

LABORATORY PROCEDURE
LINK2™ H. PYLORI RAPID TEST
For the qualitative detection of IgG antibodies to *Helicobacter pylori*
(*H. pylori*) in SERUM.

I. INTENDED USE

LINK2™ *H. pylori* Rapid Test is a qualitative immunochemical membrane assay for the detection of IgG antibodies to *H. pylori* in serum by visual interpretation. The test is for professional use as an aid in the diagnosis of *H. pylori* infection in adult symptomatic patients.

II. SUMMARY AND EXPLANATION

Helicobacter pylori (formerly called *Campylobacter pylori*) was first isolated from human gastric biopsy material in 1982.¹ Epidemiological studies indicate that *H. pylori* is present in approximately 30% of adults in the industrialized nations. Prevalence increases with age (more than 50% of individuals may be infected above the age of 50) and correlates with low socio-economic status in childhood. Transmission is most probably by the fecal-oral or oral-oral route.

Colonization by this gram-negative, microaerophilic bacterium is characterized by acute inflammatory reaction, with infiltration of the lamina propria by mononuclear (and frequently polymorphonuclear) cells. Infection is self-limiting in a few individuals, but the majority develop an active, chronic (predominantly antral) gastritis of varying severity. *H. pylori* infection is found in more than 90% of duodenal ulcer patients and in around 75% of all peptic ulcer sufferers.

H. pylori infection is also more common in gastric cancer patients. The risk of gastric cancer has been estimated to be six-fold higher in *H. pylori* infected populations than in uninfected populations.²

Although the actual mechanism by which *H. pylori* infection promotes ulcer formation is unknown, several studies have concluded that eradication of *H. pylori* in ulcer patients leads to an eight-fold reduction in the rate of recurrence found with conventional anti-ulcer therapies.³⁻⁵

An accurate diagnosis of *H. pylori* infection is of value in targeting antibiotic-based eradication regimens in symptomatic patients. Since *H. pylori* is rarely found in patients with Type A (autoimmune) or Type C (reactive) gastritis, or in reflux esophagitis, the detection of infection can aid in the differential diagnosis of peptic ulcer disease.

The **LINK2** *H. pylori* Rapid Test is an immunoassay for the detection of antibodies against *Helicobacter pylori*, the causative agent of Type B chronic gastritis.¹⁻² The test has been designed to give a positive result in patients who are or have been infected with *H. pylori*. The test is easy to perform and requires no specialized equipment or instrumentation. Visual interpretation provides an accurate qualitative result in the physician's office, hospital, or laboratory. Diagnosis of *H. pylori* infection by antibody immunoassay can reduce the number of patients requiring endoscopy.⁶

III. PRINCIPLE OF THE PROCEDURE

LINK2 *H. pylori* Rapid Test is a dry chemistry immunological test which detects antibodies to *H. pylori*.

The test device contains a strip of plastic-backed membrane held in a result "window". In contact with this strip is a porous pad containing blue latex particles which have been coated with highly purified *H. pylori* antigen. The membrane has been coated with two lines in the window area (i.e., highly purified *H. pylori* antigen on the test line and a control line which captures the blue latex particles).

When a serum sample is introduced into the test device, it solubilizes the colored latex dried into the porous pad. The serum then moves by capillary action across the membrane. Any anti-*H. pylori* present in the serum binds to the antigen on the test line. The presence of bound antibodies is revealed by the colored latex reagent.

Latex particles then migrate along the test strip where they are captured by the control line.

The presence of two lines in the result window indicates a positive test while only a single control line is a negative test.

IV. REAGENTS

The **LINK2** *H. pylori* Rapid Test kit includes:

Test Devices	15	Each foil pouch contains one test device and a blue desiccant package.
Sample Collection Combs	15	Each plastic bag contains five sample collection combs.
Test Result Interpretation Card	1	Card.

Storage Instructions: **LINK2** *H. pylori* Rapid Test kits are stable until the expiration date when stored between 15 - 30°C. Do not freeze.

Precautions: For In Vitro Diagnostic Use.

WARNING: Because no test method can offer complete assurance that HIV, hepatitis B virus, or other infectious agents are absent, SPECIMENS AND CONTROL SOLUTIONS SHOULD BE HANDLED AS THOUGH CAPABLE OF TRANSMITTING AN INFECTIOUS DISEASE. The Food and Drug Administration recommends such material be handled at a Biosafety Level 2. BSL2 is referenced in the Centers for Disease Control and Prevention/National Institutes of Health (CDC/NIH) manual, *Biosafety in Microbiological and Biomedical Laboratories*.

Do not use test components beyond the expiration date. Do not open foil pouch until just before use.

Do not use test if blue desiccant package is completely pink.

Always wear gloves when taking blood samples and performing the test. Proper attention should also be given to potential infectivity of the samples used in the test.

After completion of the test, all components should be disposed of as biohazardous waste.

V. SAMPLE COLLECTION

Fresh serum should be used as a sample type for the test.

