

# **H. PYLORI CHEK™**

An Enzyme Immunoassay for the Qualitative Detection of  
*Helicobacter pylori* Specific Antigen in Human Fecal Specimens

Catalog No. 30996 (96 Tests)  
 *In Vitro* Diagnostic Medical Device

**Made in the USA**

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**Developed and Manufactured by:**



**TECHLAB, Inc.**  
**2001 Kraft Drive**  
**Blacksburg, VA 24060-6358, USA**  
**[www.techlab.com](http://www.techlab.com)**

**Distributed by:**



**Aleré North America, LLC**  
**30 South Keller Road**  
**Orlando, Florida 32810**  
**TEL 1-877-441-7440**  
**1-321-441-7200 OUTSIDE USA**

## H. PYLORI CHEK™

### 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.

**Caution: U.S. Federal Law restricts this device to sale by or on the order of a physician.**

### EXPLANATION

It is estimated that half of the global population is infected with *H. pylori*.<sup>1</sup> The majority of those infected remain asymptomatic and do not require treatment (colonized individuals). A minority of infected individuals develop gastritis, and a fraction of those further develop gastric ulcers or gastric cancer.<sup>2</sup> The diagnosis of *H. pylori* infection is endoscopy with biopsy – the biopsied tissue is tested for the presence of *H. pylori* by culture, histology, or rapid urease test. Under current guidelines, endoscopy is still recommended for the diagnosis of *H. pylori* infection in patients with alarm symptoms (e.g. GI bleeding, sudden weight loss, excessive vomiting, anemia), or patients over the age of 55. However, for younger patients not exhibiting alarm symptoms, non-invasive tests such as the urea breath test (UBT) or fecal antigen test are recommended for diagnosis of *H. pylori* infection.<sup>3,4</sup> Following completion of a treatment regimen of antibiotics and a proton pump inhibitor (PPI), it is recommended that patients be tested to verify eradication of *H. pylori* infection.<sup>5</sup> Serum antibody tests are also available, but these are unable to distinguish between past and current infection. By detecting antigen present in fecal specimens, the *H. PYLORI CHEK*™ test allows for the non-invasive detection of *H. pylori* when endoscopy is not required.

### PRINCIPLE OF THE TEST

The *H. PYLORI CHEK*™ test uses antibodies specific to *H. pylori* antigen. 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. In the assay, an aliquot of a 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 *Substrate*, a color is detected due to the enzyme-antibody-antigen complexes that formed in the presence of antigen.

### MATERIALS PROVIDED

**MA | PLT** *Microassay Plate* – 12 strips, each consisting of 8 wells coated with antibodies to *H. pylori* antigen (stored with desiccant)

**CONJ | ENZ** *Conjugate (7 mL)* – Antibodies to *H. pylori* antigen coupled to horseradish peroxidase in a buffered protein solution containing 0.05% ProClin® 300  
Signal Word: Warning – 0.05% ProClin® 300  
H317: May cause an allergic skin reaction  
P261, P272, P280, P302, P352, P333, P313, P321, P362, P364, P501

**DIL | SPE** *Diluent (40 mL)* – Buffered protein solution. The *Diluent* is also to be used as the negative control solution (see TEST PROCEDURE) containing 0.05% ProClin® 300.



Signal Word: Warning – 0.05% ProClin® 300  
 H317: May cause an allergic skin reaction  
 P261, P272, P280, P302, P352, P333, P313, P321, P362,  
 P364, P501



**CONTROL +**

**Positive Control (3.5 mL)** - *H. pylori* antigen in a buffered protein solution containing 0.05% ProClin® 300

Signal Word: Warning – 0.05% ProClin® 300  
 H317: May cause an allergic skin reaction  
 P261, P272, P280, P302, P352, P333, P313, P321, P362, P364, P501



