A. 510(k) Number:
   K032222

B. Analyte:
   Helicobacter pylori antigens

C. Type of Test:
   Lateral flow immunoassay

D. Applicant:
   Meridian Bioscience Inc.

E. Proprietary and Established Names:
   ImmunoCard STAT! HpSA

F. Regulatory Information:
   1. Regulation section:
      21 CFR Part 866.3110 Campylobacter fetus serological reagents
   2. Classification:
      Class I
   3. Product Code:
      LYR – Campylobacter pylori
   4. Panel:
      83 (Microbiology)

G. Intended Use:
   1. Intended use(s):
      ImmunoCard STAT! HpSA is a rapid in vitro qualitative assay for the
detection of Helicobacter pylori antigen (HpSA) in human stool. The stool
antigen detection is intended to aid in the diagnosis of H. pylori infection and
to demonstrate loss of H. pylori stool antigen following treatment.
Conventional medical practice recommends that testing by any method to
confirm the loss of antigen be done at least four weeks following completion
of therapy.
   2. Indication(s) for use:
      ImmunoCard STAT! HpSA is a rapid in vitro qualitative assay for the
detection of Helicobacter pylori antigen (HpSA) in human stool. The stool
antigen detection is intended to aid in the diagnosis of H. pylori infection and
to demonstrate loss of H. pylori stool antigen following treatment.
Conventional medical practice recommends that testing by any method to
confirm the loss of antigen be done at least four weeks following completion
of therapy.
   3. Special condition for use statement(s):
      Not applicable
   4. Special instrument Requirements:
      Not applicable

H. Device Description:
The ImmunoCard STAT HpSA is a rapid lateral flow immunoassay. It consists of chromatography strips impregnated with monoclonal anti-H. pylori as the capture antibody, red latex-conjugated detector antibody and blue latex-conjugated anti-protein as the detector antibodies for tests and controls respectively. Each strip is enclosed in a plastic frame with a window. The kit also contains positive control which is a dilute suspension of inactivated H. pylori in a buffered solution containing <0.1% sodium azide as a preservative as well as specimen diluent which is a buffered salt solution.

I. **Substantial Equivalence Information:**
   1. **Predicate device name(s):**
      Premier Platinum HpSA
   2. **Predicate K number(s):**
      K983255
   3. **Comparison with predicate:**

<table>
<thead>
<tr>
<th>Item</th>
<th>Device</th>
<th>Predicate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intended use</td>
<td>Detection of H. pylori antigen in stool</td>
<td>Detection of H. pylori antigen in stool</td>
</tr>
<tr>
<td>Assay</td>
<td>Qualitative</td>
<td>Qualitative</td>
</tr>
<tr>
<td>Specimen type</td>
<td>Stool</td>
<td>Stool</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Item</th>
<th>Device</th>
<th>Predicate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Technology</td>
<td>Lateral flow chromatography</td>
<td>Enzyme-linked immunoassay</td>
</tr>
<tr>
<td>Capture antibody</td>
<td>Monoclonal anti-H. pylori</td>
<td>Polyclonal anti-H. pylori</td>
</tr>
<tr>
<td>Conjugate</td>
<td>Red-latex conjugated anti-H. pylori</td>
<td>Rabbit polyclonal antibody specific for H. pylori conjugated to horse radish peroxidase</td>
</tr>
<tr>
<td>Result Interpretation</td>
<td>Visual read; end point visual color line</td>
<td>Results read by spectrophotometer; change in optical density of the solution</td>
</tr>
</tbody>
</table>

J. **Standard/Guidance Document Referenced (if applicable):**
FDA Guidance Document on H. pylori not referenced.

K. **Test Principle:**

ImmunoCard STAT HpSA uses capture solid phase technology to detect the presence of antigen in test specimens. To perform the test, patient stool is added to the Sample Diluent using the applicator stick that is part of the Sample Diluent Vial. The diluted stool sample (approximately a 1 in 10 dilution) is dispensed through the tip of the Sample Diluent Vial into the round sample window of the device. *H. pylori* antigen, if present in the diluted sample, binds to the detector antibody-latex conjugate as the sample moves through the device. The capture monoclonal antibody, which is bound to the assay membrane at reading window, binds the antigen-antibody-
latex complex and yields a visible pink-red line. When no antigen is present, no complex is formed and no pink-red line will appear at the test position of the central window.

A control line, appearing at the control position in the test window, shows whether adequate flow has occurred through the device during a test run. The control line is a protein of nonmammalian origin. Blue latex particles conjugated with a monoclonal antibody to this protein co-migrate with the latex-bound detector antibody during the incubation step. A blue line at the control position on the device should be present each time a specimen or control is tested. If no blue control line is seen, the test is considered invalid.

