

**510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION SUMMARY
ASSAY ONLY TEMPLATE**

A. 510(k) Number:

K181379

B. Purpose for Submission:

To obtain a substantial equivalence determination for the detection of *Helicobacter pylori* antigens in human stool.

C. Measurand:

H. pylori antigen

D. Type of Test:

Qualitative membrane enzyme immunoassay

E. Applicant:

TechLab Inc.

F. Proprietary and Established Names:

H. PYLORI QUIK CHEK

G. Regulatory Information:

1. Regulation section:

21 CFR 866.3110 *Campylobacter fetus* serological reagents

2. Classification:

Class I

3. Product code:

LYR— *Campylobacter pylori*

4. Panel:

H. Intended Use:

1. Intended use(s):

The TECHLAB *H. PYLORI QUIK CHEK* test is a rapid membrane enzyme immunoassay for the qualitative detection of *Helicobacter pylori* specific antigen in a single use cassette. It is intended for use with human fecal specimens to aid in the diagnosis of *H. pylori* infection and to demonstrate loss of *H. pylori* antigen following treatment. The test can be used with unpreserved fecal specimens and fecal specimens preserved in transport media from patients suspected of *H. pylori* infection. Testing of patients to demonstrate loss of *H. pylori* antigen following treatment should be performed no sooner than 4 weeks after completion of the treatment regimen. Test results should be taken into consideration by the physician in conjunction with the patient history and symptoms.

2. Indication(s) for use:

Same as the Intended Use.

3. Special conditions for use statement(s):

For prescription use only

4. Special instrument requirements:

None

I. Device Description:

The *H. PYLORI QUIK CHEK* test utilizes antibodies specific for *H. pylori* antigen in human fecal samples. The Membrane Device contains a "Reaction Window" with two vertical lines of immobilized antibodies. The test line ("T") contains antibodies specific for *H. pylori* antigen. The control line ("C") contains antibodies to horseradish peroxidase (HRP). The "Conjugate" consists of antibodies to *H. pylori* antigen coupled to horseradish peroxidase. After incubation, the Reaction Window is examined visually for the appearance of vertical blue lines on the "C" and "T" sides of the *Reaction Window*. A blue line on the "T" side of the Reaction Window indicates a positive result. A positive "C" reaction, indicated by a vertical blue line on the "C" side of the Reaction Window, confirms that the sample and reagents were added correctly, the reagents were active at the time of performing the assay, and that the sample migrated properly through the Membrane Device. It also confirms the reactivity of the other reagents associated with the assay.

J. Substantial Equivalence Information:

1. Predicate device name(s):

ImmunoCard STAT! HpSA

2. Predicate 510(k) number(s):

K032222

3. Comparison with predicate:

Similarities		
Item	Device: <i>H. PYLORI QUIK CHEK</i> (K181379)	Predicate: ImmunoCard STAT! HpSA (K032222)
Product Code	LYR	LYR
Intended Use	The TECHLAB <i>H. PYLORI QUIK CHEK</i>™ test is a rapid membrane enzyme immunoassay for the qualitative detection of <i>Helicobacter pylori</i> specific antigen in a single use cassette. It is intended for use with human fecal specimens to aid in the diagnosis of <i>H. pylori</i> infection and to demonstrate loss of <i>H. pylori</i> antigen following treatment. The test can be used with unpreserved fecal specimens and fecal specimens preserved in transport media from patients suspected of <i>H. pylori</i> infection. Testing of patients to demonstrate loss of <i>H. pylori</i> antigen following treatment should be performed no sooner than 4 weeks after completion of the treatment regimen. Test results should be taken into consideration by the physician in conjunction with the patient history and symptoms.	ImmunoCard STAT! HpSA is a rapid in vitro qualitative assay for the detection of <i>Helicobacter pylori</i> antigen in human stool. The stool antigen detection is intended to aid in the diagnosis of <i>H. pylori</i> infection and to demonstrate loss of <i>H. pylori</i> stool antigen following treatment. Conventional medical practice recommends that testing by any method to confirm the loss of antigen be done at least four weeks following completion of therapy.
Measured analyte	Detection of <i>H. pylori</i> stool antigen	Same

Similarities		
Item	Device: <i>H. PYLORI QUIK CHEK</i> (K181379)	Predicate: ImmunoCard STAT! HpSA (K032222)
Target Population	Persons suspected of having <i>H. pylori</i> infection	Same
Type of Test	Qualitative	Same
Controls	Positive and negative control included in kit Internal Control line	Same
Storage	Refrigerated (2°C – 8°C)	Same
Reading Method	Manual/Visual	Same