**H<sub>2</sub>SO<sub>4</sub>, 0.6N**

**Stop Solution (7 mL)** – 0.6 N sulfuric acid. CAUTION: Avoid contact with skin or eyes; flush with water immediately if contact occurs

Signal Word: Danger  
 H314: Causes severe skin burns and eye damage  
 P260, P264, P280, P301, P330, P331, P303, P361, P353,  
 P363, P304, P340, P310, P321, P305, P351, P338, P501



**SUBS REAG**

**Substrate (14 mL)** – solution containing tetramethylbenzidine and peroxide

**WASHBUF 20X**

**Wash Buffer Concentrate (50 mL)** – 20X concentrate containing phosphate buffered saline, detergent, and 0.2% thimerosal

Signal Word: Warning  
 H373: May cause damage to organs through prolonged or repeated exposure P260, P314, P501



contains mercury



## ACCESSORIES

100 Disposable plastic transfer pipettes  
 1 Wash Solution Label

2 Plastic adhesive sheets  
 50 Wooden Applicator sticks

## MATERIALS AND EQUIPMENT REQUIRED BUT NOT PROVIDED

Squirt bottle for wash reagent

Vortex mixer

Timer

Discard container

Absorbent paper

Disposable gloves

950 mL distilled water for diluting wash reagent

ELISA plate reader capable of reading at 450 nm, 450/620 nm, or 450/630 nm

Small tubes for dilution of fecal specimens (e.g., plastic 2 mL conical microcentrifuge tubes)

## SHELF LIFE AND STORAGE

The expiration date of this kit is given on the box label. Expiration dates for each component are listed on the individual labels. The kit should be stored between 2°C and 8°C and should be returned to the refrigerator as soon as possible after use.

## PRECAUTIONS

1. Rx Only – Prescription Only
2. Each component in the kit should be inspected for any signs of leakage. Upon arrival, the kit should be inspected to ensure that components are not frozen or warm to the touch due to improper shipping conditions.
3. Reagents from different kits should not be mixed. Do not use a kit past the assigned expiration date.
4. Bring all components to room temperature before use to ensure proper kit reactivity. Remove the reagents from the foam insert to reduce the time needed to warm to room temperature.
5. Do not freeze the reagents. The kit should be stored between 2°C and 8°C.
6. Caps and tips are color-coded; do NOT mix or interchange!
7. When handling assay wells, avoid scratching the bottom of the wells because this may result in elevated absorbance readings.

8. Unused microwells must be placed back inside of the resealable pouch with the desiccant to protect them from moisture.
9. Hold reagent bottles vertically when dispensing to ensure proper drop size and correct volume.
10. Microbial contamination of reagents may decrease the accuracy of the assay. Avoid microbial contamination of reagents by using sterile disposable pipettes if removing aliquots from reagent bottles.
11. All reagents, with the exception of the *Wash Buffer Concentrate*, are supplied in ready-to-use bottles. Reagents can be dispensed directly from the dropper bottles or decanted for use with multichannel pipettes. If excess reagent has been decanted, the excess should be discarded. Do not pour back into the bottle. The *Substrate* should be stored in and used from the light-protected bottle in which it is supplied. If an aliquot is removed from the original bottle for any reason, do not return unused *Substrate* to the original bottle. The *Substrate* is light sensitive and should be protected from direct sunlight or UV sources.
12. Perform the washing procedure as directed to avoid high background reactions.
13. The test has been optimized for sensitivity and specificity. Do not deviate from the specified procedure. Alterations of the specified procedure and/or test conditions may affect the sensitivity and specificity of the test.
14. Fecal specimens may contain potentially infectious agents and should be handled at "Biosafety Level 2" as recommended in the CDC/NIH Manual, "Biosafety in Microbiological and Biomedical Laboratories."
15. Handle specimens and used microassay wells as if capable of transmitting infectious agents. Wear disposable gloves when doing the test.
16. Reagents contain 0.05% ProClin® 300 as a preservative. Although the concentration is low, ProClin® 300 is known to be harmful. If skin irritation or rash occurs, get medical advice/attention. Take off contaminated clothing and wash it before reuse. Handle reagents according to existing regulations for laboratory safety and good laboratory practice. Safety Data Sheets for this product are available upon request, contact technical support.
17. The 20X *Wash Buffer Concentrate* contains 0.2% Thimerosal as a preservative. Once diluted to normal use concentration this solution is classified as non-hazardous. The *Stop Solution* contains 0.6N sulfuric acid. Flush with water immediately if contact occurs. Take off contaminated clothing and wash it before reuse. Handle reagents according to existing regulations for laboratory safety and good laboratory practice. Safety Data Sheets for this product are available upon request, contact technical support.
18. Follow your national, regional, and local ordinances accordingly for waste disposal regulations. Do not place in trash, dispose of as hazardous waste.

