CENTER FOR DRUG EVALUATION AND RESEARCH

APPLICATION NUMBER:

21-092

CLINICAL AND STATISTICAL REVIEW(S)

J. Fritsch HFD-570

CLINICAL PHARMACOLCOGY / BIOPHARMACEUTICS REVIEW JUN 24 1999

NDA:	21-092
Submission Date:	3/17/99
Drug:	^{13}C-urea (Helicosol™)
Device:	Ez-HBT™ Helicobacter Blood Test
Sponsor:	Metabolic Solutions, Inc. Nashua, NH 03063
Type of Submission:	New NDA
Category:	5S
OCPB Reviewer:	Joette M. Meyer, Pharm.D.

I. PURPOSE

This new NDA was submitted for a device/drug product called the Ez-HBT Helicobacter Blood Test. This diagnostic test qualitatively detects urease activity associated with *Helicobacter pylori* organisms colonizing the lining of the human stomach using in vitro measurement of $^{13}CO_2$ in blood samples of subjects who have ingested ^{13}C-urea (Helicosol).

II. BACKGROUND

Several urea breath tests (UBTs) have been FDA approved and contain ^{13}C- or ^{14}C-urea. Pranactin was the first ^{13}C-urea diagnostic drug to be approved (1996) as part of the Meretek UBT Kit. It contains 125 mg of ^{13}C-urea. The Pylori-Check Breath Test by Alimenterics contains 100 mg of ^{13}C-urea.

The Metabolic Solutions Ez-HBT Helicobacter Blood Test also uses ^{13}C-urea (125 mg), but the ^{13}C is detected in the blood, as opposed to detection in the exhaled breath.

Reviewer's Comment: The manufacturer of ^{13}C-urea, [redacted] is the same for the Ez-HBT test and the Meretek UBT test.

Principle of the Ez-HBT Kit
The subject ingests an oral dose of ^{13}C-urea. The enzyme urease associated with gastric *H. pylori* converts urea 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 $^{13}CO_2$ in blood if *H. pylori* is present in the stomach. Analysis of the blood is performed at Metabolic Solutions, Inc. or a qualified laboratory using Gas Isotope Ratio Mass Spectrometry or equivalent instrumentation. The method involves liberation of blood $^{13}CO_2$ using acid and measuring the headspace gas for the ratio of $^{13}CO_2$ to $^{12}CO_2$ by mass spectrometry. Presence of increased levels of $^{13}CO_2$ in the blood above a cutoff value indicates the presence of *H. pylori*.

Description of Helicosol Drug Component
The Helicosol diagnostic drug component of the kit is ^{13}C-urea, a synthetic urea linked to a stable naturally occurring isotope of carbon. ^{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). It is supplied in a glass vial containing 125 mg of lyophilized powder, for reconstitution with sterile water (also provided in the kit) to produce a clear solution for oral administration.

Pharmacology
Sterile urea has been FDA approved since 1966 and is on the "generally recognized as safe" list according to the US Code of Federal Regulations (21 CFR 184.1923).

Urea is a natural constituent of many common food products as well as a natural constituent of the human body. In healthy individuals, urea production is about 30 gm/day. All urea in the body occurs as a mixture of the ^{12}C isotope (98.9%) and the ^{13}C isotope (1.1%).

At low doses of urea, less than 100 mg/kg or about 7 gm, gut bacteria convert urea into carbon dioxide and ammonia. The ammonia is either incorporated into nonessential amino acids and then reincorporated into proteins or reconverted into urea and renally excreted. The carbon dioxide is incorporated into body substrates, such as amino acids, sugars and fatty acids, or exhaled in the breath.

After exogenous administration of higher doses, 40 grams, urea acts as an osmotic diuretic. The primary mechanism is physical and related to the intravenous administration of a hypertonic urea solution which rapidly increases blood urea osmolarity. An additional pharmacologic effect is termination of midtrimester pregnancy by intra-amniotic injection of hypertonic sterile 30% urea solutions of between 30 and 180 grams of urea. It also has been given as a 30% solution at a dose of 1.0-1.5 gm/kg for IV administration in acute angle-closure glaucoma.

