lifeSign

CLSI PROCEDURE

Product Name: **Status H. Pylori**	
Item Number: 37030	**Waived for Whole Blood**

Institution:	
Prepared By:	Date:
Title:	

Accepted By:	Date:
Title:	

Accepted By:		Date:	

Discontinued By	Date:

CONTENTS

SECTION 1 - TEST NAME

Status H. Pylori

SECTION 2 - INTENDED USAGE

Status H. pylori qualitatively detects anti-Helicobacter pylori antibody in human whole blood, serum, or plasma specimens. The test is intended for use as an aid in the diagnosis of H. pylori infection in adult patients with symptoms of gastrointestinal disorders.

SECTION 3 - SUMMARY AND EXPLANATION OF TEST

Helicobacter pylori, formerly known as Campylobacter pylori, are gram-negative icroaerophilic spiral bacteria that have been identified and cultured since 1983.[1] They can colonize the gastric mucosa for years[2], and their presence is strongly associated with chronic, diffuse, superficial gastritis of the fundus and antrum.[3-5]. As a result, they are now believed to have an etiologic role in gastritis.[6,7] Recent evidence suggests that H. pylori gastritis may progress over several decades to chronic atrophic (type B) gastritis [8,9], a lesion that is a precursor of gastric carcinoma. The epidemiologic features of gastric carcinoma and H. pylori infection are similar [10], and recent studies suggest that H. pylori infection may be a risk factor for gastric carcinoma.[11,12] Until recently, diagnosis of infection with H. pylori required endoscopy and identification of the organism by means of subsequent culture of the bacteria and/or recognition of spiral organisms in histologically evaluated sections of gastric tissue. However, the expense and invasive nature of this procedure make endoscopy impractical for epidemiologic studies. Serology has become the method of choice for such studies. There is excellent correlation between a classical clinical presentation of gastritis, the presence of H. pylori in the stomach and elevated serum levels of anti-H. pylori antibodies.[13-15] Positive results can justify a short empirical trial of antimicrobial therapy in gastritis of unknown origin, and response to treatment can be serially monitored because levels of H. pylori-specific antibodies can be expected to fall significantly after successful antibacterial therapy.[16]

SECTION 4 - PRINCIPLE OF TEST

The Status H. pylori Antibody Test utilizes indirect solid-phase immunoassay technology for the qualitative detection of H. pylori antibodies. **Status H. pylori** consists of H. pylori antigen on the test membrane and H. pylori antigen plus anti-human immunoglobulin antibodies coated on gold particles in the dye pad. Thus, in principle, the results of **Status H. pylori** may differ from the results of assay using only anti-IgG as a detector. In the test procedure, patient specimen is added in the upper area of the Sample well (S) located below the Result window.

The Developer solution is then added in the Sample well. The solution mobilizes the dye conjugated to H. pylori antigen and to anti-human immunoglobulin antibodies. If any anti-H. pylori antibody is present in the sample, the dye conjugate will bind to the H. pylori antigen band impregnated on the test membrane. Visualization of the antigen band at the Test

position (T) will occur only when the anti-H. pylori antibody is present in the sample. As the antibody-dye conjugate continues to move along the test membrane, it will be captured by a species specific antibody located at the Control position (C) to generate a colored band regardless of the presence of H. pylori antibodies in the sample. The presence of two colored bands, one at the Test position and the other at the Control position, indicates a positive result, while the absence of a colored band at the Test position indicates a negative result.

SECTION 5 - KIT CONTENTS AND STORAGE

• Each **Status H. pylori** test kit contains 30 test devices
• Each **Status H. pylori** test device contains a membrane strip coated with H. Pylori antigen and a pad with indicator conjugates in a protein matrix.
• Capillary tubes (30)
• Each kit contains a dropper bottle of developer solution (contains 0.1% sodium azide).
• Instructions for Use.
• Procedure Card.

