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

A. 510(k) Number:

K181400

B. Purpose for Submission:

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

C. Measurand:

Helicobacter pylori antigen

D. Type of Test:

Qualitative enzyme immunoassay (EIA)

E. Applicant:

TECHLAB, Inc.

F. Proprietary and Established Names:

H. PYLORI 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:

   83-Microbiology

H. Intended Use:

1. Intended use(s):

   The TECHLAB *H. PYLORI CHEK* test is an enzyme immunoassay for the qualitative detection of *Helicobacter pylori* specific antigen. 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:

   A spectrophotometric plate reader capable of reading the following wavelengths: 450 nm or 450/620 nm.

I. Device Description:

The *H. PYLORI CHEK* test uses antibodies specific to *H. pylori* infection. The "Microassay Plate" in the kit contains immobilized capture antibodies against *H. pylori* antigen. The "Conjugate" consists of antibodies specific to *H. pylori* antigen conjugated to horseradish peroxidase (HRP). In the assay, an aliquot of diluted fecal specimen is transferred to a microassay well containing the Conjugate. If the antigen is present in the specimen, it will bind to the Conjugate and to the immobilized capture antibody during the incubation phase. Any unbound material is removed during the washing steps. Following the addition of the "Substrate", a color is detected due to the enzyme-antibody-antigen complexes that formed in the presence of antigen. The color if positive is a yellow color that may be read visually or by using a spectrophotometer at the following wavelengths: 450 nm or 450/620 nm.

J. Substantial Equivalence Information:

1. Predicate device name(s):

   Premier Platinum HpSA PLUS

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

   K053335

3. Comparison with predicate:

Similarities		
Item	**Device: *H. PYLORI CHEK* (K181400)**	**Predicate: Premier Platinum HpSA PLUS (K053335)**
Product Code	LYR	LYR
Intended Use	The TECHLAB *H. PYLORI CHEK* test is an enzyme immunoassay for the qualitative detection of *Helicobacter pylori* specific antigen. 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.	Premier Platinum HpSA PLUS enzyme immunoassay (EIA) is an in vitro qualitative procedure for the detection of *Helicobacter pylori* antigens in human stool. Test results are intended to aid in the diagnosis of *H. pylori* infection and to monitor response during and post-therapy in patients. Accepted medical practice recommends that testing by any current method, to confirm eradication, be done at least four weeks following completion of therapy.
Measured analyte	Detection of *H. pylori* antigen	Same
Type of Test	Qualitative	Same
Controls	Positive and negative control included in the kit	Same
Target Population	Persons suspected of having *H. pylori* infection	Same
Storage	Refrigerated (2ºC-8ºC)	Same
Reading Method	Spectrophotometric or Visual	Same

Differences		
Item	***H. PYLORI CHEK* (K181400)**	**Predicate: Premier Platinum HpSA PLUS (K053335)**
Specimen Type	Fresh or frozen formed, semi-solid, or liquid fecal specimens. Fecal specimens in Cary-Blair and C&S Transport Media	Fresh or frozen formed, semi-solid, or liquid fecal fecal specimens
Specimen Storage	Specimens may be held up to 96 hours at 2ºC-8ºC or at 20ºC-25ºC prior to testing	Specimens may be held up to 72 hours at 2ºC-8ºC prior to testing
Assay Incubation Temperature	37ºC $\pm$ 2ºC	19ºC-27ºC
Time to Result	Approximately 1 hour	Approximately 1 hour
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, May 5th, 2007.

CLSI EP15-A3. User Verification of Precision and Estimation of Bias; Approved Guideline – Third Edition. August 14th, 2015.

CLSI EP17-A2. Evaluation of Detection Capability for Clinical Laboratory Measurement Procedures; Approved Guideline – Second Edition. January 15th, 2013.

CLSI EP25-A. Evaluation of Stability of In Vitro Diagnostic Reagents; Approved Guideline. January 15th, 2013.

L. Test Principle:

Enzyme-linked immunosorbent assay

M. Performance Characteristics (if/when applicable):

1. Analytical performance:

   a. Precision/Reproducibility:

   The precision of the *H. PYLORI CHEK* test was determined using an 8-member masked fecal specimen panel. The panel consisted of 2 negative, 1 high negative (2 times below the C_5), 1 high negative (C_5), 1 low positive (C_{95}), 1 low positive (2x

higher than the C_{95}), 1 moderate positive (3x higher than the C_{95}), and 1 moderate positive (4x higher than the C_{95}). Each fecal specimen was spiked with *H. pylori* antigen (ATCC strain 43526) to achieve the desired level. The specimens were tested twice per day over a 12-day period by multiple technicians using two different kit lots. By spectrophotometric analysis using dual OD (450/620 nm wavelenght), positive specimens tested positive 98.3% of the time and negative specimens tested negative 97.8% of the time. By visual read, positive specimens tested positive 93.1% of the time and negative specimens tested negative 94.8% of the time.

