**NAME AND INTENDED USE**

The HCD’S RapidTest *H. pylori*™ Screen is an immuno-chromatographic assay for qualitative determination of antibodies to *Helicobacter pylori* (*H. pylori*) in human serum or whole blood. The test is intended for professional use as an aid in the diagnosis of *H. pylori* infections.

**SUMMARY**

*Helicobacter pylori* (also known as *Campylobacter pylori*) are gram negative bacteria which can infect gastric mucosa. *H. pylori* infection has been shown to be associated with the causative agent of type B active chronic gastritis,(1,2) gastric lesions and some cases of duodenal ulcers.(3,4) The most definitive tests for diagnosing *H. pylori* infection involve histological staining and/or culture of antral biopsies.(5) However, these techniques are invasive, and alternative, non-invasive methods, such as, the urea breath test and serological test have been developed.(6) Various serological methods have been employed, including the complement fixation test, the bacterial agglutination test, the passive hemagglutination test, the hemagglutination assay, immunoblotting techniques, and enzyme-linked immunoassays.(7-9) All of these techniques have demonstrated a correlation between the level of reactivities and the presence of *H. pylori* in the gastric antrum.

The HCD’S RapidTest *H. pylori*™ Screen is a rapid immuno-chromatographic assay for qualitative determination of anti-*H. pylori* antibodies of all isotypes (IgG, IgM, IgA, etc.). It is a presumptive test intended for use as an aid in the diagnosis of *H. pylori* infections.

**PRINCIPLE OF THE TEST**

The immuno-chromatographic device contains dye-conjugated and immobilized *H. pylori* antigens which, in the presence of the antibody, combine to produce an antigen-antibody-dye conjugate sandwich, that appears as a distinctive visual pattern in the test zone of the device. The antibody in the test specimen is detected in approximately 10 minutes.

In the test procedure the specimen is allowed to migrate through the absorbent area of the device. If the antibody against *H. pylori* is present, labeled antigen-dye conjugate binds to it, forming an antigen-antibody-dye complex. The presence of the antibody is visually determined as a pink-rose color band when immobilized antigen in the Test Zone (“T”) captures the complex forming an antigen-antibody-antigen-dye sandwich. Proper test performance is verified in the Control Zone (“C”) by the appearance of a pink-rose band, produced by a parallel immunochromatographic reaction as the immobilized reagent captures dye conjugate regardless of the antibody in the test sample.

**REAGENTS AND MATERIALS PROVIDED**

1. Test Dipsticks
   
   Contains dye-conjugated and membrane-immobilized *H. pylori* antigens in a protein matrix with sodium azide.

2. Extraction Buffer, 11.0ml
   
   Inactivated serum containing antibody against *H. pylori* and preservative, in a dropper vial.

3. Positive Control, 1.0ml
   
   Buffered solution containing serum proteins and preservative, in a dropper vial.

4. Negative Control, 1.0ml
   
   Buffer solution containing serum proteins and preservative, in a dropper vial.

5. Transfer Pipette
6. Test Instructions

Optional:

7. Test Cup
8. Test Cup Holder

**MATERIALS REQUIRED BUT NOT PROVIDED**

1. Timer.
2. Specimen collection container.

**STORAGE AND STABILITY**

Test strips may be stored at 2°C-28°C. All controls must be refrigerated at 2°C-8°C; do not freeze any test components. Refer to the expiration date for stability.

**WARNINGS AND PRECAUTIONS**

1. Follow proper laboratory procedures for handling specimens and reagents.
2. For in vitro diagnostic use only.
3. Handle all patient samples and controls as though capable of transmitting disease. Observe established precautions against microbiological hazard, including the use of disposable gloves throughout the assay procedure.
4. Clean spills thoroughly using an appropriate intermediate-to-high level disinfectant.
5. Do not pipette samples by mouth.
6. Do not use test kits or components after the expiration date printed on the label.
7. Do not mix reagents or vial covers from different lots.

**KIT STORAGE**

Store the test kit between 4-8°C., do not freeze. Refer to the expiration date for stability. Test Strips have a stability of 12 months when stored properly.

**SPECIMEN COLLECTION**

The HCD’S RapidTestH‌‌ pylori™ Screen may be performed using serum, plasma and whole blood.

1. **SERUM**

   Collect blood aseptically by venipuncture into a clean test tube without anticoagulants. Permit blood to clot for twenty to thirty minutes at room temperature. Centrifuge to obtain clear serum and transfer serum into a clean plastic or glass test tube. Specimens containing precipitate may yield inconsistent test results. Such specimens must be clarified prior to assaying.

   If specimens are not tested immediately, they should be refrigerated at 4-8°C. For storage periods greater than three days, freezing is recommended. If specimens are to be shipped, they should be packed in compliance with federal regulations covering the transportation of etiologic agents.

2. **PLASMA**

   Collect blood aseptically by venipuncture into a clean test tube containing a suitable anticoagulant, such as, Heparin or Sodium Citrate. Centrifuge to obtain clear plasma and transfer plasma into a plastic or glass test tube. Specimens containing precipitate may yield inconsistent test results. Such specimens must be clarified prior to assaying. Store plasma as noted above for serum.

3. **WHOLE BLOOD**

   Collect blood aseptically using the same procedure as stated above for plasma. Alternatively whole blood may be used from a finger aseptically punctured with a lancet.

**ASSAY PROCEDURE**

NOTE: Read all procedural instructions before running patient samples or controls.

**Procedure Notes:**

1. Bring all specimens and controls to room temperature (15-28°C) prior to testing.

2. Do not open the protective foil pouch until ready to perform the test.
3. Do not use commercial controls other than those provided with the kit as they may contain additives which interfere with test performance.

