Serim® PYLORITEK® TEST KIT

Test for *Helicobacter pylori* urease activity in gastric biopsy specimens

INTENDED USE:
The PyloriTek® Test Kit includes materials for detection of urease activity in gastric biopsy specimens for the presumptive identification of *Helicobacter pylori*. This test is for use on symptomatic patients by healthcare professionals.

For *In Vitro* Diagnostic Use

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European Pat. No.: 0633946
Canadian Pat. No.: 2,131,317
Japanese Pat. No.: 2,638,682

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SUMMARY AND EXPLANATION:

Helicobacter pylori was first identified in patients with gastritis in 1983 and was eventually shown to cause chronic active gastritis. Over 90% of patients with duodenal ulcer and about 80% of those with gastric ulcer have *H. pylori infection*. Eradication of *H. pylori* has proven effective in eliminating or reducing the recurrence of gastric and duodenal ulcers.

In addition, *H. pylori* has been associated with gastric cancer. The organism produces high levels of urease, an enzyme that reacts with urea to generate ammonia. This ammonia is detected by components of the PyloriTek® Test Kit.

DESCRIPTION:

The three components of the test kit include:

PyloriTek® Reagent Strips which contain, in separate dry reagent matrices, the substrate urea (Substrate Pad) and a pH indicator (Reaction Pad). The Reaction Pad containing the pH indicator is covered by a semi-permeable membrane which allows passage of gaseous ammonia but prevents passage of gastric tissue fluid or Hydration Reagent from the Substrate Pad.

PyloriTek® Hydration Reagent which contains a Tris buffer that is dispensed onto the Substrate Pad just prior to performing the test.

PyloriTek® Disposable Reaction Pouches or a reusable Plastic Reaction Chamber provide solid contact between the gastric biopsy and the Substrate Pad. These insure that the ammonia gas generated is directed through the membrane to the pH indicator.

PRINCIPLE OF THE PROCEDURE:

H. pylori produces the enzyme urease which is not present in mammalian tissue. The PyloriTek Test Kit detects urease activity in gastric biopsy specimens according to the following reaction:

$$\text{Urea} + H_2O \xrightarrow{\text{Urease}} 2\,NH_3 + CO_2$$

The ammonia produced is detected with a pH indicator (bromophenol blue) which turns from yellow to blue at an elevated pH.

KIT CONTENTS:

The PyloriTek Test Kit contains: 1 bottle of PyloriTek Reagent Strips
1 bottle of PyloriTek Hydration Reagent
PyloriTek Disposable Reaction Pouches
or PyloriTek Reusable Reaction Chamber

ACTIVE INGREDIENTS:

Substrate Pad: 3.3% Urea
Reaction Pad: 0.1% Bromophenol Blue, 0.2% Sulfamic Acid
Hydration Reagent: 1.8% Tris Buffer

WARNINGS & PRECAUTIONS:

The PyloriTek Test Kit is for *in vitro* diagnostic use. Normal precautions should be taken in the handling and disposal of the biopsy specimen and any material which came into contact[1] with the specimen.

H. pylori infection is believed to be spread by fecal/oral and possibly oral/oral contact. Higher incidence of infection is found in geographical areas with poor hygiene and sanitation practices. Standard medical hygiene practices should be followed with all materials used in the procedure room.

STORAGE:

- PyloriTek Test Kits must be stored in the refrigerator at 2°–7° C until the bottle is opened for use.
- Allow all kit components to come to room temperature between 15°–30° C (59°– 86° F) before opening.
- Do not use after the expiration date.
- Once opened, store the kit components at room temperature 15°–30° C (59°–86° F).
- Always write the date the bottle is opened in the space provided on the bottle label.
- Do not use strips more than three months after the bottle has been opened or after the kit expiration date, whichever occurs first.

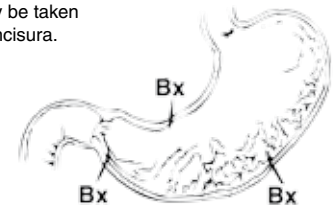
HANDLING PROCEDURES:

- When opening a kit for the first time, allow all PyloriTek Kit components to come to room temperature 15°–30° C (59°–86° F) prior to opening or use.
- After removing a PyloriTek Reagent Strip from the bottle, promptly replace and tighten the cap.
- Do not remove strips from the bottle until ready for use.
- Do not return unused strips exposed to ambient conditions to the bottle.
- Do not place test strips near sources of heat, ammonia (ammonia ampoules, ammonia-containing cleaning compounds) or formaldehyde. Extreme heat or chemical fumes may cause development of extraneous background color on the strip.
- Do not remove desiccant. Keep strips in the original bottle to protect from ambient moisture.
- Do not touch the red Positive Control spot with gloved hands as this may remove some or all of the control material.
- Do not place strips back in the bottle once Hydration Reagent has been added.
- Always use Disposable Reaction Pouches (or Reaction Chamber) to hold strips during the reaction time. Do not use tape or a paper clip.
- Do not carry the pouches or chambers with developing tests in the pockets of your clothes during the one hour development time. As biopsy tissue may contain potentially infectious organisms, the pouch or chamber and contents should be considered a potential biohazard.
- Do not remove developing strips from pouch or chamber. Removing and reopening the strip may affect the reaction.