#### PRELIMINARY PREPARATIONS

1. **All reagents must be at room temperature prior to use in the assay.**
2. **Prepare 1X Wash Solution.** The *Wash Buffer Concentrate* is supplied as a 20X concentrate (a precipitate may be noticed). It should be diluted to a total volume of 1 liter by adding 50 mL of the concentrate to 950 mL of distilled water. The 1X *Wash Solution* can be stored between 2° and 8°C for up to the expiration date of the kit.
3. **Assay Strip Preparation.** Each strip contains 8 wells coated with antibodies specific to *H. pylori* antigen. Each specimen or control will use one of these coated wells. Determine the number of wells to be used. Avoid contact with the base of the wells. Assay wells not used must be returned to the foil pouch and carefully resealed with desiccant.

## COLLECTION, HANDLING, AND STORAGE OF FECAL SPECIMENS

Acceptable Sample Type	Do Not Use
Fresh Fecal Specimens	Fecal Specimens in Formalin-based fixative (e.g., sodium acetate formalin, 10% formalin)
Frozen Fecal Specimens	Fecal Specimens in alcohol-based fixative (e.g., polyvinyl alcohol)
Specimens in Transport Media (Cary Blair, C&S)	Concentrated Fecal Specimens

Storage Condition	Recommended Storage Time
Fresh Unpreserved Samples and Samples in Cary Blair or C&S Transport Media Stored between 2°C and 8°C	96 hours
Fresh Unpreserved Samples and Samples in Cary Blair or C&S Transport Media Stored between 20°C and 25°C	96 hours
Frozen Unpreserved Samples Stored at $\leq -10^{\circ}\text{C}$	14 days

- Use standard in-house collection and handling procedures for fecal specimens. Collect fecal specimens in clean, leak-proof containers.
- Fecal specimens that are stored frozen may be thawed up to 2 times. If using frozen specimens, thaw at room temperature.
- Set up and label one test tube for each sample as necessary.
- Add 200  $\mu\text{L}$  Diluent to each tube for unpreserved specimens. For specimens in Cary Blair or C&S Transport media, add 100  $\mu\text{L}$  Diluent to each tube.**
- Obtain one disposable plastic transfer pipette (supplied with the kit) for each sample.

Transfer Pipette



- Mix all specimens thoroughly regardless of consistency - it is essential that the samples be evenly suspended before sampling.**
  - For **fresh or frozen/thawed specimens**, using the disposable plastic transfer pipette, add 50  $\mu\text{L}$  (first graduation mark) of fecal specimen to the tube containing *Diluent* and mix well. If the specimen cannot be pipetted, use an applicator stick to transfer approximately 0.05 g of feces. This is about the size of a small pea (about 3mm in diameter).
  - For specimens in **Cary Blair or C&S Transport media**, add 100  $\mu\text{L}$  (second graduation mark) of fecal specimen to the tube containing *Diluent* and mix well.
- Close each tube of diluted sample and mix thoroughly. Proper mixing can be achieved by vortexing or inverting the tube several times. Do not allow the sample to remain in the *Diluent* for  $>2$  hours.
- If using semi-automated or automated washing equipment, once diluted, specimens must be centrifuged (5000 x g for 10 minutes) to remove any particulate matter from the supernatant before transfer to assay wells.