Differences		
Item	Device: <i>H. PYLORI QUIK CHEK</i> (K181379)	Predicate: ImmunoCard STAT! HpSA (K032222)
Specimen Type	Fecal Specimens in Cary-Blair and C&S Transport Media	Unpreserved Fecal Specimen
Time to Result	30 minutes	5 minutes
Technology	Enzyme Linked Immunoassay (ELISA)	Immunochromatographic (ICT)
Antibody Format	Polyclonal/Polyclonal	Monoclonal/Monoclonal

K. Standard/Guidance Document Referenced (if applicable):

CLSI EP07-A2 Interference Testing In Clinical Chemistry; Approved Guideline – Second Edition

CLSI EP15-A3 User Verification of Precision And Estimation Of Bias; Approved Guideline - Third Edition

CLSI EP17-A2 Evaluation Of Detection Capability For Clinical Laboratory Measurement Procedures; Approved Guideline - Second Edition

L. Test Principle:

Lateral flow immunochromatographic assay.

M. Performance Characteristics (if/when applicable):

1. Analytical performance:

a. *Precision/Reproducibility:*

The reproducibility of the *H. PYLORI QUIK CHEK* test was determined using an eight member masked fecal specimen panel. The panel consisted of 2 negative, 2 high negative (just below C₅), 2 low positive (just above LoD), and 2 moderate positive (3-4x higher than the C₉₅) specimens. Each fecal specimen was spiked using a known concentration of *H. pylori* purified flagellar antigen from whole organism to achieve the desired concentration. Testing was performed at 2 independent laboratories and on-site at TECHLAB, Inc. The Specimens were tested twice a day in triplicate over a five day period by multiple technicians at each site using two different kit lots. Positive and negative controls were run with each sample panel of masked specimens. The result from each laboratory were submitted to TECHLAB Inc. and compared. The results for the positive control, negative control, true negative, low positive, and moderate positive, was consistent among all three locations with 100% reproducibility for all test panels. The high negative provided a positive result 63 out of 180 observations.

b. *Linearity/assay reportable range:*

Not applicable.

c. *Traceability, Stability, Expected values (controls, calibrators, or methods):*

Sample stability study:

The effect of specimen storage on antigen stability was evaluated for both fresh stool samples and stool samples in transport media. The following transport media were used for the study: Thermo Scientific *Protocol* Cary Blair media and Thermo Scientific *Protocol* C&S media. For the analysis, a total of 32 fecal specimens were tested with the *H. PYLORI QUIK CHEK* test. The samples were prepared using a negative fecal matrix and spiked with *H. pylori* antigen (ATCC strain 43526). The panel consisted of 2 negative, 5 high negative (C₅), 10 low positive (1-2x C₉₅), and 15 positive specimens covering the range of the test (For fresh and preserved specimens this range was 50 ng/mL – 1200 ng/mL).

Fresh samples were stored at refrigerated temperatures (between 2°C and 8°C) and room temperatures (between 20°C and 25°C) and were tested at 0, 24, 48, 72 and 96 hours. Positive and negative controls were also tested at each timepoint.

Fecal specimens added to Cary Blair and C&S media were transported as recommended in their respective package inserts; samples were stored at refrigerated temperatures (between 2°C and 8°C) and room temperatures (between 20°C and 25°C) and were tested at 24 hour intervals from 0 to 96 hours.

For fresh samples stored at refrigerated and room temperatures, the positive and negative samples gave the expected results 100% of the time. Storage in Cary Blair and C&S transport media did not affect the stability of the samples. Based on these results, the recommended storage time for fresh, Cary Blair, and C&S stored samples is up to 96 hours when stored between 2°C and 8°C and between 20°C and 25°C.

Frozen sample stability study:

Stability of frozen fecal matrix samples compared to fresh samples was established using the 32 masked fecal specimen panel prepared as described for the storage stability study. Samples were not diluted into transport media for this study. Samples were stored at $\leq -10^{\circ}\text{C}$ or $\leq -70^{\circ}\text{C}$ for 14 days. Specimens were tested at 0, 5, 10, and 14 days. The results showed that all positive samples remained positive and negative samples remained negative throughout the study.

