



# Clinical performance of the H. PYLORI QUIK CHEK™ and H. PYLORI CHEK™ assays, novel stool antigen tests for diagnosis of *Helicobacter pylori*

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## Abstract

Infection with *Helicobacter pylori* is a global health issue, and rapid and accurate testing is a key to diagnosis. We aimed to assess the performance of two novel enzyme immunoassays (EIA), the H. PYLORI QUIK CHEK™ and the H. PYLORI CHEK™ assays, for the detection of *H. pylori* antigen in stool. Patients from five geographically diverse sites across the USA, Germany, and in Bangladesh were tested for infection with *Helicobacter pylori* with the two novel stool antigen tests and two commercially available stool antigen assays. All patients provided a stool sample and underwent esophagogastroduodenoscopy for biopsy. Results were compared to a clinical diagnosis using a composite reference method consisting of histological analysis and rapid urease testing of the biopsy. A total of 271 patients, 68.2% female and mean age of 46 years, were included. The overall prevalence of *H. pylori* infection was 24.1%. The sensitivity of the H. PYLORI QUIK CHEK™ and H. PYLORI CHEK™ was 92% and 91%, respectively. The specificity of H. PYLORI QUIK CHEK™ and H. PYLORI CHEK™ was 91% and 100%, respectively. No significant cross-reactivity against other gut pathogens was observed. The H. PYLORI QUIK CHEK™ and H. PYLORI CHEK™ assays demonstrate excellent clinical performance compared the composite reference method.

**Keywords** *Helicobacter pylori* · Stool antigen test · Rapid diagnosis · Validation · Sensitivity · Specificity

## Introduction

It is estimated that half of the global population is infected with *Helicobacter pylori* (*H. pylori*), a gram-negative, spiral-shaped microaerophilic bacterium [1]. The bacterium has several unique chemical and physical properties which allow for successful infection in the very hostile environment of the gastric lumen [2]. Specifically, 4–6 polar flagella which allow

for motility in the mucus layer of the stomach and production of urease which hydrolyzes gastric acid to ammonia are important pathogenic factors [3]. Chronic infection with *H. pylori* is a potent risk factor for gastritis, peptic ulcer disease, and gastric malignancies such as gastric adenocarcinoma and lymphoma [3]. For example, it is estimated that 36–47% of gastric adenocarcinomas worldwide are solely attributable to chronic infection with *H. pylori* [4]. The infection is typically acquired in childhood [5], and while the exact method of transmission is unclear, poor sanitary conditions and high density family households are known risk factors [6]. Highly effective therapy for *H. pylori* infection is available for most patients in whom a diagnosis has been made [7]. Accurate diagnosis and timely treatment of the infection improves outcomes for patients infected with *H. pylori* [8]. For example, eradication of *H. pylori* has been shown to reduce the risk of subsequent peptic ulcer disease and gastric adenocarcinoma, both of which can lead to significant morbidity and mortality [8, 9]. *H. pylori* infection can be diagnosed on biopsies obtained from esophagogastroduodenoscopy (EGD) using either special stains on histology [10], rapid urease testing [11], culture [12], or polymerase chain reaction testing [12], but

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performing an EGD in order to diagnose *H. pylori* infection is expensive, invasive, and not universally available. Certain noninvasive tests, such as the urea-breath test and stool antigen assays, are also limited by high cost and/or a long turn-around time to diagnose the infection [12]. In addition, most patients with *H. pylori* infection require more than one test during their diagnostic and therapeutic journey as confirmation of eradication after treatment for *H. pylori* is recommended [7]. The aim of this study was to determine the performance characteristics of two new stool-based enzyme-linked assays (EIA) tests designed to rapidly detect presence of *H. pylori* antigen.

