Accutest H. pylori Urease Test

Product Insert

Note: for *in vitro* diagnostic use only.

Accutest H. pylori Urease Test is CLIA WAIVED

Facilities performing testing must have a CLIA Certificate of Waiver. 42 USA 263a(c)(2). Any laboratory eligible for a Certificate of Waiver must follow the test system instructions, including use with only the waived specimen type, instructions for limitations/intended use, and performance of QC testing as a failure-alert mechanism. (42 CFR 493.15(e).) Any modification to the test or the manufacturer's instructions will result in the test being classified as highly complex.

Treat all biopsy specimens as if capable of transmitting disease. Caution should be used in handling and disposing of these specimens at bio-safety level 2 as recommended in the Centers for Disease Control/National Institute of Health Manual, Bio-safety in Microbiological and Biomedical Laboratories, 1984. Your laboratory safety procedures should also be followed as well as any other local or state health recommendations.

INTENDED USE: Accutest H. pylori Urease Test is intended for the qualitative detection of the urease enzyme in gastric mucosal biopsy specimens for the presumptive determination of *Helicobacter pylori* in symptomatic adult patients. **SUMMARY / BIOLOGICAL PRINCIPLE:** *Helicobacter pylori* has been shown to cause active chronic gastritis and has been implicated as a primary etiologic factor in duodenal ulcer disease, gastric ulcer and non ulcer dyspepsia[1]. By causing chronic inflammation *Helicobacter pylori* may weaken the mucosal defenses and allow acid and pepsin to disrupt the epithelium.

H.pylori produces large amounts of urease enzyme[2]. Although urease primarily allows *H. pylori to* utilize urea as a nitrogen source, the breakdown of urea also produces high local concentrations of ammonia, which enable the organism to tolerate low pH (see reaction below).

$$(NH_2)CO + 2H_20 + H^+ \xrightarrow{\text{urease}} 2NH_4^+ + HCO_3^-$$

urea — ammonium ion — bicarbonate ion

Although *H.pylori* can be detected with histology or culture of gastric tissue, simple tests for the presence of urease enable more rapid and convenient diagnosis. Tests for gastric urease are specific for *H.pylori* because mammalian cells do not produce urease and very few micro-organisms survive in the stomach, except for *H. pylori.*

WARNING: POTENTIAL BIOHAZARDOUS MATERIAL

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STORAGE: Accutest H. pylori Urease Test should be stored at room temperature away from direct light. Accutest H. pylori Urease Test has a shelf life of 24 months.

Before use, each Accutest H. pylori Urease Test slide should be inspected to make sure the test surface is yellow. If the test surface is red or magenta the slide should not be used.

SPECIMEN COLLECTION AND HANDLING

Preparation of the patient: Patients should not have taken antibiotics or bismuth salts for at least three weeks prior to endoscopy. Suppression of *H. pylori by* these agents makes the organism difficult to detect by any means, and re-growth of *H. pylori* may be patchy, leading to false negative results in the first few weeks after treatment.

Taking and Inserting the Biopsy:

1. A biopsy specimen for Accutest H. pylori Urease Test may be taken as soon as the endoscopist has examined the stomach. The usual area to biopsy is the sump of the antrum, along the greater curve.
2. Biopsy an area of normal-looking tissue rather than an area affected by erosions or ulceration. This is because *H.pylori may* be present in smaller numbers if the epithelium is eroded or the mucous layer is denuded. The standard biopsy forceps will provide a specimen of sufficient size (2 - 3 mm diameter).
3. If the biopsy specimen appears to be very small, it may be worthwhile taking a second biopsy and inserting both specimens into the Accutest H. pylori Urease Test. Be careful not to contaminate the second specimen with blood from the first biopsy site.

ACCUTEST H. PYLORI UREASE TEST PROCEDURE

1. Peel back the label of the Accutest H. pylori Urease Test thus exposing the reactive yellow pad.
2. Immediately after peeling back the label, using a sterile blunt instrument, remove the specimen from the biopsy forceps and place it onto the reactive yellow pad. Make certain that the tissue is positioned to have maximum contact with the reactive pad.
3. Re-seal the test. Press the label over the reactive pad lightly with your finger to squeeze the tissue contents out of the specimens. On the label, record the name of the patient, the date and the time the specimen was inserted.
4. Accurate resealing is important to prevent the biopsy specimens from drying up.

RESULTS: Reading the Accutest H. pylori Urease Test:

1. We recommend examining the Accutest H. pylori Urease Test at intervals of 5 minutes, 30 minutes and one hour. If any of those intervals or any time in between reveal a positive result the test is positive. Usually the first attempt to read the Accutest H. pylori Urease Test is made after the endoscopy report has been completed. This allows the endoscopist to objectively report the endoscopic findings before being aware of the presence of *H. pylori.*
2. If *H. pylori* are present in the tissue, an expanding red color zone will be noted around the biopsy specimen, or the Accutest H. pylori Urease Test will gradually change to a deep orange, then red color. A red reactive pad anytime within an hour is a positive reaction.
3. A negative result is when the Accutest H. pylori Urease Test reactive pad is still yellow 1 hour after insertion of the specimen.

MATERIALS PROVIDED: Accutest H. pylori Urease Test is packaged in boxes of 50 test slides with an instruction sheet.

MATERIALS REQUIRED BUT NOT INCLUDED WITH THE TEST: Not supplied with the Accutest H. pylori Urease tests are the biopsy forceps for collecting the specimens or the blunt instrument for transferring the specimen to the test.

QUALITY CONTROL: We recommend a positive external control must be preformed when opening a new test kit. The test kit size must not exceed 50. Accutest H. pylori Urease Test controls come in two forms: Dry Paper Dot Control and Liquid Control. Either may be used.

