Ez-HBT™ Test Instructions

I. Intended Use

The Ez-HBT™ is intended for use in the qualitative detection of $^{13}CO_2$ in whole blood specimens, collected after the ingestion of ^{13}C-urea. Helicobacter pylori (*H. pylori*) organisms colonizing the lining of the human stomach, produce urease which converts ^{13}C-urea into $^{13}CO_2$ and ammonia (NH_4^+). The device is indicated as an aid in the diagnosis of *H. pylori* infection in symptomatic adult subjects, 18 years or older.

For use by health care professionals. Administer test under a physician's supervision. Metabolic Solutions, Inc. or a qualified laboratory using Gas Isotope Ratio Mass Spectrometry or equivalent instrumentation must analyze the test samples.

II. Summary and Explanation

In the last decade, the most significant advancement in gastrointestinal medicine has been the discovery of *H. pylori*'s role in causing chronic active gastritis and peptic ulcer disease (PUD).[1,2] It has been shown that eradication of the organism prevents ulcer relapse.[3,4] This evidence has led gastroenterologists to routinely test patients who exhibit symptoms of peptic ulcers for *H. pylori* infection.

Several tests for the presence of *H. pylori* include invasive biopsy collection of gastric samples by means of esophagogastroduodenoscopy (EGD). Biopsy samples can be analyzed for the presence of *H. pylori* by microbiological culture, histological examination or direct detection of urease activity in the tissue. All of these biopsy-based methods are expensive, subject the patient to discomfort and risk and may give false results due to sampling errors when colonization of the gastric mucosa is patchy.[5]

Several serology-based methods are commercially available which detect the presence of antibodies to *H. pylori* organisms. These tests are unable to distinguish between active *H. pylori* infection and previous exposures to infection and thus can not conclusively demonstrate current *H. pylori* infection.

Several breath-based tests are also available commercially. These include the use of radioisotopes as well as stable isotopes of carbon for the detection of active *H. pylori* infection. The Metabolic Solutions' Ez-HBT™ Helicobacter Blood Test (described in the next section) uses a stable or non-radioactive isotope for detection of active *H. pylori* infection and uses a single blood sample collected by standard well-established venipuncture techniques 30 minutes after ingestion of the drug component.

III. Principle of the Ez-HBT™

The patient ingests an oral dose of 125 mg of reconstituted ^{13}C-urea. In the presence of the enzyme urease associated with gastric *H. pylori*, ^{13}C-urea is converted into $^{13}CO_2$ and ammonia (NH_4^+) according to the following reaction:

$$(NH_2)_2{}^{13}CO + H_2O + 2H^+ \xrightarrow{\text{Hp Urease}} {}^{13}CO_2 + 2NH_4^+$$

The $^{13}CO_2$ is absorbed into the bloodstream. This results in an increase in the ratio of $^{13}CO_2$ to $^{12}CO_2$ in blood if *H. pylori* is present in the stomach.[6,7] Analysis of the blood for increased levels of $^{13}CO_2$ is performed at Metabolic Solutions, Inc. or a qualified laboratory using Gas Isotope Ratio Mass

Spectrometry or equivalent instrumentation. In the absence of gastric *H. pylori*, the ratio of $^{13}CO_2$ to $^{12}CO_2$ in the blood does not increase.

IV. Description of Helicosol™ Diagnostic Drug Component

The Helicosol™ diagnostic drug component of the kit is ^{13}C-urea, a synthetic urea prepared as a lyophilized, white powder for reconstitution with sterile water (also provided in the kit) to produce a clear, colorless solution for oral administration. Greater than or equal to 99% of the carbon molecules in the Helicosol drug component are in the form of ^{13}C, a stable naturally occurring, non-radioactive isotope of carbon.

Helicosol™ is supplied in a 100 ml glass vial containing 125 mg ^{13}C-urea lyophilized powder.

^{13}C-urea has the following chemical formula: $^{13}CH_4N_2O$. The drug is the diamide of ^{13}C-carbonic acid and is highly soluble in water (1 gram per mL at 25°C).

An average adult body normally produces about 30 grams per day of urea, which is a product of protein metabolism. Of this amount, about 9 grams is retained. Naturally occurring urea in the body is composed of 98.9% ^{12}C-urea and 1.1% ^{13}C-urea.