## TEST PROCEDURE

- Bring all reagents and the required number of test strips to room temperature before use.

2. **Add 1 drop (50  $\mu$ L) of Conjugate (red cap) to each well.** Gently mix the *Conjugate* in the bottle by inverting several times. Be sure to hold each bottle vertically when adding the drops. Use 1 well for each fecal specimen, 1 well for the *Positive Control* and 1 well for the negative control. Identification marks may be written directly on side of well.
3. **Using a new transfer pipette, transfer 100  $\mu$ L of diluted specimen (or supernatant from the centrifuged diluted sample if using automated washing equipment) to the assay well.** Add 1 drop (50  $\mu$ L) of the *Positive Control* (black cap) to the positive control well and 100  $\mu$ L of the *Diluent* (negative control) to the negative control well. Tap the sides of the plate to mix.
4. Cut the adhesive plastic sheet to the size necessary to cover the wells. **Cover the wells and incubate them at 37°C  $\pm$  2°C for 50 minutes.**
5. Shake out contents of assay wells into a discard pan.
6. **Wash each well using the 1X Wash Solution in a squirt bottle with a fine-tipped nozzle,** directing the *Wash Solution* to the bottom of the well with force. Fill the wells, and then shake the *Wash Solution* out of the well into a discard pan. Slap the inverted plate on a dry paper towel.  
*Note: If using semi-automated or automated washing equipment, add 350  $\mu$ L of 1X Wash Solution to each well. Wash for a total of 5 times.*
7. Repeat step 6 four additional times using a dry paper towel each time. If any particulate matter is seen in the wells, continue washing until all the particulate matter is removed.
8. After washing, completely remove any residual liquid in the wells by striking the plate onto a dry paper towel until no liquid comes out. Dispose of paper towels and specimen containers properly.
9. **Add 2 drops (100  $\mu$ L) of Substrate (blue cap) to each well.** Gently tap the wells to mix the *Substrate*. Incubate the wells at room temperature for 10 minutes. Gently tap the wells at 5 minutes.
10. **Add 1 drop (50  $\mu$ L) of Stop Solution (yellow cap) to each well.** Gently tap the wells and wait 2 minutes before reading. The addition of the *Stop Solution* converts the blue color to a yellow color which may be quantitated by measuring the optical density at 450 nm on a microplate ELISA reader. The instrument should be blanked against air. If a dual wavelength reader is used, blank against air at 620 or 630 and read at 450 nm. Wipe the underside of each well before measuring the optical density. If an ELISA reader is unavailable, the test may be read visually in good light against a white background. Read within ten minutes after adding *Stop Solution*.

#### QUALITY CONTROL

1. Positive and negative controls must be run with each series of test specimens. The positive control demonstrates that the assay is functioning properly for the detection of *H. pylori* antigen in fecal specimens. The negative control demonstrates that the assay is not reacting nonspecifically.
2. Positive and negative controls must fall within their respective ranges (below) or the test results are not valid. If these results are not observed, call Technical Services.
  - a) **Positive Control must be a visible yellow color.** If read on a spectrophotometer, the OD at 450 nm or using dual wavelength at 450/620 nm or 450/630 nm must be  $\geq$  0.500. Any well that gives a positive reading without visible color should be repositioned, wiped on the underside of the well, and read again.
  - b) **Negative Control must be visually clear.** If read on a spectrophotometer, the OD at 450 nm must be  $<$ 0.120. If read at 450/620 nm or 450/630 nm the absorbance must be  $<$  0.080. If not, the test is invalid and should be repeated, paying attention to the wash procedure.
3. Visual readings must be taken in good light against a white background.