Storage and Stability
The **Status H. pylori** test kit should be stored at 2–30°C (35–86°F) in the original sealed pouch. The storage conditions and stability dating given were established under these conditions. The kit is stable until the expiration date.

SECTION 6 - MATERIALS REQUIRED BUT NOT PROVIDED

- Vacutainer tubes for either serum or plasma procedure
- Anticoagulant (i.e., CPDA-1, heparin, or EDTA) for plasma
- Centrifuge
- Lancet
- Timer
- Latex Gloves

SECTION 7 - WARNINGS AND PRECAUTIONS

For in vitro diagnostic use only.
• Do not interchange materials from different product lots and do not use beyond the expiration date.
• Use separate clean capillary tubes for different specimens. Do not pipette by mouth.
• Do not smoke, eat or drink in areas in which specimens or kit reagents are handled.
• Wear disposable gloves while handling kit reagents or specimens and thoroughly wash hands afterwards.
• All patient samples should be handled as if they were capable of transmitting disease. Observe established precautions against microbiological hazard throughout all procedures and follow the standard procedures for proper disposal of specimens.
• Developer solution in this kit contains 0.1% sodium azide as a preservative, which may react with lead or copper in plumbing to form potentially explosive metal azides. Upon disposal, always flush with a large volume of water to prevent azide buildup in drains.

• The **Status H. pylori** device should remain in its original sealed pouch until ready for use. Do not use the test if the pouch is damaged.

SECTION 8 - PATIENT PREPARATIONS AND SPECIMEN COLLECTION

Whole Blood:

a). Anticoagulated Blood:
Whole blood collected over sodium heparin, lithium heparin, citrate or EDTA can be used. Mix whole blood by inversion and use in the test as outlined in the Test Procedure. Whole blood can be stored at 2–8°C for 24 hours.

b). Fingertip Blood:
Prick the finger and collect the blood in a capillary tube to the 25 µL mark. Follow the steps in Test Procedure.

Serum:

Collect blood sample into a tube containing no anticoagulant. Allow the blood to clot at room temperature (18–30°C) and then centrifuge at 1500 x g for ten minutes at room temperature.

Plasma:

Collect whole blood sample into a tube containing anticoagulant such as CPDA-1, heparin, or EDTA.
Remove the serum or plasma from the blood cells as soon as possible to avoid hemolysis. When possible, clear, non-hemolyzed specimens should be used. Mildly hemolyzed samples do not affect the test result, but will create an undesirable reddish background in the Result Window.
Specimens containing any particulate matter may give inconsistent test results. Such Specimens should be clarified by centrifugation prior to testing.

Storage of specimens

Refrigerate all specimens at 2–8°C until ready for testing. If serum or plasma specimens will not be tested within 48 hours, they should be frozen and stored at -20°C or below. Specimens should not be repeatedly frozen and thawed. Bring samples to room temperature (18–30°C) before testing. Frozen samples must be completely thawed, thoroughly mixed, and brought to room temperature prior to testing. If specimens are to be shipped, they should be packed in compliance with Federal and carrier regulations covering transportation of etiologic agents.

SECTION 9 - QUALITY CONTROL AND ASSURANCE

A quality control check is recommended using commercially available control sera. The frequency of Q.C. tests is determined according to your laboratory's standard Q.C. procedures. Upon confirmation of the expected results, the kit is ready for use with patient

specimens. If external controls do not perform as expected, do not use the test kits, and Contact LifeSign Technical Services at 800-526-2125.
When the test has been performed correctly and the device is working properly, a distinct colored line will always appear at the Control position (C). The colored line at the Control position (C) is considered an internal positive procedural control. If the line does not appear, a new device should be tested. If the problem persists, contact LifeSign Technical Services at 800-526-2125.
When the test has been performed correctly and the device is working properly, the background in the Result window will clear, providing a distinct test result. This clearing background in the Result Window is considered an internal negative procedural control.

