Enzyme Immunoassay for the Detection of *Helicobacter pylori*
Antigens in Stool Specimens for Diagnosis and Monitoring

REF **619096** IVD Rx Only

For Professional Use Only

INTENDED USE

The Premier HpSA Flex enzyme immunoassay (EIA) is an in vitro qualitative procedure for the detection of *Helicobacter pylori* antigens in human stool. The test is intended for use with unpreserved stool specimens or preserved stool specimens in transport media. 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.

SUMMARY AND EXPLANATION OF THE TEST

Recent studies have shown that *Helicobacter pylori* infections are a major public health concern globally.[1] It is estimated that over half of the global population is infected with *Helicobacter pylori*.[1, 3] Individuals infected with the *H. pylori* organism fall under two groups. The first group, "colonized," makes up the majority of the global population and exhibits no gastrointestinal signs and symptoms. The second group, "infected", exhibit gastrointestinal signs and symptoms which include gastritis, peptic or gastric ulcers, or gastric or duodenal cancer.[5, 9] *H. pylori* infection is considered the leading cause for chronic gastritis and is also considered an etiological agent for gastric cancer (adenocarcinoma) and peptic ulcer disease.[1-3] *H. pylori* infection has also been associated with mucosa-associated lymphoid tissue (MALT) and has been attributed to approximately 89% of gastric cancer diagnoses.[1, 4]

There are multiple methods in which to diagnose an *H. pylori* infection. Biopsy-based tests are invasive methods which include culture, polymerase chain reaction (PCR), and the rapid urease test (RUT). The urea breath test (UBT), serology, and stool antigen test are non-invasive methods of detection.[1, 6] It is recommended for clinicians to use non-invasive testing methods to aid in the diagnosis of patients who are: (i) without alarming symptoms (e.g., unexplained weight loss, progressive dysphagia, odynophagia), (ii) under the age of 55, and (iii) have a low risk of gastric cancer.[7, 8]

Premier HpSA Flex is a microwell-based enzyme immunoassay that detects *H. pylori* antigens present in human stool. No calculations are required, and the visual color change makes the interpretation of results objective and simple. In addition, the HpSA test permits the assessment of established or novel anti-*H. pylori* treatment during and post-therapy to monitor for treatment effectiveness, relapse, or eradication. Premier HpSA Flex is a modification of the Premier Platinum HpSA PLUS in which a new preserved specimen type claim has been added to the intended use of these devices. The Premier HpSA Flex assay contains the same kit components as the Premier Platinum HpSA PLUS assay, however, the Premier HpSA Flex device supports the use of both unpreserved and preserved stool specimens.

BIOLOGICAL PRINCIPLES

The Premier HpSA Flex test utilizes a plurality of monoclonal anti-*H. pylori* capture antibodies adsorbed to microwells. (Plurality is defined as a mixture of monoclonal antibodies.) Diluted patient samples and a conjugate (peroxidase-conjugated to a plurality of monoclonal antibodies) are added to the wells and incubated for one hour at room temperature. A wash is performed to remove unbound material. Substrate is added and incubated for 10 minutes at room temperature. Color develops in the presence of bound enzymes. Stop Solution I is added, and the results are interpreted visually or spectrophotometrically.

REAGENTS/MATERIALS PROVIDED

The maximum number of tests obtained from this test kit is listed on the outer box.

1. **Antibody-Coated Microwells** - Breakaway plastic wells coated with a plurality of murine monoclonal antibodies specific for *H. pylori*.
2. **Positive Control** - Inactivated *H. pylori* diluted in 10 mM phosphate-buffered solution with 0.02% Thimerosal, pH 7.2.
3. **Sample Diluent/Negative Control** - pH 7.2, 10 mM phosphate-buffered solution with 0.02% Thimerosal.
4. **Premier 20X Wash Buffer I** - pH 6.8, 180 mM phosphate-buffered solution with 0.2% Thimerosal.
5. **Enzyme Conjugate** – A plurality of murine monoclonal antibodies specific for *H. pylori* conjugated to horseradish peroxidase in a pH 7.8, 50 mM Tris-buffered solution containing 0.02% Thimerosal.
6. **Premier Substrate I** - a-buffered solution containing urea peroxide and tetramethylbenzidine. (pH 5.0)
7. **Premier Stop Solution I** – 1 M phosphoric acid. CAUTION: Avoid contact with skin. Flush with water if contact occurs.
8. Transfer pipettes (one for each test sample). Each pipette is marked to indicate 50 µL, 100 µL, 200 µL and 300 µL volumes.
9. Plate sealer
10. Wooden stick applicators.

MATERIALS NOT PROVIDED

1. Test tubes (12 x 75 mm) for dilution of sample
2. Distilled or deionized water
3. Squirt bottle
4. Graduated cylinder for making 1X Wash Buffer I
5. Transport Media (Cary Blair or C&S)
6. EIA plate reader capable of reading absorbance at 450 or 450/630 nm*
7. Semiautomated plate washer*

* **Note:** It is the operator's responsibility to validate the semiautomated plate washers and readers prior to their use with this product.

