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ALIMENTERICS

PYLORI-CHEK

BREATH TEST KIT

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 **Alimenterics Inc.**
301 American Road
Morris Plains, NJ 07950 U.S.A.

ALIMENTERICS PYLORI-CHEK BREATH TEST KIT

I. INTENDED USE

The Pylori-Chek Test System is intended for use with the LARA Laser Assisted Ratio Analyzer for the qualitative detection of urease associated with *Helicobacter pylori* infection in the human stomach and as an aid in the diagnosis of *H. pylori* infection in symptomatic adult patients. The Pylori-Chek Test system consists of a Pylori-Chek test kit for the collection of breath samples and a LARA Laser Assisted Ratio Analyzer for the measurement of the ratio of $^{13}\text{CO}_2$ to $^{12}\text{CO}_2$ in the breath sample.

For use by healthcare professionals. To be administered under a physician's supervision.

II. SUMMARY AND EXPLANATION OF THE TEST

Peptic ulcer disease is a chronic inflammatory condition of the stomach and duodenum. Despite the fact that the disease has relatively low mortality, it results in substantial suffering of those affected.

A strong association between *H. pylori*, and chronic superficial gastric and gastrointestinal disease has been well established. It is associated with type B gastritis, ^{2,3} duodenal ulcer, ^{4,5} gastric ulcer, ^{6,7} gastric cancer ⁸ and non-Hodgkin's lymphoma. ⁸

H. pylori was first cultured from human gastric mucosa in 1982.¹ The organisms, spiral gram negative bacteria, are found in the human stomach between the gastric epithelium and the mucosa. Isolates implicated in the above mentioned disease states are distinguished by the production of copious amounts of endogenous urea amidohydrolase (urease).^{9,10} The enzyme catalyzes the breakdown of urea to carbon dioxide and ammonia, which are absorbed into the bloodstream.

Several methods are employed to determine the presence of *H. pylori* in the gastrointestinal tract. Histologic staining of biopsy tissue using various stains has been shown to give adequate results with specificity of over 90%.¹¹ Mucosal biopsy samples can be cultured using non-selective enriched media. However, due to the exacting needs of the organism, culture is the least sensitive (70-80%)¹¹ of all available techniques. Direct detection of urease activity of biopsy specimens is achieved by placing tissue in Christensen's urea agar and observing a color change. Biopsy and its associated analytic techniques are invasive and often not well tolerated by patients. Non-invasive tests consist of serum assays for IgG antibodies against *H. pylori*.¹¹

However, these are not a reliable indicator of current infection. Urea breath tests using the radioactive isotope ^{14}C or the non-radioactive isotope ^{13}C labeled urea can detect current infection with *H. pylori*, and have been shown to be highly sensitive.^{12,13}

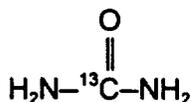
III. PRINCIPLE OF TEST

The Alimenterics Pylori-Chek Breath Test is based on the ability of the organism, *H. pylori*, to produce large amounts of the enzyme urease, which hydrolyses urea to NH_4^+ and HCO_3^- , the latter being exhaled as CO_2 . Using non-radioactive ^{13}C -labeled urea, an increase in the ratio of $^{13}\text{CO}_2$ to $^{12}\text{CO}_2$ over time is an indication of the presence of *H. pylori*. This change can be detected by isotope ratio analyzers that measure pre-ingestion and post-ingestion samples of the patient's breath.

Each Pylori-Chek test kit comes with a jar containing 100 mg of white crystalline powder which is reconstituted using water just prior to taking the test. The powder is urea (see structure and molecular weight given below), labeled with the non-radioactive isotope ^{13}C carbon (^{13}C -urea).

Name and structure:

Urea (non-radioactive ^{13}C Carbon-labeled (^{13}C), 99%) $^{13}\text{CH}_4\text{N}_2\text{O}$



Molecular Weight 61.05

The non-radioactive ^{13}C -labeled urea preparation contains no additives or bulking compounds.

The test procedure involves collection of a sample of the patient's breath to determine the baseline ratio of $^{13}\text{CO}_2$ and $^{12}\text{CO}_2$. Following ingestion of a test meal and an aqueous solution containing 100 mg non-radioactive ^{13}C -labeled urea, two additional breath samples are collected, one at thirty and one at sixty minutes. The samples are then introduced into the LARA System which measures the ratio of $^{13}\text{CO}_2$ to $^{12}\text{CO}_2$. The results of the three readings are analyzed concurrently by the LARA System. Test values are generated and a report is printed for each patient as indicated in the test report section.