## Materials and methods

### Study design, patients, and specimens

We prospectively recruited patients at five geographically diverse clinical sites from August 2017 to May 2018 in order to determine the diagnostic performance of a novel stool antigen tests in patients in the initial diagnosis of *H. pylori* infection. Three of the study sites were in North America (Minnesota, VA, USA), one in Europe (Essen, Germany), and one in Southeast Asia (Dhaka, Bangladesh). For the initial diagnosis claim, we included patients with symptoms of dyspepsia, gastritis, or peptic ulcer. All patients underwent esophagogastroduodenoscopy where at least six gastric biopsies were obtained for histological analysis. One additional gastric biopsy was obtained for rapid urease testing. All patients provided a stool sample. Exclusion criteria included asymptomatic patients, patients in whom the presence/absence of *H. pylori* was already known. Patients had to have refrained from antibiotics and bismuth compounds (e.g., Pepto-Bismol™) for 2 weeks prior to submitting a fecal sample. Proton-pump inhibitor (PPI) use was recorded if within 2 weeks of providing the fecal sample.

### Stool test

All study sites collected stool specimens from patients within 48 h before or after the reference endoscopy procedure. Stool specimens were subsequently stored at 2–8 °C for up to 14 days then frozen if not tested. All stool specimens were analyzed at the central reference laboratory. Stool samples underwent testing with H. PYLORI QUIK CHEK™ test, a rapid membrane EIA (rapid EIA), and the H. PYLORI CHEK™ test, a microwell EIA (EIA) according to the manufacturer's instructions (TechLab Inc., Blacksburg, VA). An optical density (OD) of  $\geq 0.120$  at single wavelength (450 nm) or  $\geq 0.080$  at dual wavelength (450/620 nm) were considered positive for the H. PYLORI CHEK™ test and visual results were used to

determine a positive result for the H. PYLORI QUIK CHEK™ test. In addition, all stool samples were also tested using two commercially available enzyme immunoassays following the manufacturer's instructions (Premier Platinum HpSA Plus; Meridian Bioscience (microwell) and ImmunoCard STAT! HpSA, Cincinnati, OH (rapid test)).

### Histological testing

Gastric biopsies were stained with hematoxylin and eosin or modified Giemsa stain for routine histological analysis to determine the presence or absence of *H. pylori*.

### Rapid urease test

A single gastric biopsy was placed in the gel of the CLOtest\* Rapid Urease Test (Kimberly-Clark\*) and then stored at ambient temperature. A positive test was defined as a change to the reference color within 24 h based on the manufacturer's instructions.

### Culture

*H. pylori* culture was not routinely performed at all study sites. However, results were included in a sub-analysis for completeness.

### Definition of the composite reference method

Diagnostic performance of the stool antigen tests was determined by comparing results to a composite reference method (CRM, gold standard for *H. pylori* infections) that includes 3 possible tests performed on the biopsy: culture, histology, and rapid urease test results, where a positive diagnosis is made if 2 out of 3 tests are positive [13]. For the purposes of this study, the results from rapid urease testing and histology were utilized for case definition as these two tests were performed in all patients across all study sites (Table 1). In addition, an

**Table 1** Composite reference method for diagnosis of *H. pylori* infection from gastric biopsy using histology and rapid tissue urease

CRM algorithm for specimens submitted for initial diagnosis		
Histology	Rapid urease test	CRM result (I = inconclusive)
+	+	+
+	–	I
–	+	+
–	–	–

\* Patients with a single positive urease test at baseline may be more appropriately considered infected

overall analysis which included indeterminate cases based on the CRM definition as well as data from sites which performed *H. pylori* culture based was also performed. A subgroup analysis which excluded patients in whom PPI exposure occurred within 2 weeks was performed (Table 1).

### Cross-reactivity testing

The specificity of the H. PYLORI QUIK CHEK™ and H. PYLORI CHEK™ assays was challenged by examining the reactivity of a wide range of common intestinal organisms and viruses (Tables 2 and 3). For the analysis, the bacteria were grown to early stationary phase ( $> 10^8$  CFU/mL); McFarland Standard #4 and stock cultures of viruses were purchased. The cultures were diluted 1:10 in (i) fecal matrix that was negative for *H. pylori* (negative fecal pool) or (ii) fecal sample matrix that was spiked with *H. pylori* antigen (ATCC strain 43526) at 2–3 times the amount to produce a positive results ( $C_{95}$ ; positive fecal pool). The preparations were assayed in both assays and qualitative results were reported.

### Ethical considerations

All patients provided informed consent and the study protocol received internal review board approval at each individual study site.