V. Warnings and Precautions

1. For *in vitro* diagnostic use only. The Helicosol™ drug solution is taken orally as part of the diagnostic procedure.

2. Discard Helicosol solution if not used within 4 hours after reconstitution.

3. A negative result does not rule out the possibility of H. *pylori* infection. False negative results occur at a rate of approximately 5-10% with this procedure. If clinical signs are suggestive of H. *pylori* infection, retest with a new sample or an alternate method.

4. A false positive test may occur due to urease associated with other gastric spiral organisms observed in humans such as *Helicobacter hominis*.

5. Antimicrobials, proton pump inhibitors, and bismuth preparations are known to suppress H *pylori*, and ingestion of these within two weeks prior to performing the Ez-HBT™ Helicobacter Blood Test may give false negative results.

6. Premature collection of the blood sample can lead to a false negative diagnosis for a patient with a marginally positive Ez-HBT™ Helicobacter Blood Test result.

7. A false positive test could occur in patients who have achlorhydria.[8]

8. If particulate matter is visible in the reconstituted Helicosol™ solution after thorough mixing, the solution should not be used.

VI. Shelf Life and Storage

The components of the Ez-HBT™ test kit should be stored at 25°C (77°F), excursions permitted to 15° - 30°C (59° - 86°F). See USP Controlled Room Temperature procedures for further details.

The following components of the Ez-HBT™ test kit have expiration dates: ^{13}C-Urea (Helicosol™), Ensure™ nutrition drink, Vacutainer® blood tubes and sterile water. Do not use any of these components beyond the indicated expiration date on the respective labels.

VII. Patient Preparation

1. The patient should have fasted at least four (4) hours prior to administering the Ez-HBT™ test.

2. The patient should not have taken antimicrobials, proton pump inhibitors or bismuth preparations within two weeks prior to administering the Ez-HBT™ test.

VIII. Procedure for Collection of Blood Samples

Materials Provided in the Collection Kit

- One (1) glass vial containing Helicosol™ powder (^{13}C-urea, 125 mg)
- One (1) bottle containing sterile water (75 ml)
- Two (2) Drinking Straws
- One (1) 8 oz. Ensure™ drink, vanilla flavor
- One (1) Vacutainer® 3 ml tube containing sodium heparin
- Vacutainer® Brand Blood Collection System including needle and adapter
- Gloves
- Alcohol wipe
- Bandage
- Tourniquet
- Gauze

Materials Needed But Not Provided in the Collection Kit

- A timer capable of timing an interval up to 30 minutes
- Test request forms (optionally provided by qualified laboratory)
- Specimen labels (optionally provided by qualified laboratory)
- Specimen return envelopes (optionally provided by qualified laboratory)

Note: A Gas Isotope Ratio Mass Spectrometer and related analytical equipment are required for analysis of blood samples. Analyses are performed at Metabolic Solutions or a qualified laboratory.

IX. Step-Wise Procedure

Time intervals listed in the following procedure are critical. The timer icon highlights these critical steps: ⏲

1. Verify that the patient has been prepared for the test as specified in Section VII.

2. Open the Ez-HBT™ Kit which should contain all the materials listed above. To avoid confusion, be sure to keep these items patient-specific.

3. Complete all the areas of the test request form.

4. Open the Ensure™ can.

5. Instruct the patient to drink the Ensure™ using one of the straws. (The drink acts to delay gastric emptying during the test procedure).

6. Set the timer for five (5) minutes after the patient finishes drinking Ensure™ before administering the Helicosol™ solution.

7. Prepare the Helicosol™ solution just prior (within 30 minutes) of use:
   - Snap open the bottle of sterile water and Helicosol™ bottle.
   - Pour sterile water into the Helicosol™ bottle.
   - Re-seal the Helicosol™ bottle and gently invert the bottle 10-15 times to dissolve the Helicosol™ powder (about 15 seconds). The solution should be clear and colorless with no particulate matter. Invert several times again if the solution is not clear. If the particulate matter is present after through mixing, the solution should not be used.

8. Instruct the patient to drink all the Helicosol™ solution directly from the glass vial using second straw.

9. Set a timer to 30 minutes when the patient completes drinking the Helicosol solution. The patient should sit quietly for the 30 minute interval. The patient should not eat, drink, or smoke.

10. Thirty (30) minutes after the Helicosol™ solution is consumed, collect a blood sample. Apply a tourniquet on one arm of patient. Cleanse the area of venipuncture with alcohol and dry with gauze.