L. Performance Characteristics (if/when applicable):

1. Analytical performance:
   a. Precision/Reproducibility:
   The reproducibility of ImmunoCard STAT! HpSA was determined with known negative (n = 5) and positive (n = 5) samples, that were coded and randomly sorted to prevent their identities. Two of the five positive samples were near the limit of detection for the assay. The reproducibility samples were tested on three consecutive days by three independent test sites. Intra-assay and interassay reproducibility was 100%.

<table>
<thead>
<tr>
<th>Sample Status</th>
<th>Premier OD reading</th>
<th>Referee (MBI)</th>
<th>Clinical Site #1</th>
<th>Clinical Site #2</th>
<th>Clinical Site #3</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Premier</td>
<td>Day 1</td>
<td>Day 2</td>
<td>Day 3</td>
<td>Day 1</td>
</tr>
<tr>
<td>Neg</td>
<td>0.017</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Pos</td>
<td>0.737</td>
<td>4/5</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Neg</td>
<td>0.016</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Pos</td>
<td>1.140</td>
<td>7</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Neg</td>
<td>0.028</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Low Pos</td>
<td>1.442</td>
<td>1</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Neg</td>
<td>0.042</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Low Pos</td>
<td>1.041</td>
<td>2</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Pos</td>
<td>1.493</td>
<td>5</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Neg</td>
<td>0.058</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Pos Cont</td>
<td>2.309</td>
<td>N/A</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Neg Cont</td>
<td>0.034</td>
<td>N/A</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

* The signal intensity (strength) of a positive reaction in ImmunoCard STAT! will not necessarily correlate with the OD value obtained in Premier Platinum HpSA EIA.

Legend: 0 = negative, 1-10 = semiquantitative scoring scale used in the interpretation of ImmunoCard STAT! positive test results. (A value was assigned to the intensity of color in the Test Line, where 1 is the weakest visible positive reaction and 10 is the strongest. A 4/5 means the reaction fell between a grade of 4 and a grade of 5.) w = weak (correlates with a semiquantitative reaction grade of +/-, 1 or 2)

b. Linearity/assay reportable range:
   Not applicable

c. Traceability (controls, calibrators, or method):
   Not applicable
d. Detection limit:
The lower limit of detection of this assay is 64 ng/mL in tests with sonicated antigen prepared from *H. pylori* strain TV1970. This limit does not vary from formed (solid) to semi-solid stool.

e. Analytical specificity:
The specificity of ImmunoCard STAT! HpSA was tested utilizing the following bacterial, viral and yeast strains. Positive and negative stools were spiked with $\geq 1 \times 10^8$ bacteria or yeast. None of the microorganisms tested yielded a positive result in the negative stool or interfered with detection of the positive stool. Both the negative and positive stool was positive when spiked with *Helicobacter pylori* strain 43504.

Adenovirus Type 2
Adenovirus Type 40
Coxsackie Type B1
Coxsackie Type B6
Echovirus Type 22
Feline calicivirus
Rotavirus
*Aeromonas hydrophila*
*Campylobacter coli*
*Campylobacter jejuni*
*Candida albicans*
*Citrobacter freundii*
*Clostridium perfringens*
*Clostridium difficile* (2)
*Enterobacter cloacae*
*Enterococcus faecalis* (2)
*E. coli* (2)
*E. coli* 0157:H7 (2)
*E. fergusonii*
*Helicobacter felis*
*Klebsiella pneumoniae*
*Proteus vulgaris*
*Pseudomonas aeruginosa*
*Salmonella dublin*
*Salmonella (Group B)*
*Salmonella hilsversum*
*Salmonella minnesota*
*Salmonella typhimurium*
*Staphylococcus aureus*
*Staphylococcus aureus* (Cowan I)
*Staphylococcus epidermidis*
*Serratia liquefaciens*
*Shigella boydii*
*Shigella dysenteriae*
*Shigella flexneri*
*Shigella sonnei*
*Yersinia enterocolitica*
**TESTS FOR INTERFERING SUBSTANCES**

The following substances were found to have no effect on results when present in stool at the concentrations indicated.

- Tums® Antiacid (5 mg/mL)
- Tagamet® (5 mg/mL)
- Prilosec® (5 mg/mL)
- Mylanta® Antacid (1:20)
- Pepto-Bismol® (1:20)
- Barium sulfate (5%)
- Whole Blood (50%)
- Leukocytes (50%)
- Mucin (3.4%)
- Stearic acid/palmitic acid (fecal fat) (4%)
- Hemoglobin (tarry stool) (12.5%)

**f. Assay cut-off:**

The assay cut off is 64 ng/ml of H. pylori antigen

2. **Comparison studies:**

   a. **Method comparison with predicate device:**

   **Comparative studies:** Four independent laboratories tested specimens in parallel with ImmunoCard STAT! HpSA and a reference ELISA in vitro diagnostic method, Premier Platinum HpSA (Meridian Bioscience, Inc, Cincinnati, OH). Some samples giving discordant results between the two assays were sent to and evaluated by a reference laboratory. The results of the parallel tests are given below. Corrected results are calculated following investigation of discordant samples by the referee laboratory.