Safety
Helicosol is urea synthesized with the isotope ^{13}C replacing the more abundant stable isotope ^{12}C. The only difference between the two molecules is the atomic mass. Isotope effects in biochemical reactions are negligible for ^{13}C-urea compared with ^{12}C-urea.

The average human body contains 1980 mg/kg or 138.6 gm of ^{13}C. Administration of Helicosol (125 mg ^{13}C-urea) results in less than a 0.1% increase in the ^{13}C content of the body. Therefore, it is believed that the administered dose is too low to have any therapeutic or pharmacologic effects other than as a detection substrate.

No serious adverse events occurred as a result of Helicosol administration in 615 subjects tested in the Ez-HBT clinical trials. In addition, there have been no serious adverse events reported in the literature with ^{13}C-urea administration to healthy subjects or patients with renal failure and uremia.

III. SYNOPSIS

Pharmacokinetics

Reviewer's Comment: The sponsor has not conducted pharmacokinetic studies with Helicosol. Instead, the following information on the absorption, distribution, metabolism, and elimination of urea from the human body originates from reports from the published scientific literature. The sponsor provides this data to support the safety of ^{13}C-urea at the dose level found in Helicosol (125 mg).

Absorption

There is no literature information on the absolute bioavailability of urea. The presence of *H. pylori* in the stomach would confound the results of a bioavailability study since the bacteria produce an enzyme capable of hydrolyzing urea before it is absorbed.

Reviewer's Comment: ^{13}C-urea (Helicosol) does not require absorption to act as a detection substrate for H. pylori. The purpose of administering ^{13}C-urea is so that if the bacteria are present the compound will be cleaved by the bacterial urease enzyme in the GI tract and $^{13}CO_2$ will be absorbed into the blood.

Distribution

Urea is known to be a highly soluble substance (1 gram/mL of water at 25°C) and freely passes across membranes. Therefore, the volume of distribution of urea is roughly the same as total body water, about 50-70% of body weight. Table 1 summarizes the values for volume of distribution of urea from the literature in healthy male subjects and in patients with renal failure and uremia.

Reviewer's Comment: Renal failure does not appear to affect the volume of distribution, but does affect the clearance of urea, as will be seen in the upcoming section on elimination.

The tissue distribution of urea was investigated in nine mice injected intraperitoneally with 0.5 mg of ^{14}C-urea.[7] Tissue analysis revealed uniformly distributed drug (± 25%) in the following tissues: liver, spleen, heart, muscle, brain, and blood. The kidneys contained 2-3 fold higher concentrations than in the other tissues. The authors speculated the high kidney levels were due to the concentrating action of the tubules.

Metabolism

Bacteria in the lower gastrointestinal tract degrade about 25-30% of an orally administered dose of urea prior to absorption. The remaining portion of the dose is absorbed and excreted in the urine (see Table 2).

Antibiotic pre-treatment can virtually eliminate GI degradation of urea. The percent of administered drug that is metabolized in the gut decreases from about 26% down to 9%.[2]

In septic patients, the GI metabolism of urea is almost completely eliminated. The authors believe that the rate of endogenous metabolism of urea depends mainly on the activity of the gut flora, which may be affected by dietary protein intake and clinical status of the patient.[8]

Reviewer's Comment: The Ez-HBT test will be administered to patients prior to receiving antimicrobial treatment for H. pylori.

Other factors that may increase the rate or amount of degradation of orally administered urea are obesity and *H. pylori* infection.

Elimination

The elimination of urea occurs primarily by renal clearance, as mentioned previously. The clearance and elimination half-life of urea have been reported in the literature to be about 60 mL/min for healthy male subjects and about 5 mL/min for patients with renal failure and uremia as shown in Table 3. The amount of dietary protein has also been shown to decrease the amount of urea excreted renally in patients with chronic renal disease.[11]

TABLE 1
Volume of Distribution (Vd) of Urea in Healthy Subjects and Patients with Renal Failure and Uremia