11. Insert needle into holder of the Vacutainer® Blood System. Using gloves and standard venipuncture techniques, collect a blood sample into the 3-ml Vacutainer tube.

12. After the blood collection is complete, remove the tourniquet from arm and then remove needle from vein. Apply pressure to the venipuncture site and then place a bandage over site.

13. Dispose of needle using standard universal precaution techniques.

14. Clearly mark the blood tube with regard to patient name, date and time of venipuncture. Mark this information directly on the Vacutainer® tube or on the labels provided by the qualified laboratory.

15. Review the completed test form(s) and labels for accuracy and completeness. Retain a copy of the test form.

16. Prior to shipment store samples at 15° - 30°C (59° - 86°F).

17. Send to lab within 4 days of test administration. Blood samples must arrive at the laboratory before 7 days after collection, otherwise the samples are not suitable for analysis.

X. Quality Control

Metabolic Solutions' clinical laboratory and other qualified laboratories performing the Ez-HBT blood analysis follow written policies and procedures designed to monitor and evaluate the overall quality of the total testing process. Prior to becoming a qualified testing facility, the laboratory must meet specifications for precision, accuracy, memory carryover and linearity of $^{13}CO_2$ measurements. In addition, proficiency samples provided by Metabolic Solutions are used to qualify laboratories.

As part of the Quality Assurance program, control and reference gases are used to detect persistent and sporadic errors. Persistent errors are detected using control and reference gases periodically placed throughout the analytical run to accept or reject whole runs or portion of runs. Sporadic errors, which

occur unpredictably on individual specimens, are detected by quality criteria applied to each blood tube measurement. These criteria include a certain minimum amount of CO_2 in the blood tube for measurement and analytical result within an expected range (see Section XIII).

In the event of quality criteria failure detected by the laboratory, you will be notified as soon as possible about the nature of the failure and the recommended remedial action.

XI. Test Results

A. Analytical Method

The ratio of $^{13}CO_2/^{12}CO_2$ in the blood sample is determined by Metabolic Solutions, Inc. or another qualified laboratory using Gas Isotope Ratio Mass Spectrometry or equivalent instrumentation.

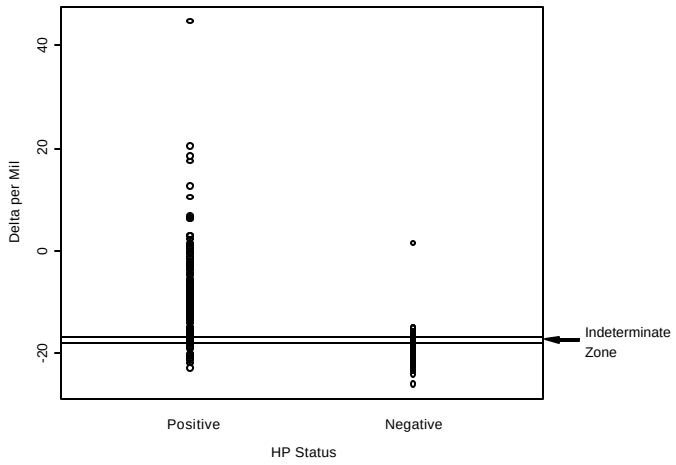
B. Calculation of Results

The result of the Ez-HBT test is provided as the delta per mil. No calculations are required by the customer. Delta per mil is the relative difference between the ratio of $^{13}CO_2$ to $^{12}CO_2$ in the sample and a reference standard.

C. Determination of the Cutoff Point

The cut-off point is the level used to discriminate between *H. pylori* infected and uninfected individuals. For the Ez-HBT™ Helicobacter Blood Test, the delta per mil cutoff point was found to be -17.5 in a controlled study of 338 patients (125 infected and 178 noninfected out of 303 with congruent results). The reference standards were histological examination of biopsy tissue and rapid urease testing. The cutoff point was evaluated by determining the Ez-HBT™ values at which histologically and urease activity negative and positive subjects were best distinguished. Figure 1 shows graphically the Ez-HBT cutoff point that distinguishes histologically positive and negative patients. An indeterminate zone of 1 per mil around the cutoff (-17.0 to -18.0) was established. No determination as to the presence or absence of H. *pylori* should be made for a subject whose Ez-HBT™ result falls within this zone. If a test result is indeterminate, a repeat test should be performed with a timed blood collection at 45 minutes, instead of the usual 30 minutes, in order to confirm or reject *H. pylori* infection.

