H. pylori Whole Blood/Serum Test Cassette

One Step Assay
Rapid Visual Results
For Qualitative In Vitro Diagnostic Use
Serum: Moderately complex
Whole Blood: CLIA-Waived

INTENDED USE

The *Accutest H. pylori* Whole Blood/Serum Cassette 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.[1-3] 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 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 of life 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*. 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.[5]

This *H. pylori* Whole Blood/Serum Cassette 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 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.

MATERIALS AND REAGENTS PROVIDED

- 20 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. Freezing to -70°C (-94°F) will not cause damage to the device.

Do not freeze and/or expose the kit to temperatures over 30℃ (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.

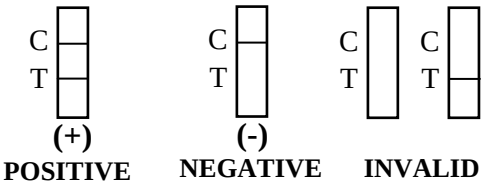
PRECAUTIONS

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

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. **DO NOT INTERPRET THE RESULTS AFTER 7 MINUTES**. For the whole blood test, a slight hemolysis might be observed, but it does not interfere with the results.

INTERPRETATION OF RESULTS



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 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 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].

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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 in house.

		Clinical confirmed results		Total
		Positive	Negative	
Accutest	Positive	137	9	146
	Negative	7	143	150
Total		144	152	296

The sensitivity of this device is 95.1% (137/144) and the specificity is 94.1% (143/152).

B. Comparison with a legally marketed device

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

		Marketed *H. pylori* Test		Total
		Positive	Negative	
Accutest	Positive	142	4	146
	Negative	3	147	150
Total		145	151	296

The agreement between these two devices is 97.9% (142/145) for positive specimens, and 97.4% (147/151) for negative specimens. This study demonstrated that the *Accutest H. pylori* Rapid Test-Whole Blood/Serum is substantially equivalent to the marketed device.

C. Comparison with *Accutest H. pylori* Serum Cassette Test

The panel of two hundred and ninety-six (296) clinical serum specimens, one hundred and forty-four (144) positive and one hundred and fifty-two (152) negative, was blind labeled and tested with Serum Test and Whole Blood/Serum Test side-by-side. The results were summarized in the table below.

		Whole Blood/Serum Test		Total
		Positive	Negative	
Serum Test	Positive	146	1	147
	Negative	0	149	149
Total		146	150	296

When compared to each other, the Whole Blood/Serum Test and the Serum Test showed a 99.3%(146/147) agreement for positive specimens and a 100% (149/149) agreement for negative specimens. These results indicate that the two formats are equivalent.

D. 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	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	15 mg/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	+	-

E. Reproducibility

Reproducibility studies were performed for *Accutest H. pylori* 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 *Accutest H. pylori* 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) border-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.

REFERENCE

1. O'Connor, JH., Helicobacter pylori and gastric cancer: a review and hypothesis. Eur. J. Gastro. Hepa. 1992; 6: 103-109.
2. Mcguigan JE., Peptic ulcer and gastritis. In Harrison's Principles of Internal Medicine, 12th Edition, 1988, Chapter 238, 1229-1248.
3. Cover TL. and Blaser MJ., Helicobacter pylori: A Bacterial Cause of Gastritis, Peptic Ulcer Disease, and Gastric Cancer, ASM News, 1995; 61: 21-26.
4. Podolsky I, Lee E, Cohen R, Peterson WL. Prevalence of C. pylori in healthy subjects and patients with peptic diseases. Gastroenteroloty 1989: 96: Suppl: A394. abstract.
5. Kist M., Immunology of Helicobacter pylori. In Helicobacter pylori in peptic ulceration and gastritis, edited by Marshall BJ., McCallum RW., and Guerrant RL., 1991, Chapter 8, 92-110.

30°C / 15°C	Temperature limitation		Use by YYYY-MM
LOT	Batch/Lot code	IVD	*In vitro* diagnostic medical device
	Manufacturer	REF	Catalog number
Σ n	Contains sufficient for < n > tests		Consult instructions for use
2	Do not reuse		Caution, consult accompanying documents

Manufactured for:
Jant Pharmacal Corporation
Encino, CA 91436 – USA

REF **ID392**