Approximately 50 µl of test sample is needed.

VI. TEST PROCEDURES

Materials Provided: See “Reagents”.

Materials Required But Not Provided: Timing device.

To use a serum sample:

1. Dip the collection comb into serum which has been separated from red blood cells after centrifugation.
2. Completely fill the collection comb with serum sample. Do not overfill the comb.
3. Carefully push the collection comb into the test device until the comb locks into place. Place the test device on a flat surface. Begin timing the test. Results are read in 5 min.

VII. INTERPRETATION OF TEST RESULT

A positive result may appear within a few minutes when the liquid reaches the far end of the test window, otherwise read the test at 5 min. Results should be interpreted at a normal reading distance.

Positive: Two distinct blue lines in the result window, one above the letter C and one above the letter T, indicate the presence of IgG antibodies to *H. pylori*.

Negative: One distinct blue line in the result window above the letter C indicates the absence of detectable IgG antibodies.

Any other result is invalid and the test should be repeated. If the sample fails to flow across the test window, the test is invalid and should be repeated.

Positive test results which appear after 5 min are not valid.

VIII. QUALITY CONTROL

Quality control testing for laboratories is good laboratory practice and is mandated by most U.S. states and the Clinical Laboratory Improvement Amendments of 1988 (CLIA). Always check with appropriate licensing or accrediting bodies to ensure that the lab is operating within established standards.

A procedural control is built into the **LINK2** *H. pylori* Rapid Test: the presence of a control line in the test result window provides assurance that sufficient sample amount was collected and that the latex particles in the strip were properly rehydrated and flowed through the test and control areas, thereby indicating that the test is performing properly and the procedure has been run correctly. If the control line does not appear, the test result is invalid.

A built-in negative background control is provided by the clearing of background color in the test window. This verifies the test has been performed correctly. This area should be straw colored to light pink within 5 min and not interfere with reading the test result. A clear background in the test window is considered an internal negative background control. If background color remains in the test window which interferes with your ability to read the test, the test result is invalid.

The **LINK2** *H. pylori* Rapid Test Quality Control Kit (see “Availability”) is designed to be used in conjunction with the **LINK2** *H. pylori* Rapid Test to verify test performance.

It is recommended that positive and negative controls be tested with each test kit, with each change in operator within a test kit, and whenever recommended storage temperature ranges have been exceeded. If controls do not perform as expected, contact Becton Dickinson Technical Services in the U.S.A. at (800) 638-8663, prompt 2, or

your local Becton Dickinson office. Test quality control samples according to the quality requirements established by your institution or laboratory.

IX. LIMITATIONS OF THE PROCEDURE

The **LINK2** *H. pylori* Rapid Test should be used only to evaluate patients with clinical signs and symptoms suggestive of gastrointestinal disease. The test is not intended for use with asymptomatic patients. Performance characteristics for persons under the age of 18 have not been established with this test.

The results obtained from this test are intended to be an aid in diagnosis only. Each physician must interpret the results in conjunction with the patient's history, physical findings, and other diagnostic procedures.

A negative test result indicates that antibodies to *H. pylori* are either not present or at levels undetectable by the test.

A positive test indicates the presence of antibodies to *H. pylori*, but determination of an active or inactive infection cannot be made.

The **LINK2** *H. pylori* Rapid Test is qualitative. The intensity of the sample test line does not necessarily correlate to the concentration of the antibody in the blood.

The effect of hematocrit on the **LINK2** *H. pylori* Rapid Test was assessed using venous blood from four volunteers. Hematocrits were altered by removing and adding plasma. Hematocrits ranged from 22.5% to 64.8%. Altering the hematocrit did not affect the accuracy of the test.

Test results are not affected by elevated levels of creatinine or bilirubin or by hemolysis.

X. EXPECTED RESULTS

Epidemiological studies indicate that *H. pylori* is present in around 20 to 30% of adults in the industrialized nations. Prevalence increases with age (more than 50% of individuals may be infected above the age 50) and correlates with low socio-economic status in childhood. Close person to person contact in childhood is an important determinant of sero-prevalence of *H. pylori* in adulthood, suggesting that the infection is transmitted directly from one person to another and may be commonly acquired early in life.⁷

A study to determine the presence of *H. pylori* in asymptomatic adults was conducted at a U.S. hospital and at a medical device manufacturer. Ninety-eight adults of varying ages participated. Each participant was evaluated using the **LINK2** *H. pylori* Rapid Test and an ELISA serum test. Of these participants, 7.1% (7 out of 98) were found to be positive by both methods.

XI. PERFORMANCE CHARACTERISTICS

Two studies were performed to evaluate the performance of the **LINK2** *H. pylori* Rapid Test.

One study was performed at four U.S. sites and one UK site with 229 patients to evaluate the performance of the **LINK2** *H. pylori* Rapid Test to the true condition of the patient. Each patient involved in this study was evaluated with the following tests for *H. pylori*: **LINK2** *H. pylori* Rapid Test, ELISA *H. pylori* antibody detection assay, CLO-test for active *H. pylori* infection, and histology analysis for detection.