Figure 1



D. Interpretation of Results

An Ez-HBT™ result greater than -17 delta per mil units is considered diagnostically positive, indicating the presence of urease associated with H. *pylori*. An Ez-HBT™ result between -17.0 and -18.0 delta per mil is considered indeterminate. An Ez-HBT™ results less than -18 delta per mil is interpreted as diagnostically negative, indicating the absence of urease associated with *H. pylori*.

XII. Limitations of the Test

1. The performance characteristics of the test have not been established for monitoring the efficacy of antimicrobial therapies for the treatment of H. *pylori* infection.

2. The performance characteristics of the test have not been established for persons under the age of 18.

3. The specimen integrity due to storage of blood samples under ambient conditions has not been determined beyond seven (7) days.

4. A correlation between the number of *H. pylori* organisms in the stomach and the Ez-HBT™ test has not been established.

5. The Ez-HBT™ Helicobacter Blood Test should be used only to evaluate patients with clinical signs and symptoms suggestive of active duodenal ulcer disease.

6. The performance of the assay was assessed using the Europa Scientific 20/20 ABCA system and the Finnegan MAT Plus. Any other systems should be validated using the manufacturers specifications.

XIII . Expected Values

Expected values for the Ez-HBT test were determined in a controlled clinical study of 338 adult patients with dyspeptic symptoms (125 infected and 178 uninfected out of 303 with congruent results by histology and rapid urease test). The range of Ez-HBT values for the uninfected group was determined to be –26.4 to 1.29 delta per mil and 23.29 to 44.57 delta per mil for the infected group. A histogram for the distribution of delta per mil values from the uninfected patients is shown in Figure 2 and from the infected patients is shown in Figure 3.

Figure 2

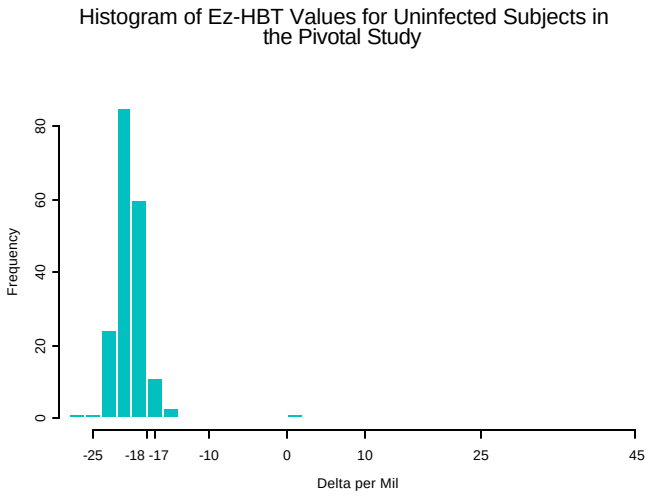
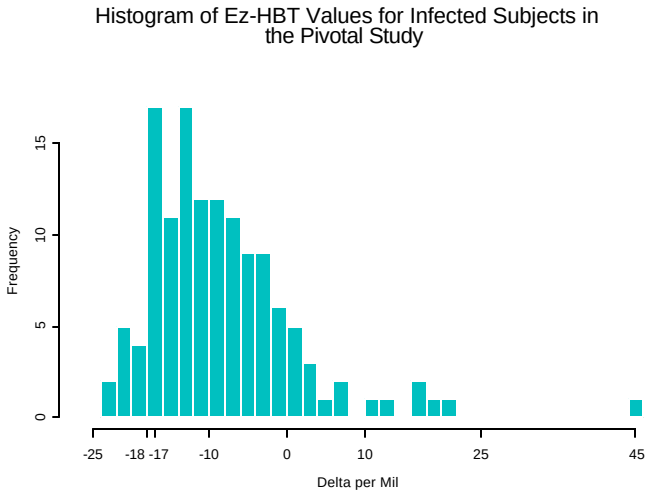


Figure 3



XIV. Interlaboratory Comparison Study

A study evaluated the variability of Ez-HBT blood measurements among three laboratories utilizing two different gas isotope ratio mass spectrometers. All laboratories met specifications for precision, accuracy, memory and linearity. A human blood pool was spiked with six different levels of sodium ^{13}C-bicarbonate to simulate blood samples of negative, positive and indeterminate values. Samples were randomized and coded to blind each laboratory. Two different proficiency blood sets were used to compare laboratory #1 both with laboratory #2 and with laboratory #3.