SPECIMEN COLLECTION & HANDLING:

The current medical consensus is that patients refrain from taking antibiotics and/or bismuth for 4 weeks prior to the endoscopy procedure. Proton pump inhibitors should also be avoided for 1 – 2 weeks prior to the procedure. These drugs have been shown to reduce urease activity of *H. pylori* and to reduce the density and number of organisms, thereby increasing the chance of a false negative test result.[2,3]

PyloriTek Reagent Strips allow individual testing of specimens from multiple areas of the stomach. Recommendations concerning multiple specimen collection vary.[4, 9] However, one specimen may be from the greater curvature of the pre-pyloric antrum. A second specimen may be taken from the antral lesser curvature at or near the incisura. In a few patients *H. pylori* is not found in the antrum and biopsies from this area show mucosal replacement by intestinal-type epithelium. In these patients, *H. pylori* infection may be found in a third biopsy from the greater curvature of the body of the stomach.[4] Avoid areas of erosion or ulceration. Up to three biopsy specimens from one patient can be tested on one PyloriTek Reagent Strip.



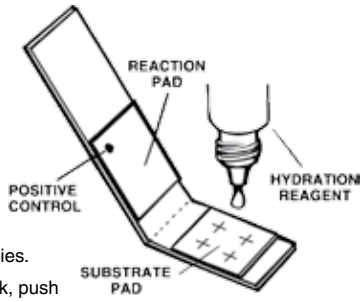
Suitable biopsy forceps may be obtained from most major gastroenterology device suppliers. A recent report[10] has indicated specimen size is not critical in obtaining satisfactory results.

SPECIMEN HANDLING PRECAUTIONS:

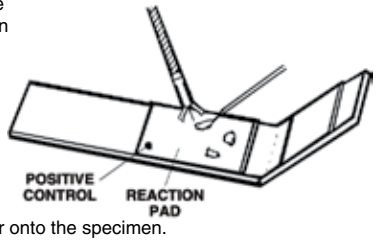
- The specimen must be transferred directly from the forceps to the Reaction Pad, as urease activity in infected specimens can be lost during transport[7] or storage prior to testing.
- Contamination of specimens with formalin (from biopsy forceps or other sources) can inhibit *H. pylori* urease activity and lead to false negative results.

TEST PROCEDURE:

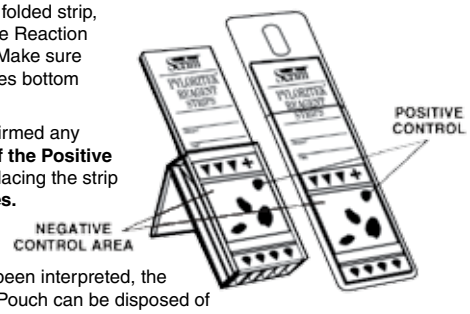
As soon as a decision is made to collect a biopsy for testing, remove a PyloriTek Reagent Strip from the bottle and replace the cap. Place the strip (printed side up) on a clean, dry, flat surface.



Step 1: Write the patient's name and the time on the Reagent Strip. Turn the strip over. **Make sure the red Positive Control Spot is visible in the upper left-hand corner of the Reaction Pad**. Crease the strip at the perforation (pads facing in). Place 3 or 4 drops of Hydration Reagent on the Substrate Pad. (To moisten evenly, it is recommended to place a drop on each quadrant.) Allow the Hydration Reagent to fully absorb before adding biopsies.



Step 2: With a clean wooden applicator stick, push the specimen from the forceps directly onto the Reaction Pad. Keep the specimen near the middle of the white, slightly raised Reaction Pad, 1⁄4" (6mm) or more from both the red Positive Control spot and the edges of the pad. If necessary, be sure to leave adequate space for a second or third specimen. (**Do not place a specimen in the upper quadrant of the Reaction Pad opposite the Positive Control spot as you may wish to use this as a negative control area**.) Fold the Substrate Pad over onto the specimen.



Step 3: Immediately insert the folded strip, with the yellow side out, into the Reaction Pouch or Reaction Chamber. (Make sure the strip is fully inserted; touches bottom of reaction device.)

A positive result may be confirmed any time **after the development of the Positive Control** within 60 minutes of placing the strip in either of **the reaction devices. Wait the full 60 minutes to confirm negative results.**

Step 4: After the results have been interpreted, the test strip/biopsy and Reaction Pouch can be disposed of in accordance with acceptable medical practice and applicable local, state and federal laws and regulations. (If the Reaction Chamber is used, the chamber can be washed in warm soapy water using a mild detergent, rinsed with clean water and dried with a fresh paper towel between uses.)