Urea Isotope	Dose	Route	Subjects (N)	Vd in L (Mean ± SD)	Vd (% of body weight)	Ref.
^{13}C-urea	24 mg 48 mg	IV IV	Healthy (1)	53.9	--	1
			Uremic (1)*	37.3	--	
^{13}C-urea or ^{14}C-urea	50 – 110 mg or 5 µCi	IV	GI disorders (4)#	40.1	70.2	2
			Uremics (2)#	38.7	54.9	
^{15}N-urea or ^{14}C-urea	25 -100 mg or 2.5 µCi	IV	Healthy (6)	39.3	61.9	3
^{14}C-urea	10 µCi	IV	Healthy (4)	43.6 ± 2.6	64.8 ± 2.4	4
			Uremics (6) 30 gm protein diet	33.5 ± 3.1	63.5 ± 3.5	
			Uremics (4) 70 gm protein diet	45.4 ± 2.3	68.0 ± 2.7	
^{14}C-urea	10 µCi	IV	Uremics (13)	43.5	63.2	5
^{14}C-urea	5 µCi	IV	Healthy (6) 40 gm protein diet	44.2 ± 2.1	60.7	6
			Healthy (6) 70 gm protein diet	43.3 ± 3.1	59.3	

* end stage renal failure, no residual kidney function
\# receiving a low protein diet (30-40 grams)

TABLE 2
Renal Excretion and Metabolism of Urea

Urea Isotope	Dose	Route	Subjects (N)	Renal Excretion (% of Urea Produced)	Metabolism in Gut (% of Urea Produced)	Ref.
^{13}C-urea or ^{14}C-urea	50 – 110 mg or 5 μCi	IV	Healthy (2)*	74	26	2
			Healthy (2)* after antibiotics	91	9	
^{15}N-urea or ^{14}C-urea	25 -100 mg or 2.5 μCi	IV	Healthy (6)	78	22	3
^{14}C-urea	5 μCi	IV	Healthy (6) 40 gm protein diet	--	29 ± 2.5	6
			Healthy (6) 70 gm protein diet	--	28 ± 3.0	
^{15}N-urea and ^{14}C-urea	250 mg	IV	Healthy (3)	78.3	21.7	8
			Septic (2)	98.4	1.6	
^{15}N^{15}N-urea	200 mg	Oral	Healthy (6)	77	--	9
^{15}N^{15}N-urea	Prime/intermittent doses (6 mg)	IV	Healthy (5)	63	37	10

* receiving a low protein diet (30-40 grams)

TABLE 3
Clearance and Half-Life of Urea in Healthy Subjects and Patients with Renal Failure and Uremia

Urea Isotope	Dose	Route	Subjects (N)	CL in mL/min (Mean ± SD)			$T_{1/2}$	Ref.
				CL_T	CL_R	CL_{NR}		
^{13}C-urea	24 mg 48 mg	IV IV	Healthy (1)	67.8	--	--	9.3	1
			Uremic (1)*	7.4	--	--	58.6	
^{13}C-urea or ^{14}C-urea	50 – 110 mg or 5 μCi	IV	GI disorders (4)*	--	--	--	6.7	2
			Uremics (2)*	--	--	--	26.8	
^{14}C-urea	10 μCi	IV	Healthy (6)	49 ± 2.8	--	--	8.3 ± 0.9	4
			Uremics (6) 30 gm protein diet	3.8 ± 0.7	--	--	65.5 ± 9.7 (n=10)	
			Uremics (4) 70 gm protein diet	9.4 ± 1.7	--	--		
^{14}C-urea	10 μCi	IV	Uremics (13)	3.5	1.4	2.1	120	5
^{15}N^{15}N-urea	28.5 mg x 1, then 6hrs later 5.5 mg Q3h x 5 doses	Oral	Healthy (6)	64.4	38.7 ± 9.2	25.7 ± 17.2	--	11

* end stage renal failure, no residual kidney function
* receiving a low protein diet (30-40 grams)

IV. SUMMARY

The Ez-HBT test is the first ^{13}C-urea blood test to seek FDA approval. The mechanism *of H. pylori* detection by this test is similar to FDA approved ^{13}C-urea breath tests. After ingestion of an oral dose of ^{13}C-urea, *H. pylori* is able to convert urea into $^{13}CO_2$ and ammonia (NH_4^+) by using its urease enzyme. The $^{13}CO_2$ is absorbed into the bloodstream and eventually exhaled in the breath. The Ez-HBT test detects the presence of increased levels of $^{13}CO_2$ in the blood.