The interlaboratory comparisons are shown graphically in Figures 4 and 5. The linear regression plots for each laboratory with the amount of ^{13}C-bicarbonate added to blood versus observed delta per mil is displayed. The slope and intercept of the linear regression plots between laboratory #1 and laboratory #2 or between laboratory #1 and laboratory #3 were not statistically different ($P > 0.05$).

Figure 4

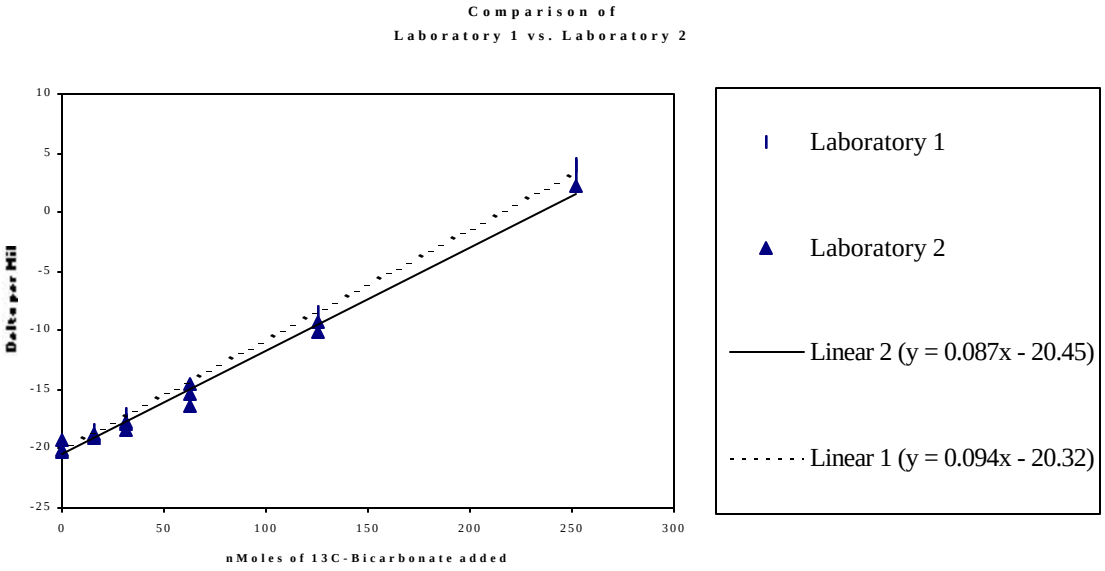
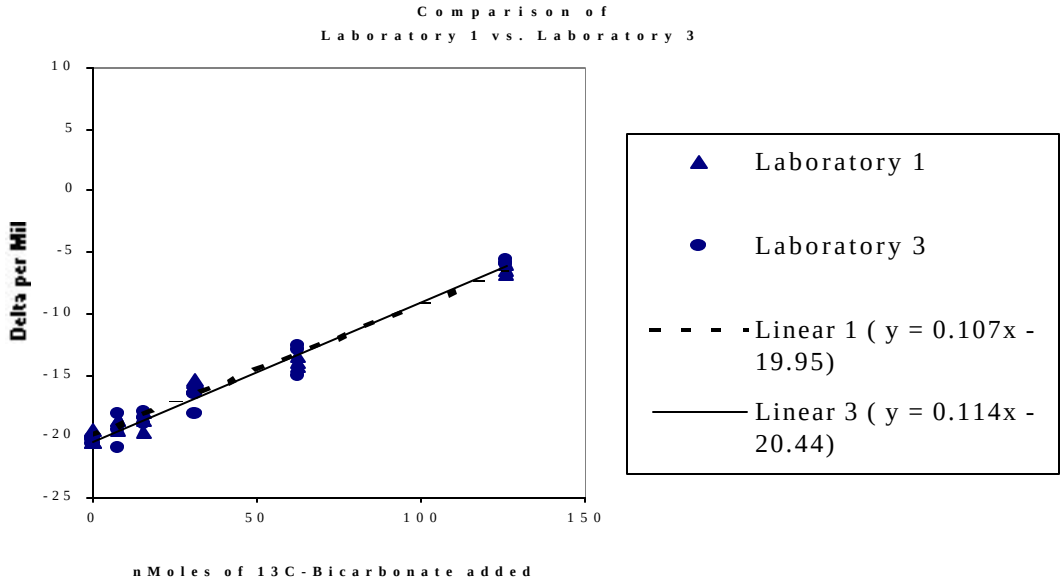


Figure 5



In summary, the results of this interlaboratory comparison indicate that a qualified laboratory using the appropriate equipment can perform the Ez-HBT analysis routinely.