PRECAUTIONS

1. All reagents are for *in vitro* diagnostic use only.
2. Patient specimens may contain infectious agents and should be handled and disposed of as potential biohazards.
3. All reagents should be mixed gently before use.
4. Do not interchange the Microwells, Enzyme Conjugate, Substrate I Reagent, or Positive Control reagents between lots. (The Sample Diluent, Premier 20X Wash Buffer I, and Premier Stop Solution I are interchangeable provided the reagents are within their assigned expiration dates when used.)
5. Allow reagents to warm to 19-27 C before use.
6. Hold reagent vials vertically at a suitable distance above the well to ensure proper drop size and delivery.
7. Do not use kit components beyond their labeled expiration date.
8. Replace colored caps on correct vials.
9. Dispose of used wash buffer and all test materials in an appropriate container. Treat waste as a potential biohazard.
10. The Positive Control reagent contains inactivated *H. pylori*. It should be handled, however, as a potential biohazard.
11. Avoid skin contact with Premier Stop Solution I (1 M phosphoric acid). Flush with water immediately if contact occurs.
12. Do not reuse microwells.
13. Unused microwells must be placed back inside resealable pouch. It is important to protect strips from moisture.
14. The transfer pipettes provided with this kit must be used for specimen preparation and transfer. Use one pipette per specimen.
15. Avoid splashing when dispensing diluted stool into microwells by placing the transfer pipette tip about halfway down the well and dispensing slowly down the side of the well.
16. Microwell washing is to be performed precisely as directed in the assay procedure. Inadequate washing may be the cause of elevated background in any EIA protocol.
17. All reagents except the Premier 20X Wash Buffer I, are provided already diluted to the proper concentration.
18. Any deviation below or above set incubation times may affect sensitivity and specificity and should be avoided.
19. Stool must be mixed thoroughly (regardless of consistency) to ensure a representative sample prior to pipetting.
20. Some precipitation may occur in Premier 20X Wash Buffer I when it is stored at 2-8 C. The precipitate will dissolve when a working dilution is made with the Wash Buffer.
21. Do not use vials that lack a label, lot number, or expiration date.

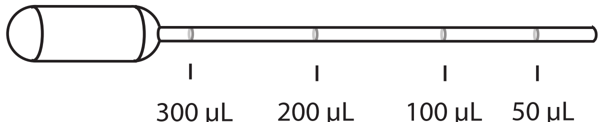
HAZARD and PRECAUTIONARY STATEMENTS

 Premier Stop Solution I	**Signal Word** Danger **Hazard Statements** H412 - Harmful to aquatic life with long-lasting effects H314 - Causes severe skin burns and eye damage Contains Phosphoric acid **Precautionary Statements - EU (§28, 1272/2008)** P260 - Do not breathe dust/fume/gas/mist/vapors/spray P264 - Wash face, hands, and any exposed skin thoroughly after handling P280 - Wear protective gloves/ protective clothing/ eye protection/ face protection P305 + P351 + P338 - IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present, and easy to do. Continue rinsing P310 - Immediately call a POISON CENTER or doctor/ physician P303 + P361 + P353 - IF ON SKIN (or hair): Remove/ Take off immediately all contaminated clothing. Rinse skin with water/shower P363 - Wash contaminated clothing before reuse P304 + P340 - IF INHALED: Remove to fresh air and keep at rest in a position comfortable for breathing P310 - Immediately call a POISON CENTER or doctor/ physician P301 + P330 + P331 - IF SWALLOWED: Rinse mouth. Do NOT induce vomiting P405 - Store locked up P501 - Dispose of contents/ container to an approved waste disposal plant.

 Premier 20X Wash Buffer I	**Signal Word** Danger **Hazard Statements** H302 - Harmful if swallowed H311 - Toxic in contact with skin **Precautionary Statements - EU (§28, 1272/2008)** P280 - Wear protective gloves/ protective clothing/ eye protection/ face protection

SHELF LIFE AND STORAGE
The expiration date is indicated on the kit label. Store the kit at 2-8 C and return the kit promptly to the refrigerator after each use.

PROCEDURAL NOTES
The Premier HpSA Flex transfer pipette is diagrammed below:



REAGENT PREPARATION
1. Bring the entire kit, including microwell pouch, to 19-27 C before use.
2. Prepare 1X Wash Buffer I as needed. For example,; 4.0 mL of Premier 20X Wash Buffer I + 76.0 mL of distilled or deionized water is sufficient to wash one strip. Place in a clean squirt bottle. The 1X Wash Buffer I can be stored at 19-27 C for up to three months.

SPECIMEN COLLECTION AND PREPARATION
This procedure is designed to be used with unpreserved stool or stool preserved in Cary-Blair or C&S transport media. The specimen should be received in an airtight transport container and stored at 2-8 C until tested. The specimen should be tested as soon as possible, but unpreserved specimens may be held for up to 72 hours at 2-8 C prior to testing; preserved specimens may be held for up to 120 hours at room temperature (19-27 C) or 2-8 C. (See SPECIMEN PREPARATION section for instructions on diluting samples.) If testing cannot be performed within the aforementioned time frame, unpreserved specimens should be frozen immediately upon receipt and stored frozen (-20 C to -80 C) until tested; preserved specimens may be frozen immediately upon receipt and stored frozen (-20 C to -80 C) for 14 days. Specimens may be frozen and thawed twice.

NOTE: Stool on swabs or in preservatives other than Cary-Blair or C&S transport media have not been evaluated for testing.

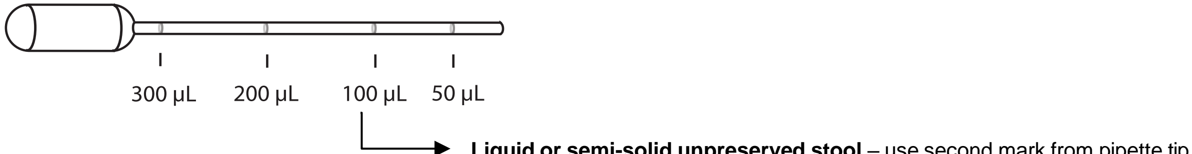
NOTE: Specimens diluted in Sample Diluent may be held at room temperature (19-27 C) for up to 11 hours or at refrigerated temperature (2-8 C) for up to 24 hours prior to further processing.