IV. WARNINGS AND PRECAUTIONS

1. For in vitro diagnostic use only. The Pylori-Chek urea solution is ingested as part of the diagnostic procedure.
2. A negative Pylori-Chek breath test result alone does not rule out the possibility of *H.pylori* infection. Typically, with this procedure, false negative results occur at a rate less than 1.5%. Always evaluate the Pylori-Chek Breath Test results along with clinical signs and patient history when diagnosing *H.pylori* related disease. If clinical signs and patient history are suggestive of *H.pylori* infection and the Pylori-Chek Breath Test result is negative, retest with a new sample or an alternative method.
3. The validity of the Pylori-Chek test depends on using the LARA to analyze breath samples captured within collectors supplied with the test kit. Since using other methods may change the precision and accuracy of the results, the manufacturer does not recommend using any alternative analytical methods or other urea breath test kits in conjunction with the Pylori-Chek Test Kit. The LARA software is only valid for analysis of Pylori-Chek tests.
4. Failure of the patient to fast as directed may affect the test results.
5. Antimicrobials, omeprazole, and bismuth preparations suppress *H.pylori*. Ingestion of these substances within four (4) weeks prior to performing the Alimenterics Pylori-Chek Breath Test may lead to false negative results.
6. False positive results may occur in patients with achlorhydria or who have gastric spiral organisms such as *Helicobacter hominis*.
7. Lack of sufficient CO₂ (less than 2.0%) or too much CO₂ (more than 6.0%) in any sample may lead to a non-evaluable test. This can be avoided by insuring that the breath collector is closed tightly after obtaining the samples.

V. SHELF LIFE, STORAGE AND HANDLING OF KIT

The shelf life for the urea powder is twenty (20) months after date of manufacture. Use the urea solution within five minutes after reconstitution.

Store the Pylori-Chek Breath Test Kit between 20°C and 25°C (68°F - 77°F). The non-radioactive ¹³C-urea and the sterile water have expiration dates. Do not use these materials beyond the expiration date stated on their labels.

VI. PATIENT PREPARATION

It is important that the patient fast 6 (six) hours before performing the Alimenterics Pylori-Chek Breath Test. In addition, in the four weeks prior to performing the test, the patient must avoid the use of antimicrobials, omeprazole, and bismuth preparations, which are known to suppress *H.pylori*.

VII. LIST OF REQUIRED MATERIALS PROVIDED

The Alimenterics Pylori-Chek Breath Test Kit, with sufficient material for one determination, consists of:

1. One vial containing 100 mg non-radioactive ¹³C-labeled urea (powder)
2. One bottle containing 50 mL purified water for reconstituting the urea.
3. Three breath collectors, each with an affixed bar code label
4. Three patient labels (labeled 1, 2, and 3)
5. One reusable pouch (containing the three breath collectors and 3 patient labels)
6. Package insert

Note: A LARA Analyzer is required for analysis of breath samples.

VIII. LIST OF REQUIRED MATERIALS NOT PROVIDED

1. One 8 oz. test meal (Ensure vanilla-flavored liquid)
2. A timer capable of timing an interval up to thirty (30) minutes

IX. STEP-BY-STEP PROCEDURE

Note: Time intervals indicated in this procedure are critical.

1. Verify that the patient has been prepared as specified in Section VII.
2. Open the Alimenterics Pylori-Chek Breath Test Kit, which should contain all materials listed in Section VIII.
3. Remove one breath collector from reusable pouch.
4. Ask the patient to provide a baseline measurement. Note that there is a narrow and a wide end of the breath collector. Instruct the patient to:

- Take a deep breath
- Pause momentarily
- Place the lips firmly around the narrow end of the breath collector
- Exhale into the breath collector
- Close the breath collector toward the end of exhalation by twisting the far end of the breath collector in a counter-clockwise direction.

Make sure the collector is closed tightly after it is removed from the mouth.