SECTION 10 - TEST PROCEDURE

Procedural notes

• Allow specimens and the **Status H. pylori** test kit to warm to room temperature (18–30°C) before testing.
• Do not open the sealed pouch until you are ready to perform the test.
• Several tests may be run at one time.
• Do not reuse a lancet.
• To avoid cross-contamination, use a new capillary tube for each test.
• To avoid contamination, do not touch the tip of the Developer Solution dropper bottle to skin or to the test device.
• Label the device with the patient's name or control number.
• When adding the Developer Solution, hold the dropper bottle in a vertical position above the lower area of the Sample Well (S).
• After testing, dispose of the Status H. pylori device and the specimen dispenser or capillary tube following good laboratory practices. Consider each material that comes in contact with the specimen to be potentially infectious.

STEP 1
Remove a device from pouch and place on flat surface.
STEP 2
For serum or plasma fill a capillary tube to the red line (10 µl). For whole blood fill a capillary tube to the black line (25 µl). Apply sample by lightly tapping the capillary on the pad of the UPPER AREA of the Sample well (S).
STEP 3
Add 2 to 3 drops of Developer Solution onto the LOWER AREA of the Sample Well (S).
STEP 4
Read result at 10 minutes. (Do not read after 15 minutes).

SECTION 11 - INTERPRETATION OF RESULTS

Positive Result

One colored band each at the Test position (T) and at the Control position (C) indicates that antibodies against H. pylori have been detected.

NOTE: The test result can be read as soon as a distinct pink-purple colored Test line (T) and a colored Control line (C) appear. Any shade of pink-purple colored Test line should be reported as a positive result.

Possible positive results :

a. Two strong colored lines at both the Test (T) and Control (C) position.
b. One strong Test line (T) and one light colored Control line(C).
c. One light colored Test line (T) and one strong colored Control line (C).

Negative
Only the colored Control line (C), with no colored Test line (T) indicates that antibodies against Helicobacter pylori have not been detected.

Invalid
A distinctive colored Control line (C) should always appear. The test is invalid if no Control line forms. Repeat the test with a new Status H. pylori test.

SECTION 12 LIMITATIONS

The results obtained by this kit should be used only to evaluate patients with other clinical symptoms of gastrointestinal disease. This assay is not intended for use with asymptomatic patients. The performance characteristics of this test with specimens from pediatric patients has not been established. A positive result only means the presence of antibodies to H. pylori and does not indicate any disease status of the patient. A positive test result does not allow one to distinguish between active infection and colonization by H. pylori. A negative result suggests that antibodies to H. pyloriare not present, or are present at a level below the detection limit. If the test result is negative and infection of H. pylori is suspected, additional testing such as culture and histological analysis is recommended.

SECTION 13 EXPECTED RESULTS

1. H. pylori is detectable in nearly 100% of adult patients with duodenal ulcer and about 80% of patients with gastric ulcer.[13,17] Status H. pylori demonstrated positive results for 94% of patients with a symptom of ulcer and positive results on 80% of gastritis patients.

2. The prevalence of H. pylori antibody increases with age, and is detectable in 5% of children, about 33% in blood donors, and approaches 50% at age 60 in the normal population of industrialized nations.[16,18] More than 25% of these infected patients are asymptomatic. Other factors such as socioeconomic status, ethnic group, different populations, geographical location and the type of clinical symptoms associated with the infection also contribute to the observed variations in prevalence.

3. Asymptomatic and untreated patients continue to test IgG seropositive as long as the H. pylori organisms are present, even after histological resolution.[16] Hence, positive results are simply consistent with the diagnosis of H. pylori-associated gastritis or duodenal ulcer; whereas, negative results are strong evidence against these diagnoses.