**Table 2** Bacteria analyzed for cross-reactivity testing

<i>Acinetobacter baumannii</i>	<i>Escherichia coli</i> EPEC
<i>Bacillus cereus</i>	<i>Escherichia coli</i> ETEC
<i>Bacillus subtilis</i>	<i>Escherichia coli</i> O157:H7 (nontoxigenic)
<i>Borrelia burgdorferi</i>	<i>Escherichia coli</i> O157:H7 (toxigenic)
<i>Campylobacter coli</i>	<i>Haemophilus influenzae</i>
<i>Campylobacter fetus</i>	<i>Lactobacillus acidophilus</i>
<i>Campylobacter helveticus</i>	<i>Listeria monocytogenes</i>
<i>Campylobacter hyointestinalis</i>	<i>Peptostreptococcus anaerobius</i>
<i>Campylobacter jejuni</i>	<i>Porphyromonas asaccharolytica</i>
<i>Campylobacter lari</i>	<i>Prevotella melaninogenica</i>
<i>Campylobacter upsaliensis</i>	<i>Proteus vulgaris</i>
<i>Candida albicans</i>	<i>Pseudomonas aeruginosa</i>
<i>Clostridium bif fermentans</i>	<i>Pseudomonas fluorescens</i>
<i>Clostridium difficile</i>	<i>Salmonella typhimurium</i>
<i>Clostridium perfringens</i>	<i>Staphylococcus aureus</i>
<i>Edwardsiella tarda</i>	<i>Staphylococcus aureus</i> (Cowan's)
<i>Enterobacter cloacae</i>	<i>Streptococcus agalactiae</i>
<i>Enterococcus faecalis</i>	<i>Yersinia enterocolitica</i>
<i>Escherichia coli</i>	
<i>Escherichia coli</i> EIEC	

**Table 3** Viruses analyzed for cross-reactivity testing

Adenovirus types 2, 40	Echovirus 9, 22
Human coronavirus	Enterovirus 70
Coxsackievirus B1, B2, B3, B6	Human rotavirus

### Statistical analysis

The demographic results were compiled and descriptive analysis, including counts and percentages were performed using SAS Software (JMP Pro 14.1.0). The clinical sensitivity and specificity for the *H. pylori* antigen assays were determined by a comparison to the composite reference method by cross-classifying each case as clinically present or absent with 95% confidence interval [14, 15]. The analysis included continuity correction.

## Results

### Demographic data

Patient demographics and clinical data are presented in Table 4. Overall, 271 patients participated in the study across five distinct geographic locations with 223 (82%) of the collected stool samples being tested following a single freeze-thaw on the assays. The mean age was 46.2 years (range 19–82) and 68.2% of participants were female. The prevalence of *H. pylori* positivity based on the CRM ranged from 0 to 69.4% depending on the geographical location. At the North American and European test sites, the prevalence of *H. pylori* in among the participants was less than 10% whereas at the South-East Asian site, nearly 70% of participants were infected.

### Diagnostic performance of the novel stool antigen test in comparison with the CRM

Detailed performance characteristics of the new stool antigen tests are presented in Tables 5, 6, and 7. Of the 271 patients, 10 patients had indeterminate results based on the CRM definition for this study, and thus, the cohort used for assay performance included 261 patients. The H. PYLORI CHEK™ assay had a sensitivity of 92% and specificity of 91% for the detection of *H. pylori* infection compared to the CRM. The H. PYLORI QUIK CHEK™ assay had a sensitivity of 91% and specificity of 100% for the detection of *H. pylori* infection compared to the CRM. Both tests had a negative predictive value of 97%. The positive predictive value for H. PYLORI CHEK™ was 76% and 98% for H. PYLORI QUIK CHEK™ (Table 5).