5. Record patient name, date and time on the No. 1 patient label and affix patient label to the breath collector that now contains the baseline specimen. **Do not cover the bar code on the breath collector.**
6. Instruct the patient to consume an entire 8 oz. test meal (Ensure-vanilla flavored liquid).
7. Prepare the Pylori-Chek solution by:
 - Carefully opening the 50 ml bottle of purified water and adding it to the jar containing the non-radioactive ^{13}C -urea powder.
 - Recapping the jar and mixing by inversion until all powder is dissolved.

Note: Reconstituted urea must be used within 5 minutes. Urea slowly decomposes in solution.

8. Instruct the patient to drink all of the Pylori-Chek Urea solution. After the patient ingests the solution, set the timer for 30 minutes. Instruct the patient to sit calmly for the 30-minute interval. The patient should not eat, drink, or smoke during this time period.
9. Remove second breath collector from the reusable pouch.
10. Thirty minutes after the patient has ingested the urea solution, ask the patient to provide a second breath sample in breath collector number 2 in the same manner as outlined in paragraph 4 above. After the patient has provided the second breath sample, re-set the timer for 30 minutes.

Instruct the patient to sit calmly for the 30-minute interval. The patient should not eat, drink, or smoke during this time period.

11. Record patient name, date and time on the No. 2 patient label and affix patient label to the breath collector that now contains the 30-minute specimen. Do not cover the bar code on the breath collector.
12. Remove third breath collector from the reusable pouch.
13. Thirty minutes after the second sample, ask the patient to provide a third breath sample in breath collector number 3 in a similar manner as outlined in paragraph 4 above.
14. Record patient name, date and time on the No. 3 patient label and affix patient label to the breath collector that now contains the 60-minute specimen. Do not cover the bar code on the breath collector.
15. Place the three breath samples in the reusable pouch and seal and deliver to laboratory for analysis.
16. Directions for analyzing the samples using the LARA Analyzer are provided in the LARA Analyzer Operator's Manual.

X. QUALITY CONTROL

As detailed in the LARA Analyzer Operator's Manual, the accuracy of system results is assured by several quality controls that are designed to eliminate or detect measurement errors. Internal calibrations are performed continuously when the system is not in use. Positive and negative control samples are run periodically to verify proper calibration of the instrument.

Both sets of controls are labeled with expected values which vary slightly from one lot to the next. Typically, positive controls have δ values above 12 and negative controls have δ values near zero. See the next section (Interpretation of Results) for a definition of delta value. Each control kit is labeled with its expected δ value. A control measurement fails when the value measured by the LARA falls outside $\pm 3.0 \delta$ units from the control's expected value. When a control measurement fails, the system will alert the operator and issue a warning if the operator attempts to run patient samples. The operator should recalibrate the LARA system and then run another control test. If the second control measurement fails, the operator should mark the instrument "Out of Service"

and call Alimenterics for repair.

Each breath specimen must contain at least 2% CO₂ and no more than 6% CO₂. Breath Specimen levels outside this range are rejected with the output message "CO₂ range error."

XI. INTERPRETATION OF RESULTS

1. Calculation of Test Results

The LARA Analyzer takes one reading from each breath collector to determine a δ value for each sample. The δ value is the ratio of ¹³CO₂ to ¹²CO₂ within gas extracted from the breath collector, expressed in parts per thousand. The baseline reading is then compared to those at 30 and 60 minutes (δ_{30} and δ_{60}).

There are three possible outcomes from a test:

- 1) A test is considered positive for the presence of urease associated with H. pylori infection when either δ_{30} or δ_{60} are greater than 6.7;
- 2) A test is considered negative for the presence of urease associated with H. pylori infection when both δ_{30} and δ_{60} are less than or equal to 5.5;
- 3) A test is considered indeterminate for the presence of urease associated with H. pylori infection when both δ_{30} and δ_{60} are between 5.5 and 6.7 or when one value is between 5.5 and 6.7 and the other is less than or equal to 5.5. If possible, the patient should be asked to return and repeat the test.