The reproducibility of the *H. PYLORI CHEK* test was determined using the same 8-member fecal specimen panel as described for the precision study. Testing was performed at 2 independent laboratories and on-site at TECHLAB, Inc. The panel members 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 results from each laboratory were submitted to TECHLAB, Inc. and compared with in-house results. The results were consistent among all three locations and produced the expected results (by dual OD) for the positive and negative samples 97.5% and 97.5% of time, respectively. By visual read, positive specimens tested positive 96.9% of the time and negative specimens tested negative 92.8% of the time

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 amples 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 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 (C_5), 10 low positive (1-2x C_{95}), and 15 positive specimens covering the range of the test (For fresh, unpreserved specimens, this range was 50 ng/mL – 1200 ng/mL).

Fresh samples were stored at refrigerated temperatures (between 2ºC and 8ºC) and room temperatures (between 20ºC and 25ºC) 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 2ºC and 8ºC) and room temperatures (between 20ºC and 25ºC) 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 96.8% and 99.1% of the time, respectively. Storage in Cary Blair transport media did not affect the stability of the samples. Storage in C&S transport media did not affect the stability of samples stored at room temperature, but a slight decrease in sample stability was observed at refrigerated temperatures. Specifically, 93.8% of samples yielded the expected results when stored in C&S transport media at refrigerated temperatures. 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 2ºC and 8ºC and between 20ºC and 25ºC.

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 $\leq$ -10ºC or $\leq$ -70ºC 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 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 CHEK* test was determined by spiking purified flagellar antigen from *H. pylori* (ATCC strain 43526) into negative fecal matrix and Cary Blair and C&S transport media. The LoD is defined as the concentration of *H. pylori* antigen that yields a positive result 95% of the time and a negative result 5% of the time. The LoD was determined as the concentration that provided the correct result 95% of the time based on testing dilutions of antigen in a negative fecal sample matrix, in replicates of 24 for two sets. LoD testing was read at both single (450 nm) and dual (450/620 nm) wavelength reads. The LoD values were similar between single wavelength reads and dual wavelength reads, however single wavelength reads trended slightly higher for some, but not all, sample types. **Table 1** lists the LoD values based on a dual wavelength reading for antigen spiked in negative fecal matrix, Cary Blair, and C&S transport media.

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

ng/mL	ng/test
Fecal Matrix	
6.70	0.13
Cary Blair	
26.57	0.33
C & S	
18.19	0.23

e. Analytical specificity:

Cross-reactivity

The *H. PYLORI 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 and fungi were 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 $>1x10^4$ CFU/mL in PBS and spiked with purified *H. pylori* flagellar antigen (ATCC strain 43526) at 2-3X LoD. No bacteria, fungi, or viruses tested showed interference with the performance of the *H. PYLORI CHEK* test.

Bacteria/Fungi

Acinetobacter baumannii
Bacillus cereus
Borrelia burgdoferi
Campylobacter coli
Campylobacter fetus
Campylobacter helveticus
Campylobacter hyointestinalis
Campylobacter jejuni
Campylobacter lari
Campylobacter upsaliensis
Candida albicans
Clostridium bifermentans
Clostridium perfringens
Edwardsiella tarda
Enterobacter cloacae
Enterococcus faecalis
Escherichia coli
Escherichia coli EIEC
Escherichia coli EPEC
Escherichia coli ETEC
Escherichia coli O157:H7 (non-toxigenic)
Escherichia coli O157:H7 (toxigenic)
Haemophilus influenzae
Lactobacillus acidophilus
Listeria Monocytogenes
Peptostreptococcus anaerobius
Porphyromonas asaccharolytica
Prevotella melaninogenica
Proteus vulgaris
Pseudomonas aeruginosa
Pseudomonas fluorescens
Salmonella typhimurium
Staphylococcus aureus
Staphylococcus aureus Cowan's
Streptococcus agalactiae
Yersinia enterocolitica

Viruses

Adenovirus Type 2
Adenovirus Type 40
Human Coxsackievirus B6
Echovirus 9

Coronavirus, Human	*Echovirus 22*
Coxsackievirus B2	*Echovirus 60*
Coxsackievirus B3	
Human Coxsackievirus B1	*Human rotavirus**