Specimen Conditions	Storage Temperature	Maximum Storage Time
Unpreserved specimens	refrigerated (2-8 C)	72 hours
	frozen (-20 to -80 C)	until tested
Preserved specimens in Cary-Blair or C&S media	room temperature (19-27 C)	120 hours
	refrigerated (2-8 C)	120 hours
	frozen (-20 to -80 C)	14 days
Unpreserved specimens diluted in Sample Diluent	room temperature (19-27 C)	11 hours
	refrigerated (2-8 C)	24 hours
Preserved specimens diluted in Sample Diluent	room temperature (19-27 C)	11 hours
	refrigerated (2-8 C)	24 hours

SPECIMEN PREPARATION
Human stool specimens, unpreserved:
1. Using a pipetting device, add **500 µL** of Sample Diluent to a clean test tube.
2. Mix unpreserved stool as thoroughly as possible.
   a. **Liquid or semi-solid unpreserved stools** - Using the supplied transfer pipette, add **100 µL** (second mark from the tip of the pipette) of unpreserved stool into Sample Diluent. Using the same pipette, gently withdraw and expel the stool suspension several times, then vortex 15 seconds. Save the transfer pipette in the sample for later use.
   b. **Formed/Solid unpreserved stools** - Using a wooden applicator stick, transfer a small portion (**5-6 mm diameter**) of thoroughly mixed unpreserved stool into Sample Diluent. Emulsify stool using the wooden applicator stick, then vortex for 15 seconds.

   Note: Unpreserved specimens diluted in Sample Diluent may be held at room temperature (19-27 C) for up to 11 hours or at refrigerated temperature (2-8 C) for up to 24 hours prior to proceeding to the test procedure.

3. Stool specimens may be centrifuged after dilution. Centrifuge at approximately 2750 x G for five minutes or until solid matter separates from liquid. Proceed with the TEST PROCEDURE after recovering supernate.

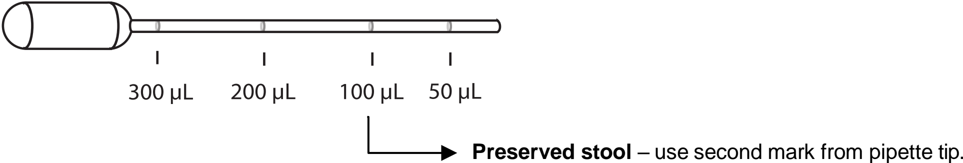


Human stool specimens, preserved in Cary-Blair or C&S transport media:

1. Using a pipetting device, add **100 µL** of Sample Diluent to a clean test tube.
2. Mix preserved stool as thoroughly as possible.
3. Using the supplied transfer pipette, add **100 µL** (second mark from tip of the pipette) of preserved stool into Sample Diluent. Using the same pipette, gently withdraw and expel the stool suspension several times, then vortex for 15 seconds. Save the transfer pipette in the sample for later use.

   Note: Preserved specimens diluted in Sample Diluent may be held at room temperature (19-27 C) for up to 11 hours or at refrigerated temperature (2-8 C) for up to 24 hours prior to proceeding to the test procedure.

4. Stool specimens may be centrifuged after dilution. Centrifuge at approximately 2750 x G for five minutes or until solid matter separates from liquid. Proceed with the TEST PROCEDURE after recovering supernate.



TEST PROCEDURE

1. After the pouch has reached temperature, break off the required number of microwells (1 well for each specimen, plus 1 positive and 1 negative control well per batch). Place the microwells in the microwell strip holder and record the location of all wells. Unused microwells must be resealed in the pouch immediately.
2. Using the specimen transfer pipette, add 100 µL of diluted stool (second mark from the tip of the pipette) to the appropriate well. (Place the pipette tip halfway into well and let the sample slowly run down the side of well.)
3. Add 2 free-falling drops of Positive Control and 100 µL of Sample Diluent /Negative Control to the appropriate wells.
4. Add 1 free-falling drop (approximately 50 µL) of Enzyme Conjugate to each well. Firmly shake/swirl the plate for 30 seconds.
5. Cut plate sealer to size and press firmly onto top of microwells to seal. Incubate the plate for 1 hour at 19-27 C.
6. Carefully remove the plate sealer and wash wells:
   a. Manual method:
      i Dump plate contents firmly into a biohazard receptacle.
      ii Bang the inverted plate on a clean stack of paper towels.
      iii Fill all wells with 1X Wash Buffer I, directing stream of buffer to the sides of the wells to avoid foaming.
      iv Repeat wash cycle (dump, bang on fresh towels, fill) 4 times for a total of 5 wash cycles. After the last fill, dump, and bang plates on fresh towels hard enough to remove as much excess wash buffer as possible, but do not allow wells to completely dry at any time.
   b. Semiautomated method using validated equipment:
      i Aspirate the contents of the well.
      ii Fill the wells to the top (approx. 300-350 µL/well) with 1X Wash Buffer I then aspirate. The washer manifold should be adjusted to ensure no foaming occurs during the filling of the wells and that the wells are thoroughly aspirated after each wash.
      iii Repeat step ii a minimum of 4 more times. Following the last wash, test wells should be thoroughly aspirated to remove as much moisture as possible.
7. Clean the underside of all wells with a lint-free tissue.
8. Add 2 free-falling drops (approx. 100 µL) of Premier Substrate Solution I to each well. Firmly shake/swirl the plate for 30 seconds. Incubate for 10 minutes at 19-27 C.
9. Add 2 free-falling drops (approx. 100 µL) of Premier Stop Solution I to each well. Firmly shake/swirl the plate for 30 seconds.
   Note: Initial color of positive reaction is blue, which changes to yellow upon the addition of Premier Stop Solution I.
10. Inspect and record reactions. Test results can be read visually or using a spectrophotometric reader.
    a. Visual Determination - Read within 15 minutes after adding Premier Stop Solution I.
    b. Spectrophotometric Determination - Zero EIA reader on air. Wipe the underside of wells with a lint-free tissue. Read absorbance at 450 nm or 450/630 nm within 15 minutes of adding Premier Stop Solution I.