Freeze/Thaw study:

A study was conducted to determine stability after 3 freeze/thaw cycles using the 32-masked fecal specimen panel described for the storage stability study. Samples were not diluted into transport media for this study. The results showed that up to 3 freeze/thaw cycles neither enhanced nor impaired the performance of the *H. PYLORI QUIK CHEK* test. Therefore, the package insert will indicate that if samples are not tested fresh, frozen stool samples may be tested and may undergo up to no more than 2 freeze/thaw cycles.

d. Detection limit:

The limit of detection (LoD) for the *H. PYLORI QUIK CHEK* test was determined by spiking purified flagellar antigen into unpreserved (raw stool) and preserved (Cary Blair and C&S media) stool samples. The concentrations are reported in ng/mL and by factoring in the dilutions and the final volume used in the assay. The LoD is the concentrations of the antigen that yields a positive result 95% of the time and a negative result 5% of the time. Table 1 lists the LoD values for antigen spiked in negative fecal matrix, Cary Blair, and C&S transport media.

Table 1. LoD Values for *H. PYLORI QUIK CHEK*

ng/mL	ng/test
Fecal Matrix	
16.1	0.24
Cary Blair	
13.0	0.20
C & S	
20.0	0.31

e. *Analytical specificity:*

The *H. PYLORI* QUIK CHEK test was evaluated for cross-reactivity with the bacteria, fungi, and viruses listed below. *H. pylori* purified flagellar antigen (ATCC strain 43526) was spiked in at 2-3 x LoD. *H. pylori* was spiked into clinical matrix that was negative for *H. pylori*. Bacteria was spiked at concentration of $>10^8$ CFU/mL and viruses at a range from $10^{3.3}$ to $10^{8.25}$ TCID₅₀ units per 0.2 mL. Due to safety concerns for *Listeria monocytogenes*, freeze-dried biomaterial from ATCC was reconstituted at $>1 \times 10^4$ CFU/mL in PBS and spiked with *H. pylori* purified flagellar antigen (ATCC strain 43526) at 2-3X LoD.

<i>Acinetobacter baumannii</i>	<i>Bacillus subtilis</i>
<i>Bacillus cereus</i>	<i>Borrelia burgdorferi</i>
<i>Campylobacter coli</i>	<i>Campylobacter fetus</i>
<i>Campylobacter concisus</i>	<i>Campylobacter fetus</i>
<i>Campylobacter hyointestinalis</i>	<i>Campylobacter helveticus</i>
<i>Campylobacter jejuni</i>	<i>Campylobacter lari</i>
<i>Campylobacter upsaliensis</i>	<i>Candida albicans</i>
<i>Clostridium bifermentans</i>	<i>Clostridium difficile</i>
<i>Clostridium perfringens</i>	<i>Edwardsiella tarda</i>
<i>Enterobacter cloacae</i>	<i>Enterococcus faecalis</i>
<i>Escherichia coli</i>	<i>Escherichia coli</i> EIEC
<i>Escherichia coli</i> EPEC	<i>Escherichia coli</i> ETEC
<i>Escherichia coli</i> O157:H7 (non-toxigenic)	<i>Escherichia coli</i> O157:H7 (toxigenic)
<i>Haemophilus influenzae</i>	<i>Lactobacillus acidophilus</i>
<i>Listeria monocytogenes</i>	<i>Peptostreptococcus anaerobius</i>
<i>Porphyromonas asaccharolytica</i>	<i>Pseudomonas aeruginosa</i>
<i>Pseudomonas fluorescens</i>	<i>Salmonella typhimurium</i>
<i>Staphylococcus aureus</i>	<i>Staphylococcus aureus</i> (Cowan's)
<i>Streptococcus agalactiae</i>	<i>Yersinia enterocolitica</i>
<i>Adenovirus</i> , 2, 40,	<i>Coronavirus</i>
<i>Coxsackievirus</i> B1, B2, B3, B6	<i>Echovirus</i> 9, 22, 70
<i>Human Rotavirus</i> *	
* tested at $10^{1.75}$	

The *H. PYLORI* QUIK CHEK test was evaluated for interfering substances with the substances and concentrations listed in Table 2. None of the substances were shown to interfere with the performance of the *H. PYLORI* QUIK CHEK test.