These possibilities are summarized in the table shown below:

δ_{30}	δ_{60}	determination
> 6.7	any value	positive
any value	> 6.7	positive
< = 5.5	< = 5.5	negative
between 5.5 and 6.7	between 5.5 and 6.7	indeterminate
between 5.5 and 6.7	< = 5.5	indeterminate
< = 5.5	between 5.5 and 6.7	indeterminate

Sample Calculation 1:

Test results: $\delta_{\text{base}} = 1.0 \delta$
 $\delta_{30} = 4.0 \delta$
 $\delta_{60} = 9.0 \delta$

Calculation: $\delta_{60} - \delta_{\text{base}} = 8.0 \delta$

Result: Positive (one or both values above 6.7δ).
Note: When one value is greater than 6.7δ , the test is automatically positive. The other value may be in or below the indeterminate zone or may not be defined in the case of a CO_2 range error.

Sample Calculation 2:

Test results: $\delta_{\text{base}} = 1.0 \delta$
 $\delta_{30} = 4.0 \delta$
 $\delta_{60} = 3.0 \delta$

Calculation: $\delta_{30} - \delta_{\text{base}} = 3.0 \delta$
 $\delta_{60} - \delta_{\text{base}} = 2.0 \delta$

Result: Negative (both values below or equal to 5.5δ)

Sample Calculation 3:

Test results: $\delta_{\text{base}} = 0.0$
 $\delta_{30} = 5.7 \delta$
 $\delta_{60} = 3.0 \delta$

Calculation: $\delta_{30} - \delta_{\text{base}} = 5.7 \delta$
 $\delta_{60} - \delta_{\text{base}} = 3.0 \delta$

Result: Indeterminate (one value in grey zone, 5.5 to 6.7δ , the other below 5.5δ)

Sample Calculation 4:

Test Results: $\delta_{\text{base}} = 0.0$
 $\delta_{30} = 5.7 \delta$
 $\delta_{60} = 6.3 \delta$

Calculation: $\delta_{30} - \delta_{\text{base}} = 5.7 \delta$
 $\delta_{60} - \delta_{\text{base}} = 6.3 \delta$

Result: Indeterminate (both values in grey zone, 5.5 to 6.7).

2. Test Report

The LARA Analyzer prints a report that records the type of test performed, the sample identification, the date and time, the δ values for the 30 and 60 minute samples and the test result; positive, negative or indeterminate. A negative test result alone does not rule out the possibility of *H.pylori* infection. For a negative result, if clinical signs and patient history are suggestive of *H.pylori* infection, and for an indeterminate result, the patient should be asked to return and repeat the test.

An unevaluable test result may be obtained when the percent CO₂ in a sample falls below 2% or above 6%. Such a result would be indicated as UAP (unable to process) on the report. These patients should be retested if possible, by obtaining fresh samples.

3. Determination of a Cut-Off Point

The cut-off point is the Alimenterics Pylori-Chek Breath Test Result (δ value) above which patients are considered to be infected with *H.pylori*. For the Alimenterics Pylori-Chek Breath Test, the delta cut-off point was determined to be 6.1 δ in a clinical study of 395 evaluable patients (including 235 infected patients and 160 uninfected patients). The reference standards were bacterial culture, histopathology and CLO test. The cut-off point was determined by calculating the breath test result (δ value) which best distinguished those patients that were determined to be negative and positive using the reference standard.

XII. EXPECTED VALUES

The range of Alimenterics Pylori-Chek Breath Test results for the uninfected group was less than 5.5 δ . Figure 1 is a histogram for the distribution of results from the uninfected and infected patients.

XIII. PERFORMANCE CHARACTERISTICS

1. Precision of the LARA Analyzer

To estimate precision, a LARA continuously measured control samples over an 8 hour period. This experiment simulates worst-case conditions of continuous operation without recalibration. Sixty (60) negative and 60 positive controls were measured alternately, at levels of 0.0 δ and 12.7 δ , respectively.

The mean, standard deviation and coefficient of variation for all measurements during the 8 hour period are shown below:

CONTROL TYPE	MEAN (δ)	STANDARD DEVIATION	COEFFICIENT OF VARIATION
Negative	-0.24	0.62	undefined
Positive	12.31	0.74	6.0%