SECTION 14 PERFORMANCE CHARACTERISTICS

Clinical specimens were collected from 207 symptomatic and asymptomatic individuals who presented for endoscopic examination. The age range was 19-83 years with a mean age of 52 years. The performance characteristics of Status H. pylori were evaluated by comparison to biopsy/histology, agglutination test and ELISA for detection of anti-H. pylori antibody. The results are summarized in tables below.

Table 1. ***Status H. pylori*** Test Result versus Biopsy/Histology

		Biopsy/Histology		
		Positive	Negative	Total
Status *H. pylori*	Positive	71	14	85
	Negative	3	115	118
	Total	74	129	203

When biopsy/histology was used as a reference, the ***Status H. pylori*** test demonstrated 95.9% sensitivity, 89.1% specificity and 91.6% agreement. Four tests were excluded in the calculation due to indeterminate results.

Table 2. ***Status H. pylori*** Test Result versus Agglutination Test

		Agglutination Test		
		Positive	Negative	Total
Status *H. pylori*	Positive	80	8	88
	Negative	6	113	119
	Total	86	121	207

When the agglutination test was used as a reference, the ***Status H. pylori*** test demonstrated 93.2% agreement.

Table 3. ***Status H. pylori*** Test Result versus ELISA

		ELISA		
		Positive	Negative	Total
Status *H. pylori*	Positive	78	10	88
	Negative	6	113	119
	Total	84	123	207

When the ELISA was used as a reference, the ***Status H. pylori*** test demonstrated 92.3% agreement.

Matrices Effect Study

Effect of specimen matrices on the result of the **Status H. pylori** test was evaluated using 59 matched specimen sets each consisting of venous whole blood, capillary whole blood, plasma and serum. Of the 59 samples tested, 46 samples were positive and 13 samples

were negative. Excellent agreement (>99%) was found between venous whole blood, capillary whole blood, plasma and serum indicating no significant effect of matrices on the test.

Reproducibility

Reproducibility of **Status H. pylori** was evaluated by testing negative, low positive and high positive samples. The samples were tested in replicates of 10 in a blind study by 4 technicians, on 3 different dates and at 4 different locations. The results showed 100% agreement with the expected results.

Proficiency (Physician Office Laboratory) Study

Status H. pylori was evaluated at 3 different physicians' office laboratories using a panel of 90 coded samples. The proficiency panel contained negative, low positive and high positive specimens in either serum or whole blood. Either technical or nontechnical personnel at three different institutions and three different days conducted the tests. The results obtained from 270 tests had a >99% agreement with the expected results. No significant differences were observed between the laboratories or personnel results.

SECTION 15 REFERENCES

1.Unidentified curved bacilli on gastric epitheliums in active chronic gastritis. Lancet 1:1273(1983).
2. Morris, A. J., et al., Long term follow-up of voluntary ingestion of Helicobacter pylori. Ann. Intern. Med. 114:662(1991).
3. Marshall BJ and Warren JR. Unidentified curved bacilli in the stomach of patients with gastritis and peptic ulceration. Lancet 1:1311(1984).
4. Dooley CP, et al. Prevalence of Helicobacter pylori infection and histologic gastritis in asymptomatic persons. N. Engl. J. Med. 321:1652(1989).
5. Buck GE, et al. Relation of Campylobacter pyloridis to gastritis a and peptic ulcer. J. Infect. Dis. 153:664(1986).
6. Blaser MJ. Helicobacter pylori and the pathogenesis of gastroduodenal inflammation. J. Infect. Dis. 161:626(1990).
7. Graham DY. Campylobacter pylori and peptic ulcer disease. Gastroenterol. 96(Suppl.2): 615(1989).
8. Blaser MJ. Pathogenesis of Helicobacter pylori-induced gastroduodenal diseases. In new antibacterial strategies, Neu, HC (ed.). London: Churchill Livingston, pp. 143-153(1990).
9. Siurala M, et. al. Chronic gastritis: Dynamic and clinical aspects. Scan. J. Gastroenterol. Suppl. 109:69(1985).
10. Sitas F, et al. Helicobacter pylori infection rates in relation to age and social class in a population of Welsh men. Gut 32:25(1991).
11. Parsonnet J, et al. Helicobacter pylori infection and the risk of gastric carcinoma. N. Eng. J. Med. 325:1127(1991).
12. Nomura A, et al. Helicobacter pylori infection and gastric carcinoma among Japanese Americans in Hawaii. N. Eng. J. Med. 325:1132(1991).
13. Musgrove C, et al. Campylobacter pylori: Clinical, histological and serological studies. J. Clin. Pathol. 41:1316(1988).
14. Booth L, et al. Clinical importance of Campylobacter pyloridis and associated serum IgG and IgA antibody response in patients undergoing upper gastrointestinal endoscopy. J. Clin. Pathol. 39:215(1986).