Table 2. Interfering Substances

Barium sulfate (5% w/v)	Mylanta (4.2 mg/mL)
Benzalkonium Chloride (1% w/v)	Naproxen Sodium (5% w/v)
Ciprofloxacin (0.25% w/v)	Nonoxynol-9 (1% w/v)
Ethanol (1% w/v)	Nystatin (1% w/v)
Hog gastric mucin (3.5% w/v)	Palmitic acid (fecal fat) (40% w/v)
Human blood (40% w/v)	Pepto-Bismol (5% v/v)
Hydrocortisone (1% w/v)	Phenylephrine (1% w/v)
Imodium (5% v/v)	Prilosec OTC (5 ug/mL)
Kaopectate (5%v/v)	Sennosides (1% w/v)
Leukocytes (0.05% v/v)	Simethicone (10% w/v)
Maalox Advanced (5% v/v)	Steric acid (fecal fat) (40% w/v)
Mesalazine (10% w/v)	Tagamet (5 ug/mL)
Metronidazole (0.25% w/v)	TUMS (50 ug/mL)
MiraLAX (7% w/v)	Human Urine (5% v/v)
Mineral Oil (10% w/v)	Vancomycin (0.25% w/v)

Inclusivity study

The reactivity of six *H. pylori* strains spanning the 3 major clades (hpEastAsia, hpEurope, and hpAfrica1) was evaluated. Samples were prepared by spiking negative fecal matrix with purified flagellar antigen from each *H. pylori* strain at 2-3x LoD. All results were read visually/manually and were positive, demonstrating that the *H. PYLORI QUIK CHEK* test can detect antigen from strains representing the major *H. pylori* clades.

***H. pylori* strains**

ATCC 700392 (hpEurope)
 ATCC 43526 (hpEurope)
 ATCC 700824 (hpAfrica1)
 ATCC 43504 (clade unknown)
 ATCC 43579 (clade unknown)
 JP26 (hpEastAsia)

f. Assay cut-off:

Not applicable.

g. Prozone/Hook Effect:

The purpose of this study was to demonstrate that a high concentration of *H. pylori* (antigen) does not interfere with a positive reaction in the *H. PYLORI QUIK CHEK* test. High concentration samples were prepared by spiking negative fecal matrix with *H. pylori* antigen at 12 ug/mL (i.e. 10x the highest concentration observed in a positive clinical sample). A total of 5 different dilutions were prepared from the contrived sample. Samples were prepared by spiking the following concentrations of

antigen into a negative fecal pool: 12 ug/mL, 6 ug/mL, 2.4 ug/mL, 1.2 ug/mL, 0.6 ug/mL and 0.12 ug/mL. Testing was performed in triplicate according to the Package Insert instructions. The results demonstrated that elevated levels of antigen did not affect the test results.

2. Comparison studies:

a. *Method comparison with predicate device:*

Not applicable.

b. *Matrix comparison:*

Not applicable.

3. Clinical studies:

a. *Clinical Sensitivity:*

Prospective study

Initial Diagnosis

The performance of the *H. PYLORI QUIK CHEK* test was evaluated by conducting a multi-site prospective clinical study. Six independent sites were included, five of which collected specimens from patients suspected of *H. pylori* infection. The sixth site, TECHLAB, did not conduct specimen collection. Of these five collection sites, three conducted testing using both the *H. PYLORI QUIK CHEK* test and the composite reference method (CRM). The remaining two sites performed CRM testing and sent samples to TECHLAB for testing using the *H. PYLORI QUIK CHEK* test. The CRM for diagnosis of *H. pylori* infection is based on endoscopy obtained gastric biopsy. The biopsied tissue was tested for the presence of *H. pylori* by histology, or rapid urease test. The sensitivity and specificity for the *H. PYLORI QUIK CHEK* test was determined using the CRM.

Prospective testing consisted of 205 stool specimens collected from the patients with symptoms of dyspepsia, gastritis, or peptic ulcer who were scheduled to undergo endoscopy with gastric biopsy as part of routine care (Initial Diagnosis Group). Of these, 83 patients were excluded either because they were on a treatment regimen [i.e., proton-pump inhibitors (PPIs), or antibiotics] or had samples with CRM results that were rapid urease positive but histology negative. The remaining 122 patients who were not taking PPIs or antibiotics at the time of specimen collection were considered for final analysis. These specimens were tested at the following five sites: Carilion Clinic, International Centre for Diarrhoeal Disease Research Bangladesh, Mayo Clinic, University of Virginia, Kliniken Essen-Mitte, and TECHLAB (internal).