INTERPRETATION OF RESULTS

The following interpretations apply to both initial diagnosis and monitoring of anti-*H. pylori* therapy.

Visual Reading

Negative = colorless to faint yellow
Positive = definite yellow color
To be called positive, a faint yellow color must be confirmed by a spectrophotometric reading. If a spectrophotometer is not available, the cut-off must be determined by an alternative method.

Spectrophotometric Single Wavelength (450 nm)

Negative:	< 0.140
Positive:	≥ 0.140
Negative Control:	< 0.140
Positive Control:	≥ 0.640

Spectrophotometric Dual Wavelength [450&630} calculated as the difference of the OD readings [A450 − A630]

Negative:	< 0.100
Positive:	≥ 0.100
Negative Control:	< 0.100
Positive Control:	≥ 0.600

If a Negative Control is < 0.000, re-blank the plate reader to air and reread the plate.

A positive result indicates the presence of *H. pylori* antigens. A negative result indicates the absence of *H. pylori* antigens, or that the level of antigens is below what can be detected by the assay. The magnitude of the OD above the cut-off is not indicative of the severity or extent of *H. pylori* infection, nor can it be correlated to an endpoint titer. Extremely strong positive reactions may yield a purple precipitate within a few minutes of stopping the reaction.

QUALITY CONTROL

This test should be performed per applicable local, state, or federal regulations or accrediting agencies.

1. At the time of each use, kit components should be visually examined for obvious signs of microbial contamination, freezing, or leakage. Do not use contaminated or suspect reagents. The Positive and Negative Controls must be used with each test batch. See the INTERPRETATION OF RESULTS section above for a description of the expected results for control reagents. Tests should be considered invalid when either control reagent does not produce the expected results. In such cases, repeat tests and controls. **If, on repeat testing, the expected reactions are still not observed and the reagents are still within their expiration date, contact Meridian's Technical Services Department at 1-800-343-3858 (US) or your local distributor.**
2. The controls are used to monitor reagent reactivity. Failure of the controls to produce the expected results can mean that one or more of the reagents are no longer reactive at the time of use, the test was not performed correctly, or that reagents or samples were not added. The Positive Control will not ensure precision at the cut-off.
3. Suspect failure of the washing method or device if the Negative Control and/or Positive Controls consistently produce out-of-specification results. Increasing the number of washes, washing more vigorously, decanting more thoroughly or recalibrating washing devices should correct the problem. **If the expected control reactions are not observed, repeat the control tests as the first step in determining the root cause of the failure. If control failures are repeated, please contact Meridian's Technical Services Department at 1-800-343-3858 (US) or your local distributor.**
4. Specimen matrix interference has not been observed in this assay as samples are significantly diluted before testing in Sample Diluent. For this reason, the positive and negative control reagents supplied as part of this assay are prepared in the matrix of the Sample Diluent. If control materials that are identical in composition to test samples are preferred, the user can prepare these by diluting known positive and negative specimens in Sample Diluent according to the SPECIMEN PREPARATION section of this insert. Add 100 µL of user-prepared controls to test wells.

EXPECTED VALUES

Recent studies have shown that *Helicobacter pylori* infections are a major public health concern globally.[1] It is estimated that over half of the global population is infected with *Helicobacter pylori*.[1, 3] In patients diagnosed with duodenal ulcers, however, it has been shown to be 90%-95%.[11] Currently, recommended eradication treatments have an overall eradication rate of 84.3% (82.1% to 86.4%).[10]

The Premier HpSA Flex test detects the presence of *H. pylori* antigens in human stool. Expected values for a given population should be determined for each laboratory. The rate of positivity may vary depending on geographic location, method of specimen collection, handling and transportation, test employed, and general health environment of the patient population under study. As demonstrated by Premier Platinum HpSA in tests conducted in the United States, Canada, and Italy, the incidence of disease ranged from 34% to 53% to 69% respectively.

LIMITATIONS OF THE PROCEDURE

1. The test is qualitative, and no quantitative interpretation should be made with respect to the values.
2. Test results should be used in conjunction with information available from the patient clinical evaluation and other diagnostic procedures.
3. Antimicrobials, proton pump inhibitors, and bismuth preparations are known to suppress *H. pylori,* and ingestion of these prior to *H. pylori* testing (culture, histology, rapid urease, UBT, antigen) may give a false negative result. If a negative result is obtained for a patient ingesting these compounds within two weeks prior to performing the Premier HpSA Flex test, it may be a false-negative result and the test should be repeated on a new specimen obtained two weeks after discontinuing treatment. A positive result for a patient ingesting these compounds, within two weeks prior to performing the Premier HpSA Flex test, should be considered accurate. As an example, patients with *H. pylori* were placed on a proton pump inhibitor (Lansoprazole) or bismuth for two weeks and tested with the Premier Platinum HpSA and a urea breath test. Patients were then taken off treatment for two weeks and retested. At the end of treatment, both assays were negative for some patients but returned to positive two weeks post-treatment (see table).

