients

One hundred thirty consecutive outpatients undergoing  $^{13}\text{C}$ -UBT to determine their post-treatment *H pylori* status at least 6 wk after the end of antimicrobial therapy, were asked to deliver a stool specimen the day after  $^{13}\text{C}$ -UBT was performed. Eight of them did not deliver it and were excluded from the study; the remaining 122 patients (46 males and 76 females; mean age  $\pm$  SD:  $54.94 \pm 13.90$  years) were definitively enrolled. They were previously given the following regimens: Proton pump inhibitor (PPI) + Clarithromycin + Amoxicillin in 49 cases; PPI + Clarithromycin + Tinidazole (or Metronidazole) in 30 cases; PPI + Levofloxacin + Amoxicillin in 13 cases; Ranitidine bismuth citrate + Clarithromycin in 4 cases; PPI + Bismuth citrate + Metronidazole + Tetracycline in 2 cases; and undetermined in 24 cases. Exclusion criteria were: use of antibiotics, histamine-2 receptor antagonists, bismuth or PPIs in the last 6 wk; chronic use of corticosteroids or non-steroidal anti-inflammatory drugs; previous gastric surgery; severe concomitant diseases; pregnancy or lactation. All patients gave their informed consent, and the study was approved by our Ethical Committee.

### Methods to assess *H pylori*

The post-treatment *H pylori* status was assessed by  $^{13}\text{C}$ -UBT that was assumed as the gold standard according to previous reports<sup>[1,10,20,21]</sup>.  $^{13}\text{C}$ -UBT was carried out at least 6 wk after the end of antimicrobial therapy according to previously validated protocols<sup>[20]</sup>. In brief, the patients were fasted overnight, and the baseline breath sample was collected. Afterwards, they drank a 200-mL water solution containing 75 mg of  $^{13}\text{C}$ -urea and 1.4 g of citric acid (BREATHQUALITY-UBT  $^{13}\text{C}$ -UREA, AB Analitica, Padova, Italy), and a further breath sample was collected 30 min later. Samples were analyzed by an infrared spectrometer and positive result was defined by a cut-off of 2.5‰. The doctor who performed the  $^{13}\text{C}$ -UBTs was blinded to the results of all the other tests.

A portion of each fresh stool sample was tested by using a rapid immunochromatographic assay commercial kit (ImmunoCard STAT HpSA, Meridian Diagnostic Inc., OH, USA) (ImmunoCard STAT) for the detection of *H pylori* antigens in stools. All tests were performed by a single unique observer (L.T.) who was unaware of the *H pylori* status. He evaluated each sample according to the method previously described<sup>[18]</sup>. A positive result was defined if both a pink-red band (test line) and a blue-colored band (control line) appeared in the reading window. If only a blue band appeared in the reading window, the result was considered negative.

The remaining portion of each stool specimen was frozen and stored, and successively tested by using a commercial kit (Amplified IDEA Hp StAR, Dako,

Table 1 Performances of Immuno Card STAT and HpStAR tests

	ImmunoCard STAT	Hp StAR
Sensitivity, % (95% CI)	58.14% (42-73)	76.74% (61-88)
Specificity, % (95% CI)	97.47% (91-100)	98.73% (93-100)
PPV, % (95% CI)	92.59% (76-99)	97.06% (85-100)
NPV, % (95% CI)	81.05% (72-88)	88.64% (80-94)
Global accuracy, % (95% CI)	83.61% (76-90)	90.98% (84-95)
False positive, <i>n</i>	2	1
False negative, <i>n</i>	18	10

CI: Confidence interval; PPV: Positive predictive value; NPV: Negative predictive value.

Glostrup, DK) (Hp StAR), widely validated in literature in both the pre-treatment and post-treatment settings<sup>[22-24]</sup>. This monoclonal enzyme immunoassay has been reported to be more accurate than polyclonal enzyme immunoassay in determining *H pylori* status after eradication treatment<sup>[25]</sup>. The Hp StAR test is an *in vitro* qualitative procedure for the detection of *H pylori* antigens in stool samples, and needs a laboratory to be performed. It is a sandwich-type enzyme immunoassay using immunoassay technology. The test was performed according to the manufacturer's instructions as previously described<sup>[18]</sup>. According to the manufacturer's guidelines, an absorbance 450 nm ( $A_{450}$ )  $\geq 0.190$  was defined as a positive and  $A_{450} < 0.190$  as a negative test result.

### Estimation of sample size and statistical analysis

Sample size calculation was performed to obtain a good accuracy power, i.e. at about 95%, and the significance study threshold was chosen at 5% (type I error: 0.05). Stool tests sensitivity, specificity, predictive values of positive and negative results, diagnostic accuracy, and their 95% confidence intervals (95% CI) were calculated using standard methods. Differences in the test performances between the two methods were analysed by using Fisher's exact test. A *P* value  $< 0.05$  was regarded as statistically significant.

## RESULTS

According to the study protocol, UBT showed eradication in 79 (64.8%) patients, and persistency of *H pylori* infection in 43 (35.2%) patients.

ImmunoCard STAT was positive in 27 cases (two false-positives), and negative in 95 (18 false-negatives), with a sensitivity and specificity of 58.14% and 97.47%, respectively. Hp StAR was positive in 34 patients (one false-positive), and negative in 88 (10 false-negatives), with a sensitivity and specificity of 76.74% and 98.73%, respectively. The overall agreement between the two tests in the evaluation of *H pylori* status was 92.6% (113 of 122 cases).