The ages of patients ranged from less than 19 years to 82 years with 100% of the specimens coming from patients were ≥ 18 years. Of the 122 patients tested 64% were female and 34% were male. No difference in test performance was observed based on patient age or gender. The results are provided in **Table 3** which shows the clinical performance of the *H. PYLORI QUIK CHEK* test for all 6 test sites combined. The results of the study show that the *H. PYLORI QUIK CHEK* test exhibited a sensitivity of 97.0%, and a specificity of 100% compared to the CRM.

Table 3. Clinical Performance of the *H. PYLORI QUIK CHEK*

Initial diagnosis	CRM Positive	CRM Negative
<i>H. PYLORI QUIK CHEK</i> Positive	32	0
<i>H. PYLORI QUIK CHEK</i> Negative	1*	89

Sensitivity (95% C.I.)	97.0% (84.7% - 99.5%)
Specificity (95% C.I.)	100%(95.9% - 100%)

*Additional testing with an FDA cleared *H. pylori* stool antigen test provided an antigen negative result.

Post Therapy Diagnosis

Eradication (post-therapy) evaluation was conducted on patients enrolled prospectively at 3 sites. A total of 9 specimens were collected at least 4 weeks after completion of the treatment regimen. Post therapy evaluation was conducted using a two-step algorithm of patient analysis. First, patients were screened for the continued presence of *H. pylori* using an FDA-cleared stool antigen test. Positive patients were reflexed to a follow-up endoscopy and biopsy analysis by rapid urease test and histology. The results are provided in **Table 4** which shows the clinical performance of the *H. PYLORI QUIK CHEK* test for all 6 test sites combined. The results of the study show that the *H. PYLORI QUIK CHEK* test exhibited a sensitivity of 100.0% compared to the CRM.

Table 4. Clinical Performance of the *H. PYLORI QUIK CHEK*

Post treatment diagnosis	CRM Positive	CRM Negative
<i>H. PYLORI QUIK CHEK</i> Positive	9	0
<i>H. PYLORI QUIK CHEK</i> Negative	0	0

Sensitivity (95% C.I.)	100% (70.1% - 100%)
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The ages of patients ranged from 33 years to 72 Years. Of the 9 patients tested, 6

were female and 3 were male.

Retrospective study

A retrospective study was conducted to evaluate performance and to augment the prospective clinical study. Testing of retrospective samples was conducted at TECHLAB.

To provide assurance that the retrospective samples are representative of wide range of OD readings, CRM positives samples and retrospective positive samples were both tested by an FDA cleared ELISA. The distribution and mean OD values obtained from the retrospective study, n = 200 samples, were compared to those values obtained from CRM positive samples, n = 46, to ensure that the use of retrospective sample results reflects the OD distribution of CRM positive samples. This analysis demonstrated that the use of retrospective and prospective clinical samples has similar distributions and no concern of bias was noted.

The performance of the *H. PYLORI QUIK CHEK* test was evaluated by testing retrospective samples at TECHLAB. Retrospective testing consisted of 200 frozen stool specimens (94 positive and 106 negative by an FDA Cleared ELISA) obtained from sample repositories. Positive percent agreement (PPA) and negative percent agreement (NPA) for the *H. PYLORI QUIK CHEK* test was determined by comparing to an FDA Cleared ELISA that was tested concurrently. The results of the study show that the *H. PYLORI QUIK CHEK* test exhibited a PPA of 98.9% and a NPA of 97.2% with an FDA Cleared ELISA. The results are shown in Table 5.

Table 5. Clinical performance of the *H. PYLORI QUIK CHEK* test on retrospective specimens.

N = 200	FDA Cleared ELISA (Positive)	FDA Cleared ELISA (Negative)
<i>H. PYLORI QUIK CHEK</i> Positive	93	3**
<i>H. PYLORI QUIK CHEK</i> Negative	1*	103
Performance		95% CI
Positive Percent Agreement	98.9%	94.2%-99.8%
Negative Percent Agreement	97.2%	92.0%-99.0%

* *H. pylori* DNA was amplified from the samples with PCR

**No *H. pylori* DNA was amplified from the sample with PCR

b. Clinical specificity:

See section M3a. above.

c. Other clinical supportive data (when a. and b. are not applicable):

See section M3a. above.

4. Clinical cut-off:

Not applicable.

5. Expected values/Reference range:

Not applicable.

N. Proposed Labeling:

The labeling supports the finding of substantial equivalence for this device.

O. Conclusion:

The submitted information in this premarket notification is complete and supports a substantial equivalence decision.