		Premier Platinum HpSA		Breath Test	
Treatment	**Time Point**	**Pos/Total**	**% Positive**	**Pos/Total**	**% Positive**
PPI	End of Treatment	15/20	75.0%	12/20	60.0%
	2 Weeks Post-Treatment	19/20	95.0%	18/20	90.0%
Bismuth	End of Treatment	15/20	75.0%	11/20	55.0%
	2 Weeks Post-Treatment	19/20	95.0%	18/20	90.0%

4. Performance characteristics have not been established for watery, diarrheal stools.
5. Performance characteristics have not been established in asymptomatic populations.
6. Histamine H2-receptor antagonists (H2 blockers) do not interfere with positive results.

SPECIFIC PERFORMANCE CHARACTERISTICS

This section contains data that was generated for multiple iterations of the current design (*Premier HpSA Flex*). The associated 510(k) numbers for this product include K980076 and K983255 (*Premier Platinum HpSA*), K053335 and K182559 (*Premier Platinum HpSA PLUS*), and K230901 (*Premier HpSA Flex*). Clinical evaluations performed with the first-generation Premier Platinum HpSA demonstrated that an ELISA-based assay could reliably and predictably detect *H. pylori* antigen in human stool in symptomatic patients. Studies also demonstrated the test can be used to monitor the efficacy of eradication therapy.

The Premier Platinum HpSA assay was evaluated on 200 symptomatic adults at one Midwestern United States location, one site in Canada, and two sites in Italy. The patients studied had a wide cross-section of gastric pathologies noted, including antral gastritis (n=81), antral gastropathy (n=25), antral erosions (n=24), esophagitis (n=21), duodenal ulcer (n=15), erosive duodenitis (n=10), GERD (n=10), "normal" (n=10), duodenitis (n=9), gastric ulcer (n=8), total stomach gastritis (n=6), hiatal hernia (n=6), Schatzki's ring (n=4), pyloric ulcer (n=2), and esophageal ulcer (n=1). HpSA test results were compared to diagnosis of *H. pylori* infection as judged by objective reference methods (culture, rapid urease, histology, and UBT). Patients were considered positive if culture was positive, or if two or more of the other three tests were positive. Nine patients with negative or no culture results, and only one other test positive, were considered unevaluable. The HpSA test exhibited 96.1% sensitivity and 95.7% specificity when compared to the reference method. Confidence intervals were calculated by the exact binomial method.

Trial Site #1

TEST		DIAGNOSIS		Sensitivity	Specificity	Pos. PV	Neg. PV	Correlation
Method	**Result**	**Infected**	**Not Infected**	**± 95% CI**	**± 95% CI**	**± 95% CI**	**± 95% CI**	**± 95% CI**
PP HpSA	Pos	17	3	94.4%	91.4%	85.0%	97.0%	92.5%
EIA	Neg	1	32	72.7-99.9%	76.9-98.2%	62.1-96.8%	84.2-99.9%	81.8-97.9%
	Equ	0	0					

Reference Methods: Histology, Rapid Urease, Breath Test. Readings Single and Dual Wavelength.

Trial Site #2

TEST		DIAGNOSIS		Sensitivity	Specificity	Pos. PV	Neg. PV	Correlation
Method	**Result**	**Infected**	**Not Infected**	**± 95% CI**	**± 95% CI**	**± 95% CI**	**± 95% CI**	**± 95% CI**
PP HpSA	Pos	9	0	100.0%	100.0%	100.0%	100.0%	100.0%
EIA	Neg	0	8	66.4-100.0%	63.1-100.0%	66.4-100.0%	63.1-100.0%	80.5-100.0%
	Equ	0	0					

Reference Methods: Histology, Rapid Urease, Culture, Breath Test. Readings Single and Dual Wavelength.

Trial Site #3

TEST		DIAGNOSIS		Sensitivity	Specificity	Pos. PV	Neg. PV	Correlation
Method	**Result**	**Infected**	**Not Infected**	**± 95% CI**	**± 95% CI**	**± 95% CI**	**± 95% CI**	**± 95% CI**
PP HpSA	Pos	44	0	97.8%	100.0%	100.0%	96.0%	98.6%
EIA	Neg	1	24	88.2-99.9%	85.8-100.0%	92.0-100.0%	79.6-99.9%	92.2-100.0%
	Equ	1	0					

Reference Methods: Histology, Rapid Urease, Culture, Breath Test. Readings Single Wavelength.

Trial Site #4

TEST		DIAGNOSIS		Sensitivity	Specificity	Pos. PV	Neg. PV	Correlation
Method	**Result**	**Infected**	**Not Infected**	**± 95% CI**	**± 95% CI**	**± 95% CI**	**± 95% CI**	**± 95% CI**
PP HpSA	Pos	29	1	93.5%	96.3%	96.7%	92.9%	94.8%
EIA	Neg	2	26	78.6-99.2%	81.0-99.9%	82.8-99.9%	76.5-99.1%	85.6-98.9%
	Equ	2	0					

Reference Methods: Histology, Rapid Urease. Readings Dual Wavelength.

