Diagnostic Automation / Cortez Diagnostics, Inc.
IMMUNO DIAGNOSTICS

CE

OneStep
H. pylori/ Serum
RapiCard™ InstaTest

REF 118561-1-19

IVD 1 Test

Specificity	**92.8 %**
Sensitivity	**95.1 %**

INTENDED USE

The *H. pylori* RapiCard™ Insta 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 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.[1-3] The exact role that *H. pylori* plays in gastrointestinal disease still needs to be precisely defined. However, the prevalence rates for *H. pylori* infection as demonstrated 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 have it.[4, 5]

In patients who present clinical symptoms relating to the gastrointestinal tract there are two major methods of investigation: invasive and noninvasive. 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. Noninvasive 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*.[5]

This device detects IgG antibodies specific to *H. pylori* infection in patient's sera and plasma. 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.

TEST PRINCIPLE

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 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 as a burgundy-colored band regardless of the presence of antibodies to *H. pylori*. The C line serves as an internal qualitative control of the test system to indicate that an adequate volume of specimen has been applied and the flow occurred.

SPECIMEN COLLECTION AND PREPARATION

1. Follow standard laboratory procedures to collect serum or plasma specimens.
2. 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.
3. Any sediment in serum specimens should be removed by centrifugation. Avoid using any turbid specimens, which may be contaminated by microorganisms.

MATERIALS AND COMPONENTS

Materials provided with the test kits

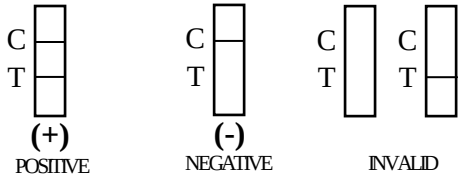
1. 25 test devices, each sealed in a pouch with a dropper pipette.
2. 1 package insert (Instructions for Use).

ASSAY PROCEDURE

1. Refrigerated specimens and other test materials, including devices, **must be equilibrated to room temperature before testing to avoid invalid results**.
2. Remove the device from the pouch and place it on a flat surface. Label the device with specimen identification.
3. Holding the dropper vertically, add four drops of serum or plasma specimen into the sample well.
4. Strong positive results may be observed in 2-3 minutes. Weak positive results may take a longer time. The results should be read within 4-7 minutes.

RESULTS

- **Positve:** 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.



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

PERFORMANCE CHARACTERISTICS

A. Sensitivity and Specificity

This device was evaluated with 296 confirmed clinical serum specimens, 144 were positive and 152 were negative. All the specimens were blind labeled. The evaluations were conducted off-site at three physician's office laboratories and one medical reference laboratory by personnel with diverse working experience and education backgrounds.

	Clinical confirmed results		Total
	Positive	Negative	
Positive	137	11	148
Negative	7	141	148
Total	144	152	296

The total positive results from the four evaluation sites were 137 (36+36+31+34) out of the 144 clinically confirmed positive specimens, indicating an overall sensitivity of 95.1% (137/144) for this device. The total negative results from the four evaluation sites were 141 (34+36+39+32) out of the 152 clinically confirmed negative specimens, indicating an overall specificity of 92.8% (141/152) for this device.

B. Comparison with a legally marketed device

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

	Marketed *H. pylori* Test		Total
	Positive	Negative	
Positive	143	4	147
Negative	2	147	149
Total	145	151	296

The agreement between these two devices is 98.6% (143/145) for positive specimens, and 97.4% (147/151) for negative specimens. This study demonstrated that the *H. pylori* Rapid Test is substantially equivalent to the marketed device.

C. Cross Reactivity and Interference

1. Other closely related microorganisms were evaluated for cross reactivity with the test. Proteins of those microorganisms were spiked into the *H. pylori* positive and negative specimens at a high concentration and tested separately. None of the microorganisms affected the test results, positive or negative.

Analytes	Conc. (mg/ml)	Specimens	
		Positive	*B. Negative*
E. coli	10	+	-
C. coli	10	+	-
C. jejuni	10	+	-
C. fetus	10	+	-
Proteus	10	+	-
N. gonorrhea	10	+	-
Streptococcus	10	+	-
Staphylococcus	10	+	-

2. Potentially cross-reactive endogenous substances including common serum components, such as lipids, hemoglobin, bilirubin, were spiked at high concentrations into the *H. pylori* positive and negative specimens and tested, separately. No cross reactivity or interference was observed to the device.

Analytes	Conc.	Specimens	
		Positive (+)	Negative (-)
Albumin	20 mg/ml	+	-
Bilirubin	10 µg/ml	+	-
Hemoglobin	10 µg/ml	+	-
Glucose	20 mg/ml	+	-
Uric Acid	200 µg/ml	+	-
Lipids	20 mg/ml	+	-

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.

Analytes	Conc. (µg/ml)	Specimens	
		Positive (+)	Negative(-)
Acetaminophen	200	+	-
Acetoacetic Acid	200	+	-
Acetylsalicylic Acid	200	+	-
Benzoylecgonine	100	+	-
Caffeine	200	+	-
DMSO	5 %	+	-
EDTA	800	+	-
Ethanol	1.0 %	+	-
Gentisic Acid	200	+	-
β - Hydroxybutyrate	20,000	+	-
Methanol	10.0 %	+	-
Phenothiazine	200	+	-
Phenylpropanolamine	200	+	-
Salicylic Acid	200	+	-

D. Reproducibility

To assess the precision of this device, a negative, a borderline positive and a high positive serum specimen were tested in ten replicates by four (4) operators independently. All results obtained were 100% in agreement with the expected results. No within-assay or between-assay discrepancy was observed.

Reproducibility studies were performed on 20 negative, 20 borderline positive and 20 positive serum specimens at three

physician's office laboratories (POL). Each specimen was run in triplicate for three days at each POL. All the intra-assay agreements were 100% except one, which was 99.4%. The inter-assay agreement was 100% at two POLs and 99.8% at one. The inter-site agreement was 99.9%.

QUALITY CONTROL

- **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 that an adequate volume of specimen 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 OF PROCEDURE

1. 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.
2. 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 60 years, while only 10-20% of adults in the third decade of life have it. People in developing countries tend to have higher prevalence[5].

PRECAUTION

1. The instructions must be followed to obtain accurate results.
2. Appropriate precautions are necessary in the collection, handling of the specimens and used assay 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.

STORAGE

1. 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
2. Exposing the kit to temperatures over 30°C may reduce the shelf life or damage the device. Freezing to -70°C (-94°F) will not cause damage to the device.

Do not expose the kit to temperatures over 30℃ (86°F). **15°** **30°C**

ISO 13485
ISO 9001

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