Urea is a natural constituent of many common food products as well as a natural constituent of the human body. Helicosol, the drug component of the Ez-HBT test, is urea synthesized with the isotope ^{13}C replacing the more abundant stable isotope ^{12}C. The dose of ^{13}C-urea in Helicosol (125 mg) is much lower than the amount of endogenous ^{13}C-urea in the body and therefore is too low to have any therapeutic or pharmacologic effects other than as a detection substrate.

The pharmacokinetics of urea have been adequately described in the literature. Although Helicosol is administered orally, the absorption of urea is not necessary for the drug to act as a detection substrate for *H. pylori.* Therefore, bioavailability is not meaningful. The volume of distribution of urea is roughly the same as total body water. Bacteria in the lower gastrointestinal tract degrade about 25-30% of an orally administered dose prior to absorption. The remaining portion of the dose is absorbed and excreted in the urine. Renal impairment significantly decreases the renal clearance of urea.

Healthy subjects and patients with renal failure have tolerated administration of doses of isotope-labeled urea similar to the 125-mg dose of ^{13}C-urea contained in Helicosol without serious adverse events.

V. REFERENCES

1. Kloppenburg WD, Wolthers BG, Stellaard, F, et al. Determination of urea kinetics by isotope dilution with [^{13}C]urea and gas chromatography-isotope ratio mass spectrometry (GC-IRMS) analysis. Clin Sci 1997;93:73-80.

2. Jones, EA, Smallwood RA, Craigie A, et al. The enterohepatic circulation of urea nitrogen. Clin Sci 1969;37:825-36.

3. Walser M, Bodenlous LJ. Urea metabolism in man. J Clin Invest 1959;38:1617-26.

4. Varcoe R, Halliday D, Carson ER, et al. Efficiency of utilization of urea nitrogen for albumin synthesis by chronically uraemic and normal man. Clin Sci Molec Med 1975;48:379-90.

5. Walser M. Urea metabolism in chronic renal failure. J Clin Invest 1974;53:1385-92.

6. Gibson JA, Park NJ, Sladen GE, et al. The role of the colon in urea metabolism in man. Clin Sci Molec Med 1976;50:51-9.

7. Leifer, E, Roth LJ, Hempelmann LH. Metabolism of ^{14}C-labeled urea. Science 1948;108:748.

8. Long CL, Jeevanandam M, Kinney JM. Metabolism and recycling of urea in man. Am J Clin Nutr 1978;31:1367-82.

9. Jackson AA, Danielsen MS, Boyes S. A Noninvasive Method for Measuring Urea Kinetics with a Single Dose of [$^{15}N^{15}N$]urea in free-living humans. J Nutr 1993;123:2129-36.

10. Hibbert JM, Forrester T, Jackson AA. Urea kinetics: comparison of oral and intravenous dose regimens. Eur J Clin Nutr 1992;46:405-409.

11. Meakins TS, Jackson AA. Salvage of exogenous urea nitrogen enhances nitrogen balance in normal men consuming marginally inadequate protein diets. Clin Sci 1996;90:215-25.

VI. RECOMMENDATION

The pharmacokinetic information submitted from the literature has been reviewed and found to be acceptable to support approval of Helicosol™ (^{13}C-urea) as part of the Ez-HBT kit for the detection of *Helicobacter pylori* in the human stomach. Please forward the labeling comments in Section VII on to the sponsor.

VII. LABELING COMMENTS FOR THE SPONSOR

1. [redacted]

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 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.

/S/ 6/24/99

Joette M. Meyer, Pharm.D.
Office of Clinical Pharmacology/Biopharmaceutics
Division of Pharmaceutical Evaluation III

/S/ 6/24/99

RD/FT signed by Funmi Ajayi, Ph.D. (Team Leader)

CPB Briefing (6/24/99) Attendees: John Lazor, Arzu Selen, Funmi Ajayi, Kellie Schoolar Reynolds, Francis Pelsor, Dennis Bashaw, Kathleen Uhl

cc: HFD-590: /NDA 21-092
/CSO/~~AndreenR~~ FritschJ
HFD-880: /BiopharmTL/AjayiF
/Biopharm/MeyerJ
/DPEIII
CDR: /MurphyB
HFZ-440 /Doria Dubois