Because *H. pylori* bacteria are sometimes difficult to collect during biopsy, the actual bacteria may not be sampled by the physician during the biopsy collection. Antibody detection tests are more likely to determine if an infection is present or has recently occurred, provided that the patient is not immuno-suppressed and is actually producing antibodies to *H. pylori* bacteria.

For this study, a “true positive” is defined as having a positive ELISA test, as well as either a positive CLO-test or a positive histology analysis.

A “true negative” is defined as having a negative ELISA test, and both the CLO-test and histology analysis must also be negative.

Any results not meeting the criteria for “true negative” or “true positive” are ruled as indeterminate.

Combining data from the four U.S. sites and the one UK site, 229 capillary samples were collected in order to compare the **LINK2** *H. pylori* Rapid Test results to the true condition of the patient. Twenty-nine samples were classified as indeterminate. The combined results for the remaining 200 samples are shown in the Table 1.

Table 1
LINK2 *H. pylori* Capillary Result

	Positive	Negative
True Positive	70	9
True Negative	18	103

Clinical Sensitivity (95% CI): 88.6% (79.5%, 94.7%)

Clinical Specificity (95% CI): 85.1% (77.5%, 90.9%)

The second study was performed with the same U.S. sites used on the first study combined with one additional U.S. site. The purpose was to compare the performance of serum to the performance of capillary blood with the **LINK2** *H. pylori* Rapid Test.

Combining data from the sites, 274 serum **LINK2** *H. pylori* Rapid Test results were compared to capillary **LINK2** *H. pylori* Rapid Test results. The combined results are shown in the Table 2.

Table 2
LINK 2 *H. pylori* Result

Capillary	Serum	
	Positive	Negative
Positive	102	8
Negative	16	148

Comparative Sensitivity (95% CI): 92.7% (86.2%, 96.8%)

Comparative Specificity (95% CI): 90.2%, (84.7%, 94.3%)

Accuracy 91.2%

Reproducibility: Reproducibility testing was performed at three external sites. Each day for 3 days, 10 blinded samples were tested at each of the three sites. These blinded samples consisted of negatives, weak positives, and positives. Additionally, three patients traveled to each site for blinded capillary blood testing.

During reproducibility testing at least 90% of negatives were found to be negative, and 100% of positives were found to be positive.

Cross Reactivity: Studies were conducted to investigate the extent to which the antibodies detected by the **LINK2** *H. pylori* Rapid Test cross-react with proteins from other related bacteria.

Crude bacterial sonicates at concentrations ranging from 0 to 2500 µg/ml were added to a *H. pylori* antigen detection assay and their inhibition effects assessed. **LINK2** *H. pylori* Rapid Test antigens were used as a comparison, since they compete in the assay and hence inhibit binding.

The results showed that proteins from the following bacteria do not inhibit the binding of **LINK2** *H. pylori* antigen and, therefore, do not cross-react with the **LINK2**TM *H. pylori* Rapid Test.

Borrelia burgdorferi *Campylobacter jejuni*
Campylobacter coli *Escherichia coli*
Campylobacter fetus

XII. AVAILABILITY

Cat. No.	Description
240905	LINK2 TM <i>H. pylori</i> Rapid Test Kit, 15 test.
240906	LINK2 TM <i>H. pylori</i> Rapid Test Quality Control Kit.

XIII. REFERENCES

1. Warren, J.R. and Marshall, B.J. 1983. Unidentified curved bacillus on gastric epithelium in active gastritis. *Lancet* *1*:1273-1275.
2. The EUROGAST Study Group: 1993. An international association between *Helicobacter pylori* infection and gastric cancer. *Lancet* *341*:8847.
3. Fiocca, R., et al. 1991. Duodenal ulcer relapse after eradication of *Helicobacter pylori*. *Lancet* *337*:1614.
4. Graham, D.Y., et al. 1992. Effect of treatment of *Helicobacter pylori* infection on the long-term recurrence of gastric or duodenal ulcer. *Ann. Intern. Med.* *116*:705.
5. Borody, T., et al. 1992. *Helicobacter pylori* reinfection 4 years post eradication. *Lancet* *339*:1295.
6. Sobola, G. et al. 1991. Screening dyspepsia by serology to *Helicobacter pylori*. *Lancet* *338*:94.
7. Webb, P.M., et al. 1994. Relation between infection with *Helicobacter pylori* and living conditions in childhood; evidence for person to person transmission in early life. *Br. Med. J.* *308*:750.

IVX. TECHNICAL INFORMATION

In the United States, telephone Becton Dickinson Microbiology System Technical Services, toll free (800) 638-8663, Prompt 2.

Approved By: _____

Date Effective: _____

Supervisor: _____

Date: _____

Director: _____

Date: _____

Review:

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