

PyloPlus+ Rapid Urease Test (RUT)

Product Insert

NOTE: for *in-vitro* diagnostic use only

PyloPlus+ Rapid 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.

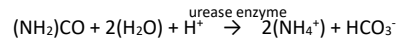
Treat all biopsy specimens as if capable of transmitting disease. Caution should be used in handling and disposing of these specimens at bio-safety level 2 as recommended in the Centers for Disease Control/National Institute of Health Manual, Bio-safety in Microbiological and Biomedical Laboratories,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

INTENDED USE:

PyloPlus RUT 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 dyspepsia¹. 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 enzyme². 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).



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:

PyloPlus RUT should be stored at room temperature away from direct light. PyloPlus RUT has a shelf life of 36 months. Before use, each PyloPlus RUT 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 PyloPlus+ RUT 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 PyloPlus RUT. Be careful not to contaminate the second specimen with blood from the first biopsy site.

PyloPlus+ RUT PROCEDURE:

1. Peel back the label of the PyloPlus+ RUT 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 PyloPlus+ RUT

1. We recommend examining the PyloPlus+ RUT 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 PyloPlus+ RUT 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 PyloPlus+ RUT 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 PyloPlus RUT reactive pad is still yellow 1 hour after insertion of the specimen.

MATERIALS PROVIDED:

PyloPlus RUT is packaged in boxes of 50 test slides with an Instructions for Use sheet.

MATERIALS REQUIRED BUT NOT INCLUDED WITH THE TEST:

Not supplied with the PyloPlus+ RUT 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 be performed when opening a new test kit. The test kit size must not exceed 50. PyloPlus+ RUT control is a liquid positive control. Additionally, if the PyloPlus+ RUT test is negative after 1 hour and there is a question if the test has functioned properly, a quality control test using a positive control is recommended.

LIQUID CONTROL:

2. Place 1 small drop of control solution directly on the testing surface. The control solution gives a positive result.
3. Reseal the PyloPlus+ RUT.
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 Gulf Coast Scientific at 813-855-1557.

LIMITATIONS:

False negative PyloPlus RUT 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 PyloPlus+ RUT can result. To reduce the occurrence of false negatives, two PyloPlus+ RUT 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 PyloPlus+ RUT, 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 PyloPlus+ RUT to avoid a false negative reading.

False positive PyloPlus+ RUT results are rare. Theoretically, false positive PyloPlus+ RUT 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 PyloPlus+ RUT, 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 PyloPlus RUT with a predicate device, PyloPlus+ RUT was shown to be 100% specific and sensitive for H-Pylori in relation to equivalency.

PyloPlus+ RUT	Histology	
	Positive	Negative
Positive	20	0
Negative	0	80

Sensitivity = 100% (95% Confidence Interval [97.5% - 100%])

Specificity = 100% (95% Confidence Interval [97.5% - 100%])

LOD = 1.0 x 10⁸ CFU

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 PyloPlus+ RUT. 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 PyloPlus RUT have been stored for a minimum of 3 years at to storage conditions in Real Time: -20 degrees Celsius and 30 degrees Celsius with a 65% relative humidity. These samples were tested monthly 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 intended use. The prepared controls were a solution of urease from Jack bean in distilled H2O. In all cases the tests performed as intended.

PERFORMANCE NOT MEETING SPECIFICATION:

If the PyloPlus RUT test does not perform as outlined in these instructions, please contact Gulf Coast Scientific at 813-855-1557 or fax 813-854-2340.

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Distributed By:

Gulf Coast Scientific, LLC.
13812 Wright Circle
Tampa, FL 33626, USA
Tel: 1(813) 855-1557
Fax: 1(813) 854-2340
Email: info@gulfcoastscientific.com
Website: www.gulfcoastscientific.com

ARJ Medical, LLC.
13812 Wright Circle
Tampa, FL 33626, USA



Obelis S.A.
Bd Général Wahis
53-1030 Brussels

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