H. pylori Rapid Test-Whole Blood/Serum (Cassette)

One Step Assay

Rapid Visual Results
For Qualitative In Vitro Diagnostic Use

INTENDED USE
The H. pylori Rapid Test is a rapid lateral flow, qualitative immunoassay. It is intended for use at point of care facilities to detect the presence of IgG antibodies specific to Helicobacter pylori (H. pylori) in human blood or serum. It provides an aid in the diagnosis of infection by H. pylori. This test has been evaluated for use with serum specimens of adults, 19 years and older.

SUMMARY AND EXPLANATION
Helicobacter pylori has been associated with a variety of gastrointestinal diseases including gastritis, duodenal and gastric ulcer, non-ulcer dyspepsia, gastric adenocarcinoma and lymphoma. The exact role that H. pylori plays in gastrointestinal disease still needs to be precisely defined and is the subject of ongoing research. However, the prevalence rates for H. pylori infection as determined by histological and bacteriological methods can approach 90% in patients who present clinical symptoms of the gastrointestinal diseases listed above. H. pylori does not appear to invade the bloodstream since no isolates yet have been detected using commercial blood culture methods. H. pylori infections occur in human populations throughout the world. In developed countries, about 50% of the population may have H. pylori infection by the age of 60 years, while only 10-20% of adults in the third decade of life have it. In patients who present clinical symptoms relating to the gastrointestinal tract there are two major methods of investigation: invasive and non-invasive. Invasive methods include culture of gastric biopsy samples, histologic examination of stained biopsy specimens, or direct detection of the urease activity in the biopsy (CLO test). These methods need to obtain a biopsy sample by endoscopy, which is expensive, and usually results discomfort and risk to the patient. Non-invasive techniques include urea breath tests and serological methods. Urea breath test requires the use of a small amount of radioactivity and a mass spectrometer. Serologic tests are employed to detect antibodies as human immune response to H. pylori. Two methods appear to be of great interest regarding their use in H. pylori routine serology, namely the ELISA and the Western immunoblot because they offer the most versatility in regards to immunoglobulin specificity and relative ease of use.

This H. pylori Rapid Test detects IgG antibodies specific to H. pylori infection in patient’s blood or serum. It is a noninvasive method and does not use radioactive isotopes; the assay procedures are easy and do not require professional training; it provides a rapid result. It is a useful on-site aid in the diagnosis of H. pylori infection.

PRINCIPLE OF THE TEST
This assay is a double antigen chromatographic lateral flow immunoassay. The test strip in the device includes: 1) a burgundy-colored conjugate pad containing colloidal gold coupled with H. pylori antigens, and 2) nitrocellulose membrane containing a test line (T line) and a control line (C line). The T line is coated with H. pylori antigens, and the C line is coated with a goat anti-H. pylori antibody. The antigens used in this device are from H. pylori cell lysate. When IgG antibodies specific to H. pylori are present in the specimen, the T line will become a burgundy-colored band. If antibodies to H. pylori are not present or are present below the detectable level, no T line will develop. The C line should always appear, regardless of the presence of antibodies to H. pylori. The C line serves as an internal quality control of the test system to indicate that an adequate volume of specimen has been applied and the flow occurred.

MATERIALS AND REAGENTS PROVIDED
- 25 test devices, each sealed in a pouch with a dropper pipette.
- 1 bottle of wash buffer-7 ml PBS diluent with 0.02% sodium azide as a preservative.
- 1 package insert (Instruction for Use).

MATERIALS NEEDED BUT NOT PROVIDED
- Lancet or other blood collection device
- Timer

STORAGE
Store kit at 15-30°C (59-86°F). Kit contents are stable for 2 years or until the expiration date printed on the label, whichever comes first.

Exposing the kit to temperatures over 30°C may reduce the shelf life or damage the device.

Do not expose the kit to temperatures over 30°C (86°F).

SPECIMEN COLLECTION AND STORAGE
1. Serum
   - Follow standard laboratory procedures to collect serum specimens.
   - Serum specimens can be stored at 9-30°C (48-86°F) for 8 hours, at 2°-8°C (36-46°F) for one week, and at ≤ -20°C (-4°F) or lower for long term storage. Repeatedly frozen and thawed specimens are not recommended for this assay.
   - Any sediment in serum specimens should be removed by centrifugation. Avoid using any turbid specimens, which may be contaminated by microorganisms.

2. Whole Blood
   - Fingerstick sampling is recommended for this assay
   - Middle or ring finger is the preferred puncture site
   - Clean patient’s finger with an alcohol swab. Wait until it is dry.
   - Puncture the fingertip with the lancet. Wipe away first sign of blood.
   - Gently rub the hand from palm to finger to help form a drop of blood over the punctured site.
   - Use the provided pipette to pick up the blood, and apply one drop of the blood to the sample well of the device. Then, follow the procedure.

PRECAUTIONS
1. The instructions must be followed to obtain accurate results.
2. Appropriate precautions are necessary in the collection, handling of the specimens and use of test materials as potentially biohazardous.
3. For each specimen, use a disposable pipette, and a test device. Do not reuse the pipette and device.
4. Do not use kit beyond the expiration date, which appears on the package label.

PROCEDURE
1. Refrigerated specimens and other test materials, including devices, must be equilibrated to room temperature before testing
2. Remove the device from its wrap pouch prior to performing the assay. Label the device with identification.
3. Add one drop of fresh blood or serum to the sample well marked "S". Allow about 30 seconds for the specimen to be absorbed totally. Discard the first three drops of wash buffer from the wash buffer squeeze bottle. Then add three drops of wash buffer into the sample well.
4. Strong positive results may be observed in 2-3 minutes. Weak positive results may take a longer time, up to 7 minutes. For the whole blood test, a slight hemolysis might be observed, but it does not interfere with the results.