*tested at $10^{1.75}$

Interfering substances
The *H. PYLORI 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 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 strain at 2-3x LoD. All results were read at dual-wavelength and were positive, demonstrating that the *H. PYLORI 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:

The following cut off-values and result interpretation criteria were established for the spectrophotometric single and dual wavelength reading methods:

Spectrophotometric Single Wavelength (450 nm)
Negative: <0.120
Positive: ≥0.120
Negative Control: <0.120
Positive Control: ≥0.500

Spectrophotometric Dual Wavelength (450/620 nm)
Negative: <0.080
Positive: ≥0.080
Negative Control: <0.080
Positive Control: ≥0.500

The following result interpretation criteria were established for the visual reading method:

Visual Reading
Negative = Colorless to very faint yellow color
Positive = Definitive yellow color

A positive result indicates that *H. pylori* antigens were detected. A negative result indicates that no *H. pylori* antigens were detected, or that the antigen levels are below what can be detected by the assay.

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 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 there was no overall hook effect, and that elevated levels of *H. pylori* 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 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 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 CHEK* test. The CRM for diagnosis of *H. pylori* infection is based on endoscopally 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 CHEK* test was determined using the CRM.

Prospective testing consisted of 176 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, 67 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 109 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). Reading of the *H. PYLORI CHEK* was conducted by dual wavelength spectrophotometric analysis and by visual reading of the "Microassay Plate" for color change.

The ages of patients ranged from 19 to 82 years with 100% of the specimens coming from patients that were ≥18 years. Of the 109 patients tested, 66% 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 CHEK* test for all 6 test sites combined. The results of the study show that the *H. PYLORI CHEK* test, by dual spectrophotometric analysis, exhibited a sensitivity of 100%, and a specificity of 96.1% compared to the CRM. Testing was also conducted by visual reading of "Microassay Plates". Visual results were the same as dual wavelength spectrophotometric results 99% of the time.

Table 3. Clinical performance of the *H. PYLORI CHEK* by dual wavelength spectrophotometric analysis.

Initial Diagnosis	CRM Positive	CRM Negative
H. PYLORI CHEK **Positive**	32	3*
H. PYLORI CHEK **Negative**	0	74
Performance		**95% CI**
Sensitivity	100%	89.3% - 98.9%
Specificity	96.1%	89.2% - 98.7%

* All three specimens tested positive initially by the *H. PYLORI CHEK* test, but negative upon re-testing with the *H. PYLORI CHEK* test .

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 ELISA . 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 CHEK* test for all 6 test sites combined. The results of the study show that the *H. PYLORI CHEK* test exhibited a sensitivity of 77.8% compared to the CRM.

Table 4. Clinical performance of the *H. PYLORI CHEK*

Post-Therapy Diagnosis	CRM Positive	CRM Negative
H. PYLORI CHEK **Positive**	7*	0
H. PYLORI CHEK **Negative**	2**	0
Performance		**95% CI**
Sensitivity	77.8%	45.3% - 93.7%

*One specimen tested positive by visual read but negative by spectrophotometric interpretation by the *H. PYLORI CHEK* test ($OD_{450/620}$ 0.034).

**One specimen tested negative initially by the *H. PYLORI CHEK* test, but positive upon re-testing with the *H. PYLORI CHEK* test.

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 a wide range of OD readings, CRM positive 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= 75 samples, were compared to those values obtained from CRM positive samples, n=42, 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 have similar distributions and no concern of bias was noted.

Thee performance of the *H. PYLORI CHEK* test was evaluated by testing retrospective samples at TECHLAB. Retrospective testing consisted of 196 frozen stool samples (75 positive and 121 negative by an FDA-cleared ELISA) obtained from sample repositories. Positive percent agreement (PPA) and negative percent agreement (NPA) for the *H. PYLORI 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 CHEK* test exhibited a PPA of 100% and a NPA of 100% with an FDA cleared ELISA. The results are shown in **Table 5**.

Table 5. Clinical perfromance of the *H. PYLORI CHEK* test on retrospective samples.

N = 196	**FDA Cleared ELISA (Positive)**	**FDA Cleared ELISA (Negative)**
H. PYLORI CHEK **Positive**	75	0
H. PYLORI CHEK **Negative**	0	121
Performance		**95% CI**
Positive Percent	100%	95.1%-100%

Agreement		
Negative Percent Agreement	100%	96.9%-100%

b. Clinical specificity:

See section M3a. above.

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

Not Applicable

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 substantial equivalence decision.