## INTERPRETATION OF RESULTS

	Spectrophotometric Reading	
	Single Wavelength at 450 nm	Dual Wavelength at 450/620 nm or 450/630 nm
<b>Negative</b>	OD < 0.120	OD < 0.080
<b>Positive</b>	OD ≥ 0.120	OD ≥ 0.080

A positive result in the *H. PYLORI CHEK*<sup>™</sup> test confirms the presence of *H. pylori* antigen in the sample; a negative result indicates the absence of antigen or insufficient levels of antigen for detection.

### Visual Interpretation

The negative control well should be colorless or have only a faint yellow color. The *Positive Control* well should give a visible yellow color. If these results are not observed, call Technical Services. A test sample is considered positive if it has an obvious yellow color when compared to the negative control well. It may be less yellow or more yellow than the color observed in the positive control well. A test sample is considered negative if the reaction is colorless or less yellow than the negative control well.

### LIMITATIONS OF THE *H. PYLORI CHEK*<sup>™</sup> TEST

1. The *H. PYLORI CHEK*<sup>™</sup> test is used to detect *H. pylori* antigen in fecal specimens. The test confirms the presence of *H. pylori* antigen in the sample, and this information should be taken under consideration by the physician in light of the clinical history and physical examination of the patient.
2. A negative test result does not preclude the possibility of the presence of *H. pylori* antigen in the specimen which may occur if the level of antigen is below the detection limit of the test.
3. False negative results may occur if a patient has used antibiotics, proton pump inhibitors (PPIs) or bismuth compounds in the 14 days prior to fecal sample collection, as these medications are known to inhibit *H. pylori*. In these cases, a new fecal sample should be collected and tested 14 days after treatment has stopped. Positive results from patients that have used antibiotics, PPIs, or bismuth compounds in the 14 days prior to fecal sample collection are still considered accurate.
4. Transferring too little sample, or failure to mix and completely suspend the sample in the *Diluent*, may result in a false-negative test result.
5. The *H. PYLORI CHEK*<sup>™</sup> test is qualitative. The intensity of the color should not be interpreted quantitatively.
6. No data exists on the effects of colonic washes, barium enemas, laxatives, or bowel preparations on the performance of the *H. PYLORI CHEK*<sup>™</sup> test. These procedures can result in extensive dilution or the presence of additives that may affect test performance.
7. Performance characteristics have not been established in asymptomatic populations.

### EXPECTED VALUES

The *H. PYLORI CHEK*<sup>™</sup> test detects the presence of *Helicobacter pylori* antigen in human fecal samples. *H. pylori* infection is a global phenomenon with reported prevalence rates in adults ranging from 20% to 95%.<sup>1</sup> In addition to geographical location, factors such as age, ethnicity, and socioeconomic status also affect the prevalence rate.<sup>6,7</sup> *H. pylori* is purportedly the cause of 70%-85% of gastric ulcers and 90%-95% of duodenal ulcers.<sup>8</sup> Historically, treatment regimens to eradicate *H. pylori* infection reported success rates ranging from 76%-94%, but the efficacy of standard treatment regimens has declined due

to factors such as the increased prevalence of antibiotic resistant *H. pylori* strains.<sup>9</sup> The effectiveness of eradication therapy can improve significantly when a tailored regimen is prescribed.<sup>10</sup>

### PERFORMANCE CHARACTERISTICS

The performance of the *H. PYLORI CHEK*<sup>™</sup> test was evaluated at 6 independent sites. Patients were recruited that were undergoing endoscopy as part of routine care. A composite reference method (CRM) comparison was used in the evaluation consisting of rapid urease and histology of the biopsy samples. The following table shows a summary of the clinical performance data. The results of the study show that the *H. PYLORI CHEK*<sup>™</sup> test, by dual wavelength spectrophotometric analysis, exhibited sensitivity of 100% and specificity of 96.1% with CRM biopsy results. Testing was also conducted by visual reading of plates. Visual results were the same as dual wavelength spectrophotometric results 99% of the time.