INTERPRETATION OF RESULTS

IMPORTANT: Do not interpret the results after 7 minutes. The T Line should always be interpreted independently of the C Line.

C T
(+) C T C T C T
POSITIVE NEGATIVE INVALID

Positive:
If both the C line and T line appear, the result indicates that the IgG antibodies specific to H. pylori are detected and the result is positive.

A faint line in test region indicates a borderline specimen, which should be re-tested using an alternative method for confirmation.

Negative:
If only the C line appears in the control region, the test indicates that no antibodies to H. pylori are detected and the result is negative.

Invalid:
When no control line appears within 5 minutes, repeat the test with a new test device.

QUALITY CONTROL PROCEDURE
- Built-in Control Features
  This test contains a built-in quality control feature, the C line. The appearance of the burgundy C line indicates that an adequate volume of specimen and wash buffer has been applied and the flow occurred.
- External Quality Control
  External controls are recommended, positive and negative, to monitor the performance of the assay.

LIMITATIONS
This test is a qualitative assay for professional in vitro diagnostic use only. A positive result does not distinguish active infection from colonization of H. pylori. Therefore, positive results should always be evaluated with other confirmatory methods available to the physician. This assay has not been established for patients less than 19 years of age.

Literature references have suggested cross reactivity of IgG antibody with other closely related organisms such as Borrelia burgdorferi and Pseudomonas species. However, performance of this assay has not been evaluated with these organisms. Therefore, the specificity of this device is not known if this organism is encountered.
EXPECTED VALUES

*H. pylori* infections occur in human populations throughout the world, but the prevalence of infection in the population varies with age, standards of hygiene, geographical regions, and probably socioeconomic status. In developed countries, about 50% of the population may have *H. pylori* infection by the age of 80 years, while only 10-20% of adults in the third decade of life have it. People in developing countries tend to have higher prevalence.

PERFORMANCE CHARACTERISTICS

A. Sensitivity and Specificity

This device was evaluated with 296 confirmed clinical serum samples, 144 were positive and 152 were negative. All the specimens were blind labeled. The evaluations were conducted in house.

<table>
<thead>
<tr>
<th>Serum Test</th>
<th>Positive</th>
<th>Negative</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive</td>
<td>146</td>
<td>1</td>
<td>147</td>
</tr>
<tr>
<td>Negative</td>
<td>0</td>
<td>149</td>
<td>149</td>
</tr>
<tr>
<td>Total</td>
<td>146</td>
<td>150</td>
<td>296</td>
</tr>
</tbody>
</table>

The agreement between these two devices is 97.9% (142/145) for positive specimens, and 97.4% (141/152) for negative specimens. This study demonstrated that the *H. pylori* Rapid Test-Whole Blood/Serum is substantially equivalent to the marketed device.

B. Comparison with a legally marketed device

A side-by-side comparison study between the *H. pylori* Rapid Test-Whole Blood/Serum and a marketed device was conducted. Two hundred and ninety-six (296) clinical serum specimens were evaluated with the *H. pylori* Rapid Test-Whole Blood/Serum and the marketed device. The results were summarized in the table below.

<table>
<thead>
<tr>
<th>Analytes</th>
<th>Conc. (g/ml)</th>
<th>Specimens</th>
</tr>
</thead>
<tbody>
<tr>
<td>Albumin</td>
<td>20</td>
<td>Positive</td>
</tr>
<tr>
<td>Bilirubin</td>
<td>10</td>
<td>Negative</td>
</tr>
<tr>
<td>Hemoglobin</td>
<td>15</td>
<td>Positive</td>
</tr>
<tr>
<td>Cholesterol</td>
<td>20</td>
<td>Negative</td>
</tr>
<tr>
<td>Uric Acid</td>
<td>200</td>
<td>Positive</td>
</tr>
<tr>
<td>Lipids</td>
<td>20</td>
<td>Negative</td>
</tr>
</tbody>
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3. Some other Common Biological Analytes were spiked into the *H. pylori* positive and negative specimens and tested separately. No significant interference was observed at the levels listed in the table below.

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<th>Specimens</th>
</tr>
</thead>
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<tr>
<td>Acetaminophen</td>
<td>200</td>
<td>Positive</td>
</tr>
<tr>
<td>Acetylsalicylic Acid</td>
<td>200</td>
<td>Negative</td>
</tr>
<tr>
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<td>Positive</td>
</tr>
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E. Reproducibility

Reproducibility studies were performed for the *H. pylori* Rapid Serum Test at three physician office laboratories (POL). Sixty (60) clinical serum specimens, 20 negative, 20 borderline positive and 20 positive, were used in this study. Each specimen was run in triplicate for three days at each POL. The intra-assay agreements were 100% at two sites, and 99.4% at one site. The inter-assay agreement was 100% at two sites and 99.8% at one. The inter-site agreement was 99.9%.

In addition, the *H. pylori* Rapid Whole Blood/Serum Test was evaluated with a panel of sixty (60) clinical serum specimens and a panel of sixty (60) whole blood samples spiked with different levels of IgG antibodies specific to *H. pylori*. In each panel there were twenty (20) negative, twenty (20) borderline-line positive, and twenty (20) positive, accordingly. The samples were blind labeled and tested at three physician’s office laboratories (POL). In summary, results obtained were the same as expected for all specimens at all evaluation sites except that one borderline positive was missed at one site. The agreement for clinical serum specimens was 100% at all three evaluation sites. The agreement for spiked whole blood was 100% at two sites and 98.3% at one site.

REFERENCES