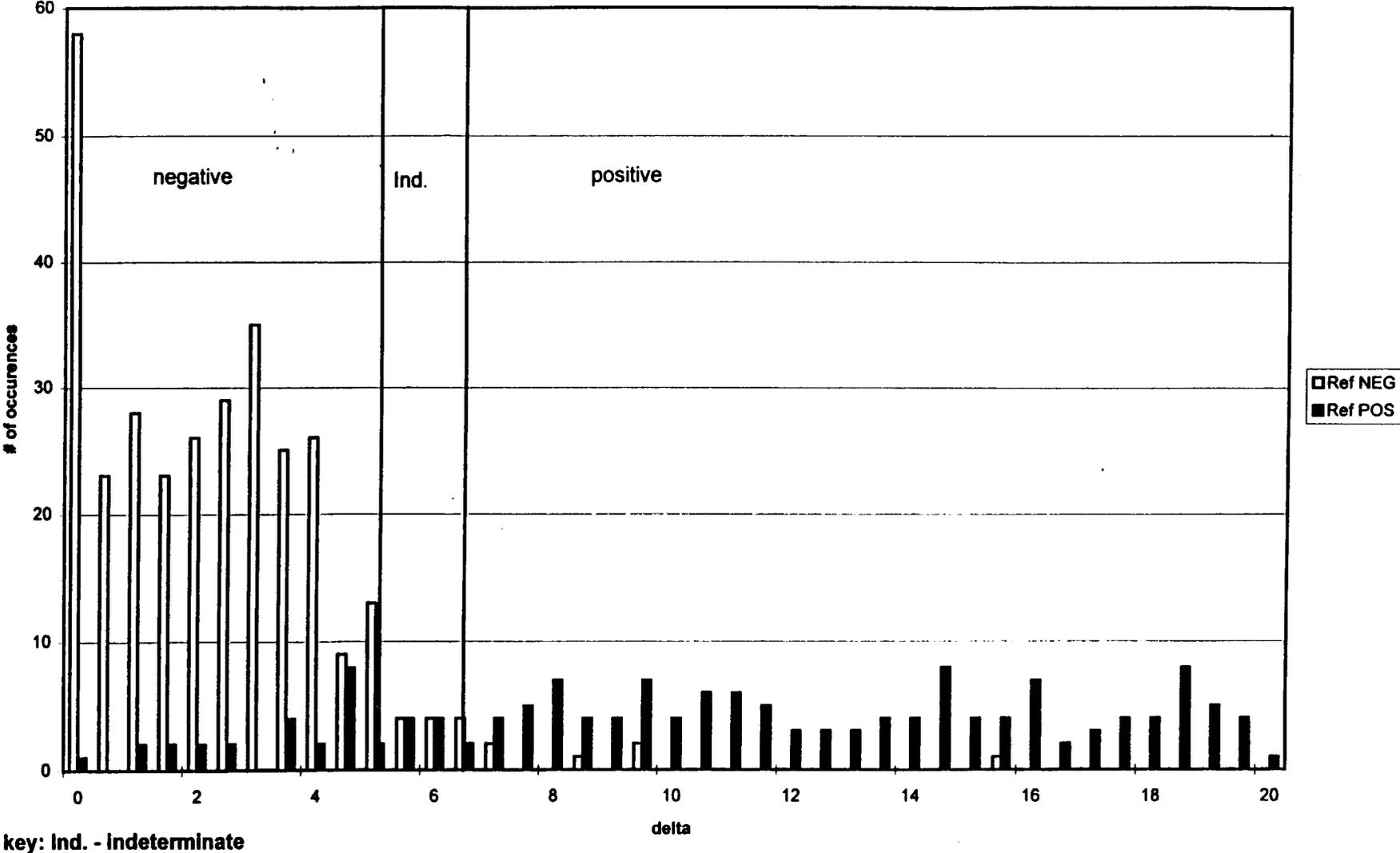
2. Clinical Trials

A. Study Protocol

The data presented in this section were collected from a clinical trial conducted at seven sites in both Europe and the United States. Patients who were referred for upper gastrointestinal endoscopy were eligible to enter the study, regardless of whether the patients had a history of ulcer. Of the 432 patients who were enrolled in the study, 398 successfully completed the Pylori-Chek breath test. Of these, 31 were diagnosed with duodenal ulcers, 19 were diagnosed with gastric ulcers, 393 were diagnosed with gastritis and 42 were diagnosed as normal. Including the breath test, patients who entered the study were tested for *H.pylori* infection using the following four diagnostic methods:

Histopathology. Two biopsy specimens obtained from endoscopy were evaluated by an experienced pathologist using hematoxylin-eosin stain and the Warthin-Starry methods.