15. Fox JG, et al. Campylobacter pylori-associated gastritis and immune response in a population at increased risk

SECTION 16 TECHNICAL ASSISTANCE

Technical assistance is available from the distributor of Status H. Pylori, LifeSign, LLC, Skillman, New Jersey, between the hours of 8:30 a.m. and 4:45 p.m. E.S.T.
Phone: 1-800-526-2125
Fax: 1-732-246-0570
Email: info@lifesignmed.com

Helpful CLIA brochure links to explain Clinical Laboratory Improvement Amendments (CLIA) regulation requirements

Individualized Quality Control Plan- IQCP

http://www.cms.gov/Regulations-and-Guidance/Legislation/CLIA/Downloads/CLIAbrochure11.pdf

Proficiency Testing

https://www.cms.gov/Regulations-and-Guidance/Legislation/CLIA/downloads/CLIAbrochure8.pdf

Proficiency Testing Providers

http://www.cms.gov/Regulations-and-Guidance/Legislation/CLIA/Downloads/ptlist.pdf

Personnel Competency Assessment

http://www.cms.gov/Regulations-and-Guidance/Legislation/CLIA/Downloads/CLIA_CompBrochure_508.pdf

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Test Validation Form

Account Name: _____

Address: _____

_____

Telephone: _____

Test Name: _____ Lot # : _____

Start Date: _____

Supervisor Signature: _____

Sample Number	Expected Results	Test Result	Tester's Initials	Comments

Reviewed by: _____

Corrective Action Form

Problem /Error	Corrective Action

Laboratory Technologist: _____ Date: _____

Laboratory Director: _____ Date: _____

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Certification of Training

This is to verify that personnel responsible for running _____ test at _____ have been thoroughly in-serviced on the test and the test procedure(s).

This has included:

Review of the package insert
Demonstration of the product assay
Successful performance of the test and interpretation of results

Names of the personnel who have been trained with the above test and are responsible for reporting patient results:

Print Name	Signature	Date

Signature(s) of those responsible for personnel and testing:

_____ _____
Signature Date

_____ _____
Signature Date

_____ _____
Signature Date

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Quality Control

Name of Facility _____

Use this cover sheet with each new shipment and/or with each new kit lot

Product _____ Lot# _____ Exp Date _____

Date Received _____ Rec'd By _____

	Date	Positive Control	Negative Control	Initials
Initial QC				
Additional QC				
Additional QC				
Additional QC				
Additional QC				
Additional QC				
Additional QC				

Reviewed by _____ Date _____

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Testing Personnel Competency Assessment

Test _____

Procedure	Satisfactory	Unsatisfactory	Not Applicable	Comments/Corrective Action(s)
Observation of Test performance				
Patient Sample Preparation				
Specimen Handling/Processing				
Testing				
Recording/Reporting Results				
Assessment of Test Performance Using Known Samples				
Review of Records				
Patient/Quality Control Log Sheet Records				
Proficiency Testing Records				
Assessment of Problem Solving Skills				

(Attach all supporting documents)

Evaluator: _____ Date: _____

Testing Personnel: _____ Date: _____