The diagnostic performances of ImmunoCard STAT and Hp StAR are reported in Table 1. Despite Hp StAR seemed to work better than ImmunoCard STAT, no significant difference was observed between the two stool tests.

## DISCUSSION

Rapid tests for the detection of *H pylori* antigens in stool can be very useful in clinical practice, as they are cheap, easy and quickly performed, and can be done in the doctor's office within 10 min. Several studies demonstrated their high diagnostic accuracy in untreated patients<sup>[14-18]</sup>, and they can reliably replace UBT in this setting. Conversely, their clinical usefulness to evaluate *H pylori* status after eradication therapy has been scarcely investigated. Our study demonstrated that sensitivity, negative predictive value, and global accuracy of rapid stool test were rather low (58.1%, 81%, and 83.6%, respectively), so this test cannot be regarded as a reliable tool in the post-treatment setting. On the contrary, several authors reported that rapid stool tests have post-treatment performances similar to the pre-treatment ones, and are also indicated to assess the success of eradication therapy<sup>[26-33]</sup>. However, other authors reported lower performances of rapid stool tests after eradication therapy in either adults<sup>[34]</sup> or children<sup>[14]</sup>, and Gisbert *et al*<sup>[35]</sup> suggested that they cannot be recommended to confirm *H pylori* eradication after treatment. In a multicenter trial investigating the same commercial kit used in our study, Kato *et al*<sup>[14]</sup> found a sensitivity of only 75% in the post-treatment setting, but in this study only frozen stools were tested. Conversely, we tested fresh stools, as this option better reflects what happens in the near-patient environment, such as the doctor's office. Using fresh stools, we expected results better than (or at least similar to) those obtained by Kato *et al*<sup>[14]</sup>. On the contrary, they were worse because sensitivity of Immuno Card STAT was 58.14%, resulting in wrong diagnosis in 20 of 122 patients, with a global accuracy of 83.6%.

In our study, all tests were read by a single unique observer, who in all cases was able to assess the positivity or negativity of the test. Indeed, the test was classified as positive even if the intensity of the pink-red band appearing in the reading window (test line) was very weak, according to the manufacturer's instructions. Conversely, other authors observed that the test line was so weakly visible that they judged the result as equivocal, in a percentage ranging from 5% to 11.9%<sup>[15,26]</sup>.

We compared the rapid stool test with another, well-known, monoclonal enzyme immunoassay (Hp StAR), which has to be performed in laboratory. No significant difference was observed between the two tests, and their concordance was 92.6%, similar to that reported in our prior study investigating the same commercial kits in the pre-treatment setting<sup>[18]</sup>. It follows that in our study the performances of the laboratory monoclonal test provided unsatisfying also results, with a sensitivity lower than 80% and a global accuracy of 90.98%, and we think it cannot be recommended to confirm *H pylori* eradication after treatment. Our opinion is supported by a recent systematic review on the role of stool antigen test for the diagnosis of *H pylori*, which showed relatively low accuracy in some post-treatment studies with polyclonal stool antigen test, and suggested that its use in clinical practice is yet to be defined<sup>[23]</sup>. Indeed, the Maastricht 2-2000 Consensus Conference also recommended UBT as the most reliable,

non-invasive test to assess eradication efficacy<sup>[1]</sup>.

In our study, we did not use invasive methods (such as histology or rapid urease test) to evaluate *H pylori* status, as we and our Ethical Committee judged it unethical that patients with no indications to gastroscopy had to undergo invasive procedures to assess *H pylori* eradication. However, <sup>13</sup>C-UBT was assumed as the gold standard to evaluate *H pylori* status, as it is considered the method of choice to monitor success of therapy in both adults and children, and is recommended by current guidelines<sup>[1,36,37]</sup>. Indeed, strong evidences of sensitivity and specificity of <sup>13</sup>C-UBT close to 100% emerge from some good reviews<sup>[20,38,39]</sup>, and these performances remain very high (quite over 90%) also using low doses of <sup>13</sup>C-urea<sup>[40]</sup>.

In conclusion, our study suggests that rapid stool test is not very accurate in the post-treatment setting, and it cannot be recommended to evaluate the success of eradication therapy, as well as the laboratory monoclonal test. However, the conflicting results reported in literature about this topic make the planning of wide multicenter trials quite necessary, to reach a definitive answer to this controversial question.

## COMMENTS

### Background

In the last years, several stool antigen tests have been put on the market and approved by the U.S. Food and Drug Administration for detection of *H pylori* before and after therapy. These tests are based on an enzyme immunoassay carried out in laboratory, and this limit delays the diagnostic report.

### Research frontiers

Recently, some rapid stool tests not requiring laboratory assay have been put on the market. These near-patient tests are cheap, easy and fast to be performed, and have good diagnostic accuracy in the pre-treatment setting. Several authors reported that rapid stool tests have post-treatment performances similar to the pre-treatment ones, and are also indicated to assess the success of eradication therapy.

### Innovations and breakthroughs

Our study suggests that sensitivity, negative predictive value, and global accuracy of rapid stool test are rather low, so it cannot be regarded as a reliable tool in the post-treatment setting.

### Applications

Our study suggests that rapid stool test is not very accurate in the post-treatment setting, and it cannot be recommended to evaluate the success of eradication therapy.

### Peer review

This is a well-designed paper and the results are correctly presented. Through comparison with the well-known and validated laboratory stool test (Amplified IDEA Hp StAR, Dako), the authors suggest that rapid stool test cannot be regarded as a reliable tool in the post-treatment setting. Also Hp StAR cannot be recommended to confirm *H pylori* eradication after treatment.

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