**Table 4** Patient characteristics

Clinical site (location)	University of Virginia (Charlottesville, VA)	Carillion Clinic (Roanoke, VA)	ICCDR (Dahka, Bangladesh)	Mayo Clinic (Rochester, MN)	University of Duisberg, Essen, Germany	Total
Number of participants	8	20	72	77	94	271
Mean age (SD) in years	58.1 (6.53)	57.5 (11.3)	33.2 (6.1)	48.6 (16.0)	51.1 (15.3)	46.2 (15.2)
Female, <i>N</i> (%)	5 (62.5)	14 (70.0)	40 (55.6)	58 (75.3)	68 (72.3)	185 (68.2)
PPI use, <i>N</i> (%)	6 (85.7)	17 (85.0)	0 (0)	34 (44.1)	24 (25.5)	81 (29.9)
<i>H. pylori</i> infection based on CRM* (%)	0 (0)	1 (5.0)	50 (69.4)	7 (9.1)	5 (5.3)	63 (23.2)

\* Histology and rapid urease test

### Diagnostic performance of the novel stool antigen test in comparison with other commercially available immunoassays

In the *N* = 261 study population, the Premier Platinum HpSA Plus assay had a sensitivity of 87% and specificity of 87%. The Immunocard STAT had a sensitivity of 92% and a specificity of 97% (Table 5).

### Diagnostic performance in patients with no PPI exposure

Among patients in whom no PPI exposure occurred (*N* = 182), the H. PYLORI CHEK™ assay had a sensitivity of 95% and specificity of 90% for the detection of *H. pylori* infection compared to the CRM. The H. PYLORI QUIK CHEK™ assay had a sensitivity of 93% and specificity of 99% for the detection of *H. pylori* infection compared to the CRM (Table 6).

### Detailed comparison of histopathological, culture, and rapid urease results with stool assays

Table 7 shows detailed results of comparison of the endoscopic-based tests with the stool-based assays in the entire study cohort (*N* = 271).

**Table 5** Performance characteristics of *H. pylori* antigen assays

<i>H. pylori</i> antigen test	<i>N</i>	Sensitivity % (95% confidence interval)	Specificity % (95% confidence interval)	Positive predictive value % (95% confidence interval)	Negative predictive value % (95% confidence interval)
H. PYLORI QUIK CHEK™	261	91% (96–80)	100% (100–97)	98% (100–90)	97% (99–93)
H. PYLORI CHEK™	261	92% (97–82)	91% (94–86)	76% (85–65)	97% (85–65)
Premier Platinum HpSA Plus	259	87% (94–76)	87% (91–81)	68% (78–57)	96% (98–91)
ImmunoCard STAT! Hpsa	261	92% (99–82)	97% (99–93)	91% (96–80)	97% (99–94)

### Cross-reactivity

A total of 38 common intestinal bacteria (Table 2) and 6 common intestinal viruses (Table 3) were used to challenge the specificity of the H. PYLORI QUIK CHEK™ and H. PYLORI CHEK™ tests. No cross-reactivity was observed with all the negative results remaining negative and spiked positive samples remaining positive.

### Discussion

The H. PYLORI QUIK CHEK™ and H. PYLORI CHEK™ tests are new stool antigen tests within this current study that showed excellent sensitivity and specificity for the diagnosis of *H. pylori* infection. The clinical performance of these assays was superior to the Premier Platinum HpSA Plus test that had a high false-positive rate lowering the positive predictive value to 68% in this study population. A highly specific and sensitive noninvasive test for *H. pylori* is clinically important as the high positive and negative predictive values aid in preventing misclassification and hence under or over treatment of this important condition.

In addition to aiding initial diagnosis, these rapid assays may offer a useful tool to assess response to therapy and predict noninvasively those patients requiring

**Table 6** Performance characteristics of *H. pylori* antigen assays in patients without PPI use

<i>H. pylori</i> antigen test	<i>N</i>	Sensitivity % (95% confidence interval)	Specificity % (95% confidence interval)	Positive predictive value % (95% confidence interval)	Negative predictive value % (95% confidence interval)
H. PYLORI QUIK CHEK™	182	93% (83–98)	99% (95–100)	98% (89–100)	97% (92–99)
H. PYLORI CHEK™	182	95% (85–99)	90% (83–95)	82% (71–90)	97% (92–99)
Premier Platinum HpSA Plus	181	90% (79–96)	84% (76–90)	73% (61–82)	94% (88–98)
ImmunoCard STAT! Hpsa	182	95% (85–99)	97% (91–99)	93% (83–98)	98% (92–99)

retreatment. *H. pylori* antigen in stool may be present for weeks following treatment and current guidelines recommend that stool antigen testing not be done until at least 4 weeks after completion of antibiotic therapy to confirm eradication [7].