XV. Performance Characteristics

Method Comparisons in Clinical Trials

Experimental Design

The method comparison data presented here were collected from a double blind clinical field trial. The study had 338 adult patients with gastrointestinal symptoms who were enrolled in the study at 7 clinical sites around the United States and evaluated by three diagnostic methods:

1. Histopathology

Biopsy specimens, fixed with 10% buffered formalin, were cut into 4-mm sections, stained with hematoxylin and eosin and Warthin-Starry stains and examined by an experienced pathologist.

2. PyloriTekÒ (Serim Research Corp.)

Biopsy specimens were tested for urease activity with the PyloriTek® test method according to the instructions on the package insert.

3. Ez-HBT™ Helicobacter Blood Test (Metabolic Solutions, Inc.)

The Ez-HBT™ Helicobacter blood test was performed in accordance with the procedures described in this package insert.

Results:

Method comparison results are presented in two-way contingency tables. Tables 1 and 2 compare the Ez-HBT™ to histological examination and the PyloriTek® test method respectively. In Table 3, the Ez-HBT™ is compared to a congruent result from the two biopsy-based methods (histology and PyloriTek®).

Table 1: Comparison to Histological Examination

	Ez-HBT™ Helicobacter Blood Test			
Histology	Positive	Negative	Indeterminate	Total
Positive	119	12	8	139
Negative	11	177	7	195
Total	130	189	15	334

SENSITIVITY: 90.8% [95 % CI (85.9 : 95.8)]
SPECIFICITY: 94.1% [95 % CI (90.8 : 97.5)]

Table 2: Comparison to PyloriTek®

Ez-HBT™ Helicobacter Blood Test

PyloriTek®	Positive	Negative	Indeterminate	Total
Positive	117	16	8	141
Negative	12	171	6	189
Total	129	187	14	330

SENSITIVITY: 88.0% [95 % CI (82.4 : 93.5)]
SPECIFICITY: 93.4% [95 % CI (89.9 : 97.0)]

Table 3: Comparison to Congruent Endoscopic Methods (Histlogy, PyloriTek®)

Ez-HBT™ Helicobacter Blood Test

Congruent Endoscopy	Positive	Negative	Indeterminate	Total
Positive	115	10	7	132
Negative	9	169	5	183
Total	124	179	12	315

SENSITIVITY: 92.0% [95 % CI (87.2 : 96.8)]
SPECIFICITY: 94.9% [95 % CI (91.7 : 98.2)]

XVI. References:

1) Marshall, B.J., Warren, J.R., Unidentified curved bacilli on gastric epithelium in active chronic gastritis, Lancet, June 4: 1273-1275; 1983.
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3) Hentschel E, Brandstatter G, Dragosics B, et al. Effect of Ranitidine and Amoxicillin plus Metronidazole on the eradication of *Helicobacter pylori* and the recurrence of duodenal ulcers, (1993) New Eng. J. Med., 328: 308-312.
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5) Northfield TC, Mendall M, Goggin PM (Eds), Helicobacter pylori Infection. Pathophysiology, Epidemiology, and Management, Kluwer Academic Publisher (1993).
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7) Kim M.J., Michener, R., Triadafilopoulos, G., Serum ^{13}C-bicarbonate assay for the diagnosis of gastric *Helicobacter pylori* infection and response to treatment, Gastroenterology 1997;113:31-37.
8) Borriello SP, Reed PJ, Dolby JM, Barclay FE, Webster ADB. Microbial and metabolic profile of achlorhydric stomach: comparison of perniciousanaemia andhypogammaglobulinaemia. J Clin Pathol 1985;38:946-953.

The Ez-HBT™ Helicobacter Blood Test is manufactured for Metabolic Solutions, Inc., Nashua, NH 03063 by Medikmark, Inc., Buffalo Grove, IL 60089. Helicosol™ is manufactured by Lyophilization Services of New England, Inc., Manchester, NH 03103.