<table>
<thead>
<tr>
<th></th>
<th>Initial Trial Results</th>
<th>Corrected Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total samples tested</td>
<td>457</td>
<td>457*</td>
</tr>
<tr>
<td>Concordant test results</td>
<td>433</td>
<td>436</td>
</tr>
<tr>
<td>Positive samples</td>
<td>102</td>
<td>105</td>
</tr>
<tr>
<td>Negative samples</td>
<td>331</td>
<td>331</td>
</tr>
<tr>
<td>Discordant test results</td>
<td>21</td>
<td>20</td>
</tr>
<tr>
<td>Premier +, ImmunoCard -</td>
<td>6</td>
<td>6</td>
</tr>
<tr>
<td>Premier -, ImmunoCard +</td>
<td>15</td>
<td>14</td>
</tr>
<tr>
<td>Indeterminant</td>
<td>3</td>
<td>1</td>
</tr>
<tr>
<td>Premier Equivocal, ImmunoCard +</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>Premier Equivocal, ImmunoCard -</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>% correlation</td>
<td>95%</td>
<td>N/A</td>
</tr>
</tbody>
</table>

* Two discordant samples were QNS for follow up analysis.

   b. **Matrix comparison:**

   Not applicable

3. **Clinical studies:**

   a. **Clinical sensitivity:**

   **Clinical studies:** Stool samples from 227 consecutive dyspeptic patients, who were not using acid suppressant therapy or antibiotics, and who were referred for endoscopy were tested with ImmunoCard STAT! HpSA. Biopsy specimens were taken for histology, rapid urease test and culture. Patients were defined as infected with *H. pylori* if histology and urease tests were
positive, or if culture was positive. Eighty five of the 227 patients were found *H. pylori* positive. The results are summarized in the following table.

**Diagnostic accuracy of ImmunoCard STAT! HpSA before and after *H. pylori* eradication treatment.**

<table>
<thead>
<tr>
<th></th>
<th><em>H. pylori</em> status by endoscopy/biopsy/gold standard</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>True Positive</td>
<td>True Negative</td>
</tr>
<tr>
<td>IC STAT! HpSA +</td>
<td>77</td>
<td>12</td>
</tr>
<tr>
<td>IC STAT! HpSA -</td>
<td>8</td>
<td>130</td>
</tr>
<tr>
<td>Total</td>
<td>85</td>
<td>142</td>
</tr>
</tbody>
</table>

Estimated clinical sensitivity (95% CI) 90.6% (84.9 to 97.1%)
Estimated clinical specificity (95% CI) 91.5% (87.5 to 96.5%)
Predictive value, positive test (95% CI) 86.5% (79.9 to 94.1%)
Predictive value, negative test (95% CI) 94.2% (90.1 to 97.9%)
Correlation (CI 95%) 91.2% (87.3 to 94.7%)

**Correlation of ImmunoCard STAT! HpSA test results with eradication treatment**

<table>
<thead>
<tr>
<th></th>
<th><em>H. pylori</em> status by endoscopy/biopsy/gold standard</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>True Positive</td>
<td>True Negative</td>
</tr>
<tr>
<td>IC STAT! HpSA +</td>
<td>21</td>
<td>0</td>
</tr>
<tr>
<td>IC STAT! HpSA -</td>
<td>1</td>
<td>63</td>
</tr>
<tr>
<td>Total</td>
<td>22</td>
<td>63</td>
</tr>
</tbody>
</table>

Estimated clinical sensitivity (95% CI) 95.4% (86.0 to 100%)
Estimated clinical specificity (95% CI) 100%
Predictive value, positive test (95% CI) 100%
Predictive value, negative test (95% CI) 98.4% (94.5 to 100%)
Correlation (CI 95%) 98.8% (96.8 to 100%)

b. **Clinical specificity:**
Refer to (a) above

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

4. **Clinical cut-off:**
See assay cut off above

5. **Expected values/Reference range:**
Studies on the epidemiology of *H. pylori* have shown that this organism is present worldwide. Gastritis caused by *H. pylori* has been shown to correlate with age, ethnic background, family size and socioeconomic class. The prevalence of *H. pylori* infection in a given population can vary from 20% to 90%. In patients diagnosed with duodenal ulcers, however, it has been shown in every age group to be approximately 80%. Currently recommended eradication treatments have an efficacy rate between 75% and 90%.
The ImmunoCard STAT! HpSA 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 positives may vary depending on geographic location, method of specimen collection, handling and transportation, test employed and general health environment of patient population under study.

**M. Conclusion:**

The ImmunoCard STAT! HpSA is substantially equivalent in performance to the predicate device for the detection of Helicobacter pylori antigen in human stool.