READING AND INTERPRETATION OF RESULTS:

Within 60 minutes, observe the upper right-hand corner of the yellow Reaction Pad to confirm the appearance of the intense purple or blue Positive Control spot. A similar color over the specimen indicates that the specimen is infected with *H. pylori*. Occasionally a patient's specimen may display a positive result before the Positive Control Spot develops. Regardless, do not interpret the result of the patient's test until the Positive Control Spot has developed. If necessary **wait** the full 60 minutes.

The appearance of a pale blue or faint gray haze over the specimen at 60 minutes is considered a negative result. Disregard color formed more than 60 minutes after insertion of the strip into either reaction device. Refer to the "PyloriTek Quick Reference Guide" for a visual guide to interpreting reaction patterns (available upon request from Serim).

For definitive diagnosis, histological examination using Giemsa, Genta or Warthin-Starry stain is required.

QUALITY CONTROL:

Implementing routine quality control procedures will increase user proficiency, minimize procedural errors and protect against the inadvertent use of outdated product or product that has deteriorated due to improper storage or handling.

Each PyloriTek Reagent Strip has built-in **internal** controls that run concurrently with each PyloriTek test. An **external** positive control (Serim® PyloriTek® Positive Control - Product Code 5146) is also available to assist facilities in meeting regulatory agency requirements for accreditation. Each facility should determine the frequency of QC testing and the optimal procedures for its own Quality Control Program.

Internal Control

PyloriTek internal controls measure both the process and reactivity of the test; the addition of the Hydration Reagent & folding of the strip starts the test reaction, thus it serves as a process control. The built-in Positive Control Spot is reactive in that it is composed of the actual substance being tested (urease) and verifies the system's ability to detect the analyte. (When looking at the back of a PyloriTek Reagent Strip, the internal Positive Control is the small red spot located in the upper left-hand corner of the Reaction Pad.)

Within 60 minutes of starting a PyloriTek test, check the controls:

- Positive Control – Observe the yellow Reaction Pad on the front of the reagent strip for development of an intense purple or blue spot beneath the + sign.
- Negative Control – The absence of purple or blue color in any other area of the yellow Reaction Pad serves as a negative control since urease is present only in the Positive Control Spot and in infected biopsy specimens.

Failure of the Positive or Negative Control indicates that the test has not developed properly. The test is invalid and the results should not be reported. Disregard any color developed more than 60 minutes after starting the test.

External Control

Serim® PyloriTek® Positive Control (Product Code 5146) consists of urease-impregnated paper that is tested is the same manner as a gastric biopsy.

- Positive Control - Follow the "Test Procedure" using a PyloriTek® Positive Control paper in place of a gastric biopsy. Within 5 to 10 minutes of starting the test, an intense purple or blue color should develop over the Positive Control paper. Color should also develop over the internal Positive Control Spot (+).
- Negative Control - Run a PyloriTek test without any external control or biopsy. After 60 minutes, the purple or blue color should have developed ONLY over the internal Positive Control Spot (+); the rest of the Reaction Pad should remain yellow.

Should the controls react incorrectly, the test is invalid and the results should not be reported. If you are unable to resolve the problem, contact Serim at 800-542-4670 or e-mail: customerservice@serim.com for advice.

LIMITATIONS OF THE PROCEDURE:

PyloriTek Reagent Strips detect ammonia generated by H. pylori urease-catalyzed hydrolysis of urea in the Substrate Pad. Ammonia can also be produced by tissue autolysis or can be found in blood as a waste product. However, these non-specific reactions normally occur at a slower rate than can be detected visually in 60 minutes by the PyloriTek test. It is possible that abnormal increases in these reactions may produce a pale blue or faint gray color over the specimen, which is a negative result.

Negative results may occur if an area is sampled which has not been colonized, particularly if the patient has been on antibiotic and/or bismuth salt therapy.

A positive test does not distinguish between colonization and a symptomatic infection.

The PyloriTek Test Kit is to be used to aid in diagnosis only with those patients with clinical symptoms of H. pylori infection.

PERFORMANCE CHARACTERISTICS:

Biopsy specimens were collected and tested with PyloriTek Reagent Strips at three different facilities. At two of the facilities an additional biopsy was histologically examined after staining.[4] At the third site, three test procedures (in addition to PyloriTek) were performed on 103 patients. If two of the additional tests were positive, the patient was classified as "true positive." The additional tests were ^{5}C-Urea Breath Test, gel-based rapid urease test, and serological analysis.[8]

Additional clinical studies have recently been published.[11 - 15]

	# Specimens	TP	FP	TN	FN	Sensitivity %	Specificity %	PPV%	NPV%
Site 1	170	98	3	62	7	93.3	95.4	97.0	89.9
Site 2[5]	193	73	3	112	5	93.6	97.4	96.1	95.7
Site 3[6]	103	51	3	47	2	96.2	94.0	94.4	95.9

TP=true positive, FP=false positive, TN=true negative, FN=false negative, PPV=positive predictive value, NPV=negative predictive value.

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