### Age and Gender Distribution

Age and gender information was available for 109 patients. The ages ranged from 19 to 82 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.

### Initial Diagnosis *H. PYLORI CHEK*<sup>™</sup> test versus Composite Reference Method (CRM)

N = 109	CRM Positive	CRM Negative
<i>H. PYLORI CHEK</i> <sup>™</sup> Positive	32	3*
<i>H. PYLORI CHEK</i> <sup>™</sup> Negative	0	74

	95% Confidence Limits	
Sensitivity	100.0%	89.3% - 98.9%
Specificity	96.1%	89.2% - 98.7%

\*All three specimens tested positive initially by the *H. PYLORI CHEK*<sup>™</sup> test, but negative upon re-testing with the *H. PYLORI CHEK*<sup>™</sup> test.

### Post-Therapy

For Eradication (post-therapy), there were 9 samples from patients being tested post therapy. The results show that the *H. PYLORI CHEK*<sup>™</sup> test exhibited a sensitivity of 77.8% with the composite reference method.

N = 9	CRM Positive	CRM Negative
<i>H. PYLORI CHEK</i> <sup>™</sup> Positive	7*	0
<i>H. PYLORI CHEK</i> <sup>™</sup> Negative	2**	0

	95% Confidence Limits	
Sensitivity	77.8%	45.3% - 93.7%

\* One specimen tested positive by visual read but negative by spectrophotometric interpretation (OD<sub>450/620</sub> 0.034).

\*\* One specimen tested negative initially but positive upon re-testing with the *H. PYLORI CHEK*<sup>™</sup> test.

### Retrospective Sample Study

A supplemental retrospective sample study was performed comparing the *H. PYLORI CHEK™* test to an FDA cleared commercial ELISA. For this study, 196 samples (75 positive and 121 negative by the commercial ELISA) were evaluated. There was 100% correlation of results between the assays.

N = 196	FDA Cleared Commercial ELISA Positive	FDA Cleared Commercial ELISA Negative
<b><i>H. PYLORI CHEK™</i> Positive</b>	75	0
<b><i>H. PYLORI CHEK™</i> Negative</b>	0	121

		95% Confidence Limits
<b>Percent Positive Agreement</b>	100.0%	95.1% - 100.0%
<b>Percent Negative Agreement</b>	100.0%	96.9% - 100.0%

### REPRODUCIBILITY

The reproducibility of the *H. PYLORI CHEK™* test was determined using 8 fecal specimens that were coded to prevent identification during testing. Testing was performed at 2 independent laboratories and on-site at TECHLAB, Inc. The samples were tested in triplicate twice a day over a 5-day period by multiple technicians at each site using 2 different kit lots. The results were consistent among the different locations, and exhibited a correlation of 97.5%.

### CROSS REACTIVITY

The *H. PYLORI CHEK™* test was evaluated for cross-reactivity with common intestinal organisms and viruses listed below. None of the organisms or viruses were shown to interfere with the performance of the *H. PYLORI CHEK™* test.

*Acinetobacter baumannii*

*Bacillus cereus*

*Bacillus subtilis*

*Borrelia burgdorferi*

*Campylobacter coli*

*Campylobacter fetus*

*Campylobacter helveticus*

*Campylobacter hyointestinalis*

*Campylobacter jejuni*

*Campylobacter lari*

*Campylobacter upsaliensis*

*Candida albicans*

*Clostridium bifermentans*

*Clostridium difficile*

*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*

Adenovirus Types 2, 40  
Human Coronavirus  
Coxsackievirus B1, B2, B3, B6

Echovirus 9, 22  
Enterovirus 70  
Human Rotavirus

### INCLUSIVITY STUDY

The following strains, which include isolates representing described *H. pylori* populations, were tested for reactivity with the *H. PYLORI CHEK™* test. All strains tested generated a positive result.