**Test Procedure:**

1. Remove the Test Dipstick from its foil wrapper by tearing along the “notch”.
2. Add 2 drops of sample (Take approximately 50 ul of specimen and place in the sample well. For optimal results, a hanging drop of blood (between 50 and 100 ul) should be used). and 6 drops of Extraction Buffer to an appropriate cup using a transfer pipette. Swirl gently to mix.
3. Place a Test Dipstick in the cup. Allow the specimen to be absorbed into the sample area.

**NOTE:** The level of the sample solution should not exceed the maximum fill line on the test strip.

4. Wait 10 minutes and read the results.

**NOTE:** To avoid an incorrect reading or invalid result, do not interpret test results after more than 10 minutes.

   Read test between 5-10 minutes after application of specimen. Test results **should not** be interpreted after 15 minutes.

**INTERPRETATION OF RESULTS**

1. **Positive.** Two pink-rose bands appear one in control (C) and one in Test Zone (T). The sample should be considered positive for the presence of antibodies to *H. pylori*.  
2. **Negative.** One rose-pink band appears in the Control Zone (C), with no apparent band in the Test Zone (T). The sample should be considered negative for antibodies to *H. pylori*.  
3. **Invalid.** There are no color bands on the membrane, or a band is visible in the test zone but not in the control zone the test is invalid. The specimen should be re-tested using a new device If no rose-pink band appears in the Control Zone, or if a band appears in the Test Zone but not in the Control Zone, then the test is invalid. It is recommended that the specimen be retested using a new device.
**LIMITATIONS OF THE TEST**

1. The test is limited to the detection of antibodies to *H. pylori* in human serum.
2. The test is for in vitro diagnostic use only.
3. Although the test is very accurate, a low incidence of false results can occur.
4. If negative or questionable results are obtained, the test should be repeated on a fresh specimen.
5. As with all diagnostic tests, a definitive clinical diagnosis should not be based on the results of a single test, but should only be made by the physician after all clinical and laboratory findings have been evaluated.
6. The test is designed to detect anti-*H. pylori* antibodies. It is not designed to differentiate between different types of infection (current, ongoing, etc).

**SPECIFICITY AND SENSITIVITY**

A study was performed with 439 patient serum samples which included both symptomatic GI disorders and samples from non-symptomatic patients. The RapidTest *H. pylori*™ Screen and a traditional enzyme immunoassay were tested on all specimens. Of these, 173 were confirmed *H. pylori* positive and 266 were confirmed *H. pylori* negative using the traditional enzyme immunoassay. Fourteen samples tested negative with the RapidTest *H. pylori*™ Screen and positive with the enzyme immunoassay. In addition, 9 samples tested negative with the enzyme immunoassay and positive with the RapidTest *H. pylori*™ Screen. The results of this comparison evaluation are shown in the table below:

<table>
<thead>
<tr>
<th>HCD’S’s RapidTest <em>H. pylori</em>™ Screen</th>
<th>Positive</th>
<th>Negative</th>
</tr>
</thead>
<tbody>
<tr>
<td>Elisa Test</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Positive (173)</td>
<td>159</td>
<td>14</td>
</tr>
<tr>
<td>Negative (266)</td>
<td>9</td>
<td>257</td>
</tr>
</tbody>
</table>

Compared with a conventional enzyme immunoassay for the detection of antibodies to *H. pylori* from serum specimens, the RapidTest *H. pylori*™ Screen demonstrated a relative sensitivity of 92% (159/173) and a relative specificity of 96.6% (257/266). The fourteen positive *H. pylori* antibody results which initially tested negative with the RapidTest *H. pylori*™ Screen were retested by enzyme immunoassay with equivocal results. The 9 negative *H. pylori* antibody results which initially tested positive with the RapidTest *H. pylori*™ Screen were retested by enzyme immunoassay; 5 samples were weak positive.

**PRECISION**

A. Intra-assay precision was determined by assaying 11 replicates of confirmed negative, low and high antibody positive *H. pylori* patient samples.

<table>
<thead>
<tr>
<th>Number of Determinations</th>
<th>Negatives</th>
<th>Low</th>
<th>Positives</th>
</tr>
</thead>
<tbody>
<tr>
<td>Expected Results</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Observed Results</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

A. Inter-Assay Precision was determined by assaying the 11 replicates of confirmed negative, low and high antibody positive *H. pylori* patient samples at 3 independent test sites with 3 separate lots of reagent. Negative specimens tested negative after 10 minutes each time with all 3 reagent sets at all 3 test sites. Low and high antibody *H. pylori* patient samples tested positive within 10 minutes each time with all 3 lots at all 3 test sites. The HCD’S RapidTest *H. pylori*™ Screen demonstrates excellent reproducibility.

**CROSS-REACTIVITY**

To test the cross-reactivity of the HCD’S RapidTest *H. pylori*™ Screen, the following organisms were harvested on blood agar plates:

- *Campylobacter jejuni*
- *Campylobacter fetus*
- *Campylobacter coli*
- *Escherichia coli*

The membranes were coated with the extract of these organisms. The assays were then performed simultaneously with membrane coated with extract of *H. pylori*. Forty serum samples were used for this experiment and yielded negative results with membranes coated with the above organisms. Thirty of the forty serum samples tested positive with the HCD’S RapidTest *H. pylori*™ Screen and ten of forty serum samples tested negative with the HCD’S RapidTest *H. pylori*™ Screen. These results indicate that no cross-reactivity was observed. A second set of cross-reactivity experiments was performed using 10µg of each of the extraction organisms identified above to absorb the positive patient serum samples. The patient sera were then analyzed by the HCD’S RapidTest *H. pylori*™ Screen. Only the antigen from *H. pylori* absorbed out the antibodies observed.

**BIBLIOGRAPHY**