Combined Data From All Sites

TEST		DIAGNOSIS		Sensitivity	Specificity	Pos. PV	Neg. PV	Correlation
Method	**Result**	**Infected**	**Not Infected**	**±95% CI**	**±95% CI**	**±95% CI**	**±95% CI**	**±95% CI**
PP HpSA	Pos	99	4	96.1%	95.7%	96.1%	95.7%	95.9%
EIA	Neg	4	90	90.4-98.9%	89.5-98.8%	90.4-98.9%	89.5-98.8%	92.2-98.2%
	Equ	3	0					

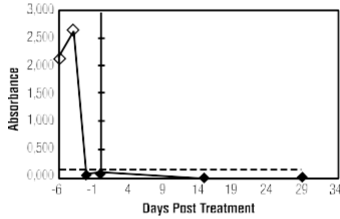
Therapeutic Monitoring:
Four sites examined the utility of the stool antigen test for monitoring anti-*H. pylori* treatment in 97 patients who initially tested positive by endoscopy (culture, histology, and rapid urease). Premier Platinum HpSA testing and endoscopic biopsies were performed four weeks after completion of physician-prescribed, *H. pylori* eradication therapy. The test results are compared in the following table. Culture, histology, and rapid urease were used to determine eradication as defined by FDA guidelines.[22]

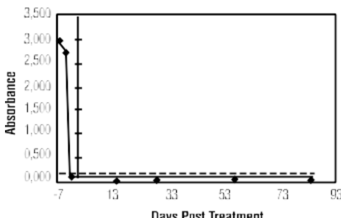
Overall: HpSA vs. 4-Week Scope		
HpSA	**4 Weeks Post Treatment**	
Result	**Infected**	**Eradicated**
Positive	18	3
Negative	1	73
Statistic	Value	95% CI
Sensitivity	94.7%	74.0-99.9%
Specificity	96.1%	88.9-99.2%
Positive PV	85.7%	63.7-97.0%
Negative PV	98.6%	92.7-100%
Correlation	95.8%	89.6-98.8%

Premier Platinum HpSA correctly identified 18/19 (94.7%) of the infected and 73/76 (96.1%) of the eradicated patients. Two of the 97 stools were equivocal by HpSA (2%). The false negative stool was from a patient that was positive by culture, histology, and rapid urease. Three false positive HpSA results were obtained from patients that were negative by all other methods (culture, histology, and rapid urease).

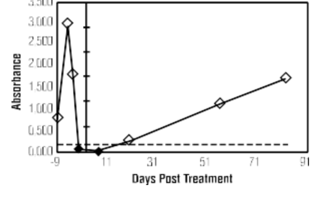
Response to treatment is generally noted by a negative HpSA test within 5 to 7 days after initiating treatment. Positive results at this time, or later, indicate ineffective therapy or recurrence. Recurrence can result from lack of patient compliance with the drug regimen, ineffective drugs, resistant strains of *H. pylori*, improper dosage, etc. Recurrent *H. pylori* infection generally occurs four weeks after termination of therapy. Occasionally, however, infections will remain cryptic beyond four weeks. This observation supports accepted medical practice that determination of eradication utilizing any diagnostic method should be done at least four weeks following completion of therapy. The figures below are typical response profiles for successful and unsuccessful eradication therapies. The vertical bar indicates completion of therapy (Day 0). Days to the left of Day 0 reflect the period that patients were taking drugs. The positive cut-off is shown as horizontal dashed lines.

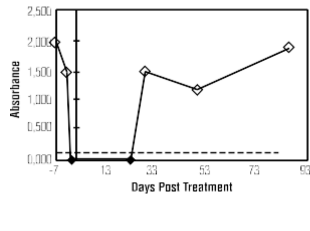
Eradicated Patient Profiles

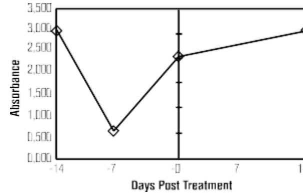




Non-Eradicated (Infected) Patient Profiles







Comparison of Premier Platinum HpSA PLUS to Premier Platinum HpSA:
Tests with 291 samples from symptomatic patients collected either prior to or following treatment were used to demonstrate that Premier Platinum HpSA PLUS performed similarly to Premier Platinum HpSA. Thirty-three of these samples were originally evaluated in an earlier trial to demonstrate the effectiveness of Premier Platinum HpSA. Test performance including 95% confidence intervals is detailed in the following table.

PP HpSA PLUS	PP HpSA (Predicate)		
	Positive	**Negative**	**Indeterminate**
Positive	94	10	3
Negative	0	183	1
Agreement	**Positive Test**	**Negative Test**	**Overall**
	94/94 = 100%	183/193 = 94.8%	277/287 = 96.5%

Eight of the 10 samples that were positive by Premier Platinum HpSA PLUS, but negative by Premier Platinum HpSA, were positive by CLO, histology or UBT testing. The three samples that were positive by Premier Platinum HpSA PLUS but indeterminate by Premier Platinum HpSA were positive by CLO, histology, or UBT testing. The one sample, which was negative by Premier Platinum HpSA PLUS but indeterminate by Premier Platinum HpSA, was negative by CLO, histology, or UBT testing.

Comparison of Modified Premier Platinum HpSA PLUS to Premier Platinum HpSA PLUS (Predicate) (unpreserved):
One hundred and fifty-nine (159) archived, unpreserved stool samples from symptomatic patients were analyzed for *H. pylori* antigen by the modified Premier Platinum HpSA PLUS and Premier Platinum HpSA PLUS (Predicate) to demonstrate that changes to the microwell and conjugate antibodies do not affect assay performance. Test performance including 95% confidence intervals is detailed in the following table.