Figure 1 - Histogram for the Distribution of Results from the Uninfected and Infected Patients



Bacterial Culture. Biopsy tissue was cultured using selective and nonselective media at 37 °C. Samples were examined every 3 days for 12 days. *H.pylori* were identified on the basis of gram morphology and production of cytochrome oxidase, catalase and urease.

CLOtest®. A biopsy specimen obtained from endoscopy was tested for urease activity in accordance with the CLOtest® instructions.

Alimenterics Pylori-Chek Breath Test. The breath test was performed in accordance with the instructions described in this package insert.

B. Study Results

This section compares the results of the Alimenterics Pylori-Chek Breath Test to the results obtained with other reference standards: histology, bacterial culture and CLOtest® for urease activity. Tables 1 and 2 compare the Alimenterics Pylori-Chek Breath Test results to histology and CLOtest®, respectively. Table 3 compares the Alimenterics LARA™ Breath Test results to the result determined by a combination of reference methods (i.e., a patient was considered positive if either the culture was positive or both the CLOtest® and histology were positive).

**Table 1
Comparison of Pylori-Chek Breath Test
to Histology**

Histology Results	Pylori-Chek Breath Test Results			
	Positive	Negative	Indeterminate	Total
Positive	215	12	4	231
Negative	6	143	8	157
Total	221	155	12	388 (Note 1)

Sensitivity: 94.7% CI(97.2-90.9%)
 Specificity: 96.0% CI(98.5-91.4%)

Note 1 - Different numbers of patients successfully completed histopathology, bacterial culture and CLOtest®. Depending on the definition of the reference, this excluded

some of the 398 patients who successfully completed the breath test.

Table 2
Comparison of Pylori-Chek Breath Test
to CLOtest®

CLOtest® Results	Pylori-Chek Breath Test Results			
	Positive	Negative	Indeterminate	Total
Positive	219	10	3	232
Negative	6	146	9	161
Total	225	156	12	393 (Note 1)

Sensitivity: 95.6% CI(97.9-92.1%)
 Specificity: 96.1% CI(98.5-91.6%)

Note 1 - Different numbers of patients successfully completed histopathology, bacterial culture and CLOtest®. Depending on the definition of the reference, this excluded some of the 398 patients who successfully completed the breath test.

Table 3
Comparison of Pylori-Chek Breath Test
to Combined Reference Methods

Culture Positive or Histology and CLO Test® Positive	Pylori-Chek Breath Test Results			
	Positive	Negative	Indeterminate	Total
Positive	221	14	4	239
Negative	5	144	8	157
Total	226	158	12	396 (Note 1)

Sensitivity: 94.0% CI(96.7-90.2%)
 Specificity: 96.6% CI(98.9-92.3%)

Note 1 - Different numbers of patients successfully completed histopathology, bacterial culture and CLOtest®. Depending on the definition of the reference, this excluded some of the 398 patients who successfully completed the breath test.

XIV. UNABLE TO PROCESS

An "Unable to Process" result may occur when insufficient CO₂ (less than 2%) is found in the patient breath collector.

This condition is usually caused by either improper usage or closure of the breath collector by the patient.

In a study of 430 patients, the results for 30 patients or 7% were unavailable because of "Unable to Process" samples. Nearly half of those samples were from baseline breath collectors where patients were most unfamiliar with their use.

In a few instances, too much CO₂ (greater than 6%) was provided by the patient, again yielding an "Unable to Process" result. This can be avoided by insuring that the patient does not smoke before or during the test.

If an "unable to process" result occurs, the physician may ask the patient to return for a second test.

XV. LIMITATIONS OF THE TEST

1. A correlation between the number of *H. pylori* organisms in the stomach and the δ values has not been established.
2. The performance characteristics of the test have not been established for monitoring the efficacy of antimicrobial therapies for the treatment of *H. pylori* infection.
3. The performance characteristics of this test for persons under the age of 18 and over the age of 75 have not been established.
4. The breath specimen integrity due to storage of breath samples in breath collectors under ambient conditions has not been determined beyond 30 days.
5. Pylori-Chek Breath Test Kit should be used only to evaluate patients

with clinical signs and symptoms suggestive of gastrointestinal disease and is not intended for use with asymptomatic patients.

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Alimenterics Inc.
301 American Road
Morris Plains, N.J. 07950

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