HFD-590
J. Friesch

CLINICAL AND STATISTICAL REVIEW

JUL 26 1999

NDA:	21-092
Submission Date:	3/17/99
Drug:	Helicosol™ (125 mg ^{13}C-urea)
Device:	Ez-HBT™ Helicobacter Blood Test
Sponsor:	Metabolic Solutions, Inc. 460 Amherst Street Nashua, NH 03063
Lead Center:	CDRH
Consulting Center:	CDER, Division of Special Pathogens and Immunologic Drug Products
Material Reviewed:	2 Volumes

Table of Contents:

I. **PURPOSE** 2

II. **SUMMARY OF CLINICAL DATA** 2

III. **REVIEWERS' RECOMMENDATIONS** 5

IV. **REVIEW OF INDIVIDUAL STUDY REPORTS**

A. **Protocol HBT-01** 6

B. **Protocol HBT-02** 10

C. **Protocol HBT-03-Cutoff** 15

D. **Protocol HBT-03** 23

E. **Protocol HBT-02 Amend 1** 40

F. **Protocol HBT-04** 47

APPENDIX 1 – Database Tabulation (Protocol HBT-03-Cutoff) 51

APPENDIX 2 – Database Tabulation (Protocol HBT-03) 52

I. PURPOSE

To evaluate a new device/drug product called the Ez-HBT Helicobacter Blood Test. This is a non-invasive, non-radioactive method for detecting *H. pylori* infection by orally administering 125 mg of ^{13}C-urea (Helicosol) and detecting $^{13}CO_2$ in a single blood sample obtained 30 minutes after administration. Presence of $^{13}CO_2$ in the blood above a cutoff value indicates the presence of *H. pylori.*

The Ez-HBT diagnostic test and Helicosol (drug product) is intended for use in the qualitative detection of urease activity associated with *H. pylori* organisms colonizing the lining of the human stomach. The test kit and drug product are intended to aid in the diagnosis of *H. pylori* infection in adult subjects. The test kit containing the drug product is to be administered only by prescription and under a physician's supervision. The testing of the blood sample is only to be performed by a qualified laboratory with a gas isotope ratio mass spectrometer.

II. SUMMARY OF CLINICAL DATA

Six separate studies employing 615 subjects were conducted to show the safety and efficacy of the device/drug product. These studies included the evaluation of both symptomatic (peptic ulcer-like symptoms) and asymptomatic subjects. A variety of reference methods (UBT, serology, histology, and urease) were employed to evaluate efficacy. Two of the studies also investigated the effects of: varying sample draw times, varying blood sample volumes, sample storage times, sample reproducibility, and air transportation on the results obtained from the test.

A summary of each of the clinical studies follows. More detailed information and results can be found in the review of individual reports in Section IV (page 6).

Protocol HBT-01: Comparison of [13C] urea breath test to [13C] urea blood test for the detection of *H. pylori*

Seventy-one (71) symptomatic subjects were examined using the Ez-HBT test in order to ascertain the cut-off point for the test. These subjects were given both the urea breath test (research test conducted and analyzed by the sponsor) and the Ez-HBT test at 2 U.S. university hospitals. The 43 subjects who were confirmed negative by UBT were next examined for their Ez-HBT values. Using this approach, a negative cut-off of –17.0 delta per mil was established. These results led to Protocol HBT-02 in order to refine the cut-off point.

Protocol HBT-02: An Investigation of a Blood Test (Ez-HBT Helicobacter Blood Test) for Diagnosis of Active Helicobacter pylori Infection)

Fifty-five (55) asymptomatic subjects were enrolled at a clinical research organization in the U.S. The Ez-HBT test was investigated in comparison to four commercially available serologic tests [redacted] Again, the cut-off point for the Ez-HBT test was determined to be –17.0 delta per mil. This was determined by assessing the Ez-HBT value of the 23 confirmed serologic negative subjects (as determined by 3 out of 4 negative tests).