Modified PP HpSA PLUS	PP HpSA PLUS (Predicate)		
	Positive	**Negative**	**Total**
Positive	57	0	57
Negative	0	102	102
Total	57	102	159
			95% CI
Positive Agreement	57/57	100.0%	93.7-100.0%
Negative Agreement	102/102	100.0%	96.4-100.0%

Comparison of Premier HpSA Flex Modification to an FDA-cleared Commercial Assay (preserved):
Method comparison testing was done to compare the performance of the Premier HpSA Flex modification to that of an FDA-cleared comparator device. There were 200 archived stool specimens enrolled in the study from patients with signs and symptoms of gastroenteritis for whom a practicing physician had ordered a diagnostic *H. pylori* antigen test. Of those 200, viable Standard of Care (SoC) data was available for 182, all of which were evaluable specimens. Specimens were preserved in Cary-Blair or C&S transport media prior to testing with the Premier HpSA Flex assay and the comparator device in a central laboratory. Clinical performance (positive and negative percent agreement) for archived specimens against the FDA-cleared comparator is presented in the following table. There were no observable differences in the performance of the Premier HpSA Flex assay with respect to preserved media type (i.e., Cary-Blair and C&S), kit lot, or patient characteristics (i.e., age, sex, and race).

Premier HpSA Flex	Comparator Device		
	Positive	**Negative**	**Total**
Positive	49	2*	51
Negative	0	131	131
Total	49	133	182
			95% CI
Positive Agreement	49/49	100.0%	[92.7% - 100.0%]
Negative Agreement	131/133	98.5%	[94.7% - 99.6%]

* 1/2 Premier HpSA Flex false positive specimens produced a positive result by the Standard of Care (SoC) testing using an FDA-cleared commercial assay.

REPRODUCIBILITY
Reproducibility of Modified Premier Platinum HpSA PLUS
Assay precision, intra-assay variability, and inter-assay variability were assessed with a 10-member reference panel prepared from moderately positive samples (n=3), low positive samples (n=3), high negative samples (n=3) and a true negative sample (n=1). In addition, the positive and negative kit controls were run when each panel was tested. Each panel was tested once a day by two technicians, at three different laboratory sites, for 5 consecutive days. Overall, 100% (300/300, 98.7-100%, 95% CI) of results obtained with the Premier Platinum HpSA PLUS were as expected. There were no invalid results generated during the study (0.0%; 0/300; 0.0-1.3%, 95% CI).

Reproducibility of Premier HpSA Flex Modification
The reproducibility of the Premier HpSA Flex assay was determined by testing preserved contrived stool samples across three independent laboratories. Samples were created with *H. pylori* antigen spiked into pooled negative stool matrix at high negative, low positive, and moderate positive concentrations, along with a true negative sample. Ten panels consisting of 12 blinded samples were provided to each of the three laboratories for a total of 360 samples. Testing was conducted at each laboratory over 5 different days. Each day two separate operators tested a separate panel while alternating between kit lots. Testing included three different kit lots (2 lots per site). In addition, the positive and negative kit controls were run when each panel was tested. For preserved stool samples, the overall agreement between the Premier HpSA Flex assay result and the expected assay result was 100.0% (95% CI: 98.9% - 100.0%).

CROSSREACTIVITY

The Premier HpSA Flex assay was evaluated for cross-reactivity and microbial interference with the organisms listed below. Each organism was tested at a minimum concentration of 1.0 x 10^7 CFU/mL for bacteria/fungi, or 1.0 x 10^5 $TCID_{50}$/mL for viruses. None of the organisms showed cross-reactivity or microbial interference in the Premier HpSA Flex assay.

Aeromonas hydrophila
Bacillus subtilis
Borrelia burgdorferi
Campylobacter coli
Campylobacter fetus
Campylobacter jejuni
Campylobacter lari
Candida albicans
Citrobacter freundii
Clostridium difficile
Clostridium perfringens
Enterobacter cloacae
Enterococcus faecalis
Escherichia coli O157:H7 (toxigenic)
Escherichia coli 9637
Escherichia coli 8739
Escherichia coli
Escherichia fergusonii
Escherichia hermanii
Escherichia hermanii
Haemophilus influenzae
Klebsiella pneumoniae
Lactococcus lactis
Listeria monocytogenes
Peptostreptococcus anaerobius
Proteus vulgaris
Pseudomonas aeruginosa
Pseudomonas fluorescens
Salmonella enterica susb. enterica serovar Dublin
Salmonella enterica susb. enterica serovar Hilversum
Salmonella enterica susb. enterica serovar Typhimurium
Salmonella Heidelberg (Group B)
Salmonella minnesota
Serratia liquefaciens
Serratia marcescens
Shigella boydii
Shigella dysenteriae
Shigella flexneri
Shigella sonnei
Staphylococcus aureus
Staphylococcus aureus (Cowan's)
Staphylococcus epidermidis
Yersinia enterocolitica

Adenovirus 41
Rotavirus

ANALYTICAL SENSITIVITY

Analytical sensitivity studies were performed to determine the analytical limit of detection (LoD) of quantified *H. pylori* antigen in human stool matrix. The LoD is defined as the lowest concentration of the target analyte that produces positive results ≥ 95% of the time.

The Premier HpSA Flex test can detect ≥ 4.66 ng *H. pylori* protein/mL of unpreserved stool or ≥ 12.00 ng *H. pylori* protein/mL of preserved (Cary-Blair or C&S) stool.