If the Accutest H. pylori Urease Test is negative after 1 hour and there is a question if the test has functioned properly, perform the following positive control test:

LIQUID CONTROL

1. Peel back the label on the Accutest H. pylori Urease Test so that the yellow ring is visible.
2. Place 1 small drop of control solution directly on the testing surface. The control solution gives a positive result.
3. Reseal the Accutest H. pylori Urease Test.
4. Observe the ring for a positive color change to magenta. The color change should occur within 30 seconds but may take up to 1 hour. **Please Note**: In some cases you may notice the center of the test changing to magenta before the outer ring. This is NORMAL and caused by the introduction of liquid onto the filter paper. Any change in color from yellow to magenta on any part of the test surface should be considered POSITIVE.
5. If after 1 hour there is no change in the color, please contact Jant Pharmacal Corporation Customer Service at 800-676-5565.

DRY PAPER DOT CONTROL

1. Peel back the label on the Accutest H. pylori Urease Test so that the yellow ring is visible.
2. Place 1 - 2 'Accutest H. pylori Urease Test 'Positive Control Dots on the ring with one drop of distilled water. The Control Dot gives a positive result.
3. Reseal the Accutest H. pylori Urease Test.
4. Observe the ring for a positive color change to magenta. The color change should occur in about 5 minutes but may take up to 1 hour. **Please Note**: In some cases you may notice the center of the test changing to magenta before the outer ring. This is NORMAL and caused by the introduction of liquid onto the filter paper. Any change in color from yellow to magenta on any part of the test surface should be considered POSITIVE.
5. If after 1 hour there is no change in the color, the test is Invalid. Please contact Jant Pharmacal Corporation Customer Service at 800-676-5565.

LIMITATIONS: False negative Accutest H. pylori Urease Test results may occur when very low numbers *of H. pylori* are present or the bacterium has a patchy distribution. For example, in 1-5% of patients the bacterium is present in the body of the stomach but not in the antrum, or vice versa. In patients with widespread intestinal metaplasia, an area of intestinal epithelium may be biopsied. As *H. pylori* does not colonize intestinal mucosa, a false negative Accutest H. pylori Urease Test can result. To reduce the occurrence of false negatives, two Accutest H. pylori Urease tests should be performed, one with a sample from the antrum and one from the body of the stomach.

All tests for *H.pylori,* including Accutest H. pylori Urease Test, will be less sensitive if the patient has recently taken antibiotics or bismuth. Re-growth of *H.pylori* may be patchy after suppression with antibiotic. Again, an extra biopsy may be taken for Accutest H. pylori Urease Test to avoid a false negative reading.

False positive Accutest H. pylori Urease Test results are rare. Theoretically, false positive Accutest H. pylori Urease Test results could occur in patients who have achlorhydria (for example patients with pernicious anemia, previous gastric surgery, or who have recently taken antacid or large doses of H2 receptor antagonists). When acid is absent, commensal organisms such as *Proteus spp.* may grow in the stomach and produce urease. False

positive reactions due to bacteria other than *H. pylori* will not usually react before 3 hours because these bacteria produce much less urease than *H. pylori.*
If there are factors which might adversely affect the performance of Accutest H. pylori Urease Test, the physician is advised to consider other diagnostic measures, such as culture with Gram stain and/or histology, in order to confirm or disprove a diagnosis of *H. pylori* infection.

USERS WITH COLOR BLINDNESS
Users with color blindness should seek assistance in interpreting the results of this test.
PERFORMANCE CHARACTERISTICS
During a clinical study conducted in 2006 comparing samples of Accutest H. pylori Urease Test with histology, Accutest H. pylori Urease Test was shown to be 100% specific and sensitive for H. pylori in relation to histology.

		Histology +	Histology -
Accutest H. pylori Urease Test	**+**	20	0
	-	0	80

Sensitivity=100%
(95% Confidence Interval* = .9705 -.1000)
Specificity=100%
(95% Confidence Interval* = .9705 -.1000)

WAIVER STUDIES
During 2008 and 2009 a study was conducted to demonstrate an insignificant risk of an erroneous result and support the issuance of a CLIA waiver by the FDA for the product Accutest H. pylori Urease Test. In order to effectively evaluate this test a total of 300 actual patient biopsies and 140 contrived biopsies were tested at 3 separate sites with no less than 3 users per site. The users were blinded as to the results and asked to perform the test using only the provided quick reference instructions. For clinical biopsy specimens, the results showed a positive agreement of 91.2% and a negative agreement of 98.7% compared to histology. For contrived specimens, the results showed positive agreement of 97.2% and a negative agreement of 95.5% compared to expected results.

FLEX STUDIES
Samples of Accutest H. pylori Urease Test have been stored for a minimum of 2 years in a various conditions and temperatures ranging from 40 degrees Fahrenheit to 85 degrees Fahrenheit. One of the testing sites was a non-air conditioned, non-humidity controlled warehouse, which allowed for continuously varying temperatures as well as varying humidity levels up to above 80% relative humidity. These samples were tested on a monthly basis and compared to duplicate samples stored in a humidity controlled and temperature controlled environment. Samples were given a pass rating if the results corresponded with the duplicate samples as well as prepared controls. The prepared controls were a solution of urease from Jack bean in distilled H20. In all cases the tests performed as intended.

PERFORMANCE NOT MEETING SPECIFICATIONS: If the Accutest H. pylori Urease Test test does not perform as outlined in these instructions, please contact Jant Pharmacal Corporation Customer Service at 800-676-5565.
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Manufactured for:

JANT Pharmacal Corporation
16530 Ventura Blvd., Suite 512
Encino, CA 91436
Phone: (800) 676-5565