ATCC 700392  
ATCC 43526  
ATCC 700824

JP26  
ATCC 43504  
ATCC 43579

### INTERFERING SUBSTANCES (U.S. FORMULATION)

The following substances had no effect on positive or negative *H. PYLORI CHEK™* test results analyzed at the concentrations indicated:

Barium sulfate (5% w/v), Benzalkonium Chloride (1% w/v), Ciprofloxacin (0.25% w/v), Ethanol (1% w/v), Hog gastric mucin (3.5% w/v), Human blood (40% v/v), Hydrocortisone (1% w/v), Imodium® (5% v/v), Kaopectate® (5% v/v), Leukocytes (0.05% v/v), Maalox® Advanced (5% v/v), Mesalazine (10% w/v), Metronidazole (0.25% w/v), MiraLax® (7% w/v), Mineral Oil (10% w/v), Mylanta® (4.2 mg/mL), Naproxen Sodium (5% w/v), Nonoxynol-9 (1% w/v), Nystatin (1% w/v), Palmitic Acid/Fecal Fat (40% w/v), Pepto-Bismol® (5% v/v), Phenylephrine (1% w/v), Prilosec OTC® (5 µg/mL), Sennosides (1% w/v), Simethicone (10% w/v), Stearic Acid/Fecal Fat (40% w/v), Tagamet® (5 µg/mL), TUMS® (50 µg/mL), Human Urine (5% v/v), and Vancomycin (0.25% w/v).

### ANALYTICAL SENSITIVITY

The Limit of Detection (LoD) for the *H. PYLORI CHEK™* test was established at 6.70 ng/mL in fecal matrix (0.13 ng/test) for *Helicobacter pylori* antigen using cell lysate antigen prepared from *H. pylori* strain ATCC 43526. For specimens in Cary Blair media, the LoD was established at 26.57 ng/mL (0.33 ng/test). For specimens in C&S media, the LoD was established at 18.19 ng/mL (0.23 ng/test).

### PRECISION – INTRA-ASSAY

For the determination of intra-assay performance, 8 fecal samples were analyzed by the *H. PYLORI CHEK™* test. The samples included 2 negative, 2 high negative, 2 low positive, and 2 moderate positive samples. Each specimen was assayed a total of five times using two different kit lots. Positive specimens tested as expected and negative specimens consistently tested negative.

### PRECISION – INTER-ASSAY

For the determination of inter-assay performance, 8 fecal samples were analyzed by the *H. PYLORI CHEK™* test. The samples included 2 negative, 2 high negative, 2 low positive, and 2 moderate positive samples. The samples were tested twice a day by multiple technicians over a 12-day period using 2 different kit lots. The positive samples tested as expected 98.3% of the time and the negatives tested as expected 97.8% of the time.

### FRESH VERSUS FROZEN SAMPLES

The effect of long term frozen specimen storage on antigen stability was evaluated. For the analysis, a total of 32 fecal specimens was tested with the *H. PYLORI CHEK™* test. The fecal specimens consisted of 2 negative fecal samples, 5 high negative fecal samples, 10 low positive fecal samples, and 15 positive fecal samples covering the range of the test (50 ng/mL – 1200 ng/mL). Samples were prepared and stored ≤ -10°C and ≤ -70°C and tested at 0, 5, 10, and 14 days. No conversion of positive-to-negative or negative-to-positive was observed in any of the samples at the specified time points.

## PROZONE

To ensure that a high concentration of *H. pylori* antigen does not interfere with a positive reaction in the *H. PYLORI CHEK™* test, high positive samples were prepared by spiking a negative fecal pool at concentrations up to 10 times the highest concentration of antigen observed in a positive clinical specimen. A total of 5 different dilutions of *H. pylori* antigen was prepared and tested in triplicate. The results demonstrated that there was no overall prozone effect, that elevated levels of antigen did not affect the detection of the antigen.

## REFERENCES

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