Specimen Type	LoD (ng/mL)
Unpreserved Stool	≥ 4.66
Preserved Stool (Cary-Blair or C&S transport media)	≥ 12.00

TESTS FOR INTERFERING SUBSTANCES

The chemical and interfering substances listed below were evaluated at the indicated concentrations for interference in the Premier HpSA Flex assay. None of the substances showed interference with the Premier HpSA Flex assay performance.

TUMS® (10 mg/500 µL)
Mylanta® (11.5 mg/500 µL)
Pepto-Bismol® (0.44 mg/500 µL)
Tagamet® (cimetidine) (1 mg/500 µL)
Prilosec OTC® (omeprazole) (1 mg/500 µL)
Barium Sulfate (25 mg/500 µL)
Whole Blood (250 µL/500 µL)
Leukocytes (white blood cells) (250 µL/500 µL)
Mucin (17 mg/500 µL)
Hemoglobin (62.5 mg/500 µL)
Stearic Acid (5.3 mg/500 µL)
Palmitic Acid (2.65 mg/500 µL)
NSAID, Ibuprofen (0.25 mg/500 µL)

REFERENCES

1. Hooi James KY, Lai Wan Ying, Ng Wee Khoon, Suen Michael MY. Global Prevalence of *Helicobacter pylori* Infection: Systematic Review and Meta-Analysis, Gastroenterology. 2017; Vol 153, No. 2.
2. Malfertheiner P, Megraud F, O'Morain C, et al. Current concepts in the management of *Helicobacter pylori* infection: the Maastricht III Consensus Report. Gut 2007;56:772–781.
3. Wang C, Yuan Y, Hunt RH. The association between *Helicobacter pylori* infection and early gastric cancer: a meta-analysis. Am J Gastroenterol. 2007;102:1789–1798.
4. International Agency for Research on Cancer *Helicobacter pylori* Working Group. *Helicobacter pylori* Eradication as a Strategy for Preventing Gastric Cancer. (IARC Working Group Reports, No. 8). Lyon, France: International Agency for Research on Cancer, 2014.
5. Guadalupe Ayala, Escobedo-Hinojosa, Wendy Itzel, de la Cruz-Herrera Carlos Felipe, Romero Irma. Exploring alternative treatments for *Helicobacter pylori* infection, World J Gastroenterology. 2014;February 14; 20(6): 1450-1469
6. Garza-González Elvira, Perez-Perez Guillermo Ignacio, Maldonado-Garza Héctor Jesús, Bosques-Padilla Francisco Javier. A review of *Helicobacter pylori* diagnosis, treatment, and methods to detect eradication, World J Gastroenterology. 2014;February 14; 20(6): 1438-1449
7. Moayyedi Paul M, MB, ChB, PhD, MPH, FACG 1 , Lacy Brian E, MD, PhD, FACG 2, Andrews Christopher N, MD 3, Enns Robert A, MD 4, Howden Colin W, MD, FACG 5 and Vakil Nimish, MD, FACG, ACG and CAG Clinical Guideline: Management of Dyspepsia, AM J Gastroenterol advance online publication., 2017;20 June doi 10.1038/ajg.2017.154.
8. Fashner Julia, MD, Gitu Alfred C. MD, Diagnosis and Treatment of Peptic Ulcer Disease and *H. pylori* Infection, Am Fam Physician. 2015;91(4):236-242.
9. Logan Robert PH, Walker Marjorie M. Epidemiology, and diagnosis of *Helicobacter pylori* infection, BMJ. 2001 Oct 20; 323(7318): 920–922.
10. Gatta, Luigi, Vakil, Nimish, Vaira, Dino, et al. Global eradication rates for *Helicobacter pylori* infection: systematic review and meta-analysis of sequential therapy BMJ 2013;347:f4587 doi: 10.1136/bmj.f4587, 7 August 2013.
11. Testerman, Traci L., Morris, James, Beyond the stomach: An updated view of *Helicobacter pylori* pathogenesis, diagnosis, and treatment, World J Gastroenterol. 2014 Sep 28; 20(36): 12781–12808.

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SYMBOL USAGE
You may see one or more of these symbols on the labeling/packaging of this product:
Key guide to symbols

Symbol	Meaning	Symbol	Meaning
	Use By	CONTROL +	Positive control
LOT	Batch Code	CONTROL –	Negative control
IVD	In vitro diagnostic medical device	EC REP	Authorized Representative in the European Community
CE	Meridian products carrying the European Conformity (CE) mark fulfill the requirements of Directive 98/79/EC or the Regulation 2017/746 on in-vitro diagnostic medical devices	SMP PREP DIL SPE	Sample Preparation Apparatus containing Sample Diluent
REF	Catalogue number		Do not freeze
	Consult Instructions for Use	BUF RXN	Reaction Buffer
	Manufacturer		For IVD Performance Evaluation Only
Σ	Contains sufficient for <n> tests	SOLN STOP	Stopping Solution
	Temperature limitation	CONJ ENZ	Enzyme Conjugate
SN	Serial number	CONTROL	Assay Control
TEST	Test Device	REAG	Reagent
	Date of manufacture	BUF WASH	Wash Buffer
BUF	Buffer		Warning
CONJ	Conjugate	DIL SPE	Specimen Diluent (or Sample Diluent)
SUBS	Substrate	BUF WASH 20X	Wash Buffer Concentration: 20X
Rx Only	Prescription Use Only	DET REAG	Detection Reagent
	Do not use if package is damaged	TUBE	Empty Tube
	CAUTION: Risk of Danger	CH REP	Swiss Authorized Representative
2	Single Use Only	PIPETTE	Transfer Pipette

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