The strengths of this study include the robust performance of the stool antigen test across multiple diverse geographic sites. Furthermore, the diagnostic performance of the new stool assays was compared with a rigorous CRM consisting of gastric biopsies in combination with histology and rapid urease testing. We also compared the new stool assays to other commercially available immunoassays to assess performance. Specificity of the new assays was challenged using gut bacteria and viruses. In addition, a subgroup analysis showed that the overall performance of this assay did not appear to be significantly impacted by recent PPI exposure, although further work is needed to determine whether the current recommendation to hold PPI use prior to testing for *H. pylori* is needed. Limitations of this study include the high, but not

unexpected, variability in *H. pylori* positivity between the different study sites and the female predominant study population. We did not collect data on additional pathological findings on biopsies beyond *H. pylori* positivity. In addition, *H. pylori* culture was not performed at all study sites. Finally, our study criteria of avoiding bismouth compounds and antibiotics for 2 weeks prior to testing could theoretically have led to some false-negative tests; however, the impact of this is unlikely to change the overall conclusions about the diagnostic performance of the assays.

In conclusion, the H. PYLORI QUIK CHEK™ and H. PYLORI CHEK™ assays demonstrate excellent clinical performance compared the composite reference method. Potential advantages of these new assays include the high accuracy and rapid availability of results for initial diagnosis and assessing response to therapy. Further studies need to examine the impact of same day results on initiation of therapy and eradication rates in the treatment of *Helicobacter pylori*.

**Table 7** Biopsy, culture, and rapid urease results compared with the *H. pylori* stool antigen testing

<i>H. pylori</i> assay		H. PYLORI QUIK CHEK™		H. PYLORI CHEK™		Premier Platinum HpSA		ImmunoCard STAT! Hpsa	
Patients <i>N</i> =271	% <i>N</i>	Positive	Negative	Positive	Negative	Positive	Negative	Positive	Negative
Histopathology 99.6% (270)									
Positive	17.7% (48)	13.7% (37)	4.1% (11)	14.5% (39)	3.3% (9)	13.3% (36)	4.5% (12)	13.7% (37)	4.1% (11)
Negative	82% (222)	7.8% (21)	74.4% (201)	13.7% (37)	68.5% (185)	16.7% (45)	64.8% (175)	10.0% (27)	72.2% (195)
Not performed	0.3% (1)		0		0		0.7% (2)		0
Culture 14.7% (67)									
Positive	11.4% (31)	40.3% (27)	5.9% (4)	40.3% (27)	5.9% (4)	40.3% (27)	5.9% (4)	41.8% (28)	4.5% (3)
Negative	13.3% (36)	28.4% (19)	25.4% (17)	28.4% (19)	25.4% (17)	26.9 (18)	26.9 (18)	32.8% (22)	20.9% (14)
Not performed	75.3% (204)		0		0		0		0
Rapid urease test 99.3% (269)									
Positive	23.2% (63)	21.2% (57)	2.2% (6)	21.6% (58)	1.9% (5)	20.4% (55)	3.0% (8)	21.6% (58)	1.9% (5)
Negative	76.0% (206)	0.4% (1)	76.2% (205)	6.6% (18)	69.9% (188)	9.7% (26)	66.2% (178)	2.2% (6)	74.3% (200)
Not performed	0.8% (2)		0		0		0.7% (2)		0

**Author contributions** 1. Halland—data collection, analysis, drafting, and review of manuscript

2. Haque—data collection, analysis, and critical review of manuscript

3. Langhorst—data collection, analysis, and critical review of manuscript

4. Boone—data collection, analysis, drafting, and review of manuscript

5. Petri—data collection, analysis, and critical review of manuscript

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**Data availability** All authors had access to the data and study materials.

## Compliance with ethical standards

**Conflict of interest** J Boone is a Senior Scientist at TechLab. No other author reports any conflict of interest.

**Ethics approval** The study was approved by IRB at each institution.

**Consent to participate** All participants gave informed consent for participation in the study.

**Consent for publication** Not applicable.

**Code availability** Not applicable.

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