Protocol HBT-03-Cutoff: An Investigation of a Cut-off Level for a Blood Test (Ez-HBT Helicobacter Blood Test) for Diagnosis of Active *Helicobacter pylori* Infection

One hundred twenty-one (121) subjects with dyspeptic symptoms at 5 clinical sites around the U.S. were given the Ez-HBT test. Each subject underwent an endoscopy with 4 biopsy tissue collections and an Ez-HBT test. An experienced pathologist at each clinical site examined a biopsy specimen from two collection sites. The other two specimens were tested by rapid urease test (PyloriTek® or CLOtest®). Sensitivity, specificity, and accuracy were measured versus reference methods histology and rapid urease (PyloriTek) independently as well as the two methods congruently. The cut-off point was confirmed as –17.0 delta per mil.

The performance characteristics of the test in this study are shown below.

Performance Characteristics	Histology (N=121)	Rapid Urease Test (N=121)	Congruent Methods (N=111)*
Sensitivity	89%	92%	94%
Specificity	96%	93%	98%
Accuracy	93%	93%	96%
Positive Predictive Value	94%	90%	98%
Negative Predictive Value	91%	94%	96%

* Ten samples (10/121) had non-agreement between the histology and rapid urease test results

Protocol HBT-03: Clinical Study to Evaluate the Safety and Efficacy of the Ez-HBT in Subjects referred for EGD

The safety and efficacy of the Ez-HBT test was studied in a large clinical study of 343 adult, symptomatic subjects complaining of upper or lower gastrointestinal problems at 7 monitored clinical sites around the U.S. The design was identical to the HBT-03-Cut-off study, with the exception that biopsy specimens obtained for histopathology were stained with [redacted] stains and sent to a central laboratory (University of Michigan Medical Center) for examination by experienced pathologists. The remaining two biopsy specimens were tested at the time of endoscopy for urease activity by a single manufacturer's rapid urease test, Pyloritek®.

Subjects with impaired gastric emptying or with concomitant use of antibiotic, bismuth-containing products or proton-pump inhibitors were excluded. Sensitivity, specificity, and accuracy were measured versus reference methods histology and rapid urease (PyloriTek) independently as well as the two methods congruently.

To summarize:

- 343 signed consent
- 339 without protocol violations (4 were removed from FDA analysis)
- 334 Evaluable for Ez-HBT (5 were withdrawn prior to Ez-HBT administration)
- 319 evaluated versus histology (of the 334, 15 had indeterminate results of Ez-HBT)
- 316 evaluated versus rapid urease test (of the 319, 3 rapid urease tests not performed)
- 303 evaluated for congruent results (of the 316, 13 had incongruent results)

The performance characteristics of the test in this study are shown below.

Performance Characteristics	Histology (N=319)	Rapid Urease Test (N=316)	Congruent Methods (N=303)
Sensitivity	90.8%	88.0%	92.0%
Specificity	94.1%	93.4%	94.9%
Accuracy	92.8%	91.1%	93.7%
Positive Predictive Value	91.5%	90.7%	92.7%
Negative Predictive Value	93.7%	91.4%	94.4%

Using the data from this study, a modified cut-off of –17.5 delta per mil was determined and an indeterminate zone of ± 0.5 around the cut-off point was established.

Twenty false results (11 false negatives and 9 false positives) were obtained from comparison of the Ez-HBT to congruent endoscopic methods and were investigated further to determine if any correlation could be made between demographic parameters (gender, race, weight, alcohol and tobacco consumption), medical history, concomitant drug therapy, protocol deviations and the results. No correlation was found. The sponsor believes that the false results are due to investigator inexperience with administering the test since 11/20 (55%) of the false results (8 false negatives and 3 false positives) came from among the first 15 samples done at any site.

Nine adverse events were reported in the 341 subjects who received Helicosol. Four of these events were considered mild, 3 moderate, and 2 severe. None was associated with Helicosol. The adverse events are summarized below. The causes cited were concurrent medication, Ensure, or concurrent disorders.

Protocol HBT-02 Amend 1: Investigations of External Events Effecting the Performance of the Ez-HBT

Multiple aliquots of blood were collected from 10 asymptomatic subjects and subjected to varying conditions of blood volume, sample drawing and holding times, and temperature. The conclusion was that blood samples can be drawn 30-60 minutes after Helicosol administration, but not < 20 minutes. The recommended blood volume for testing is 3 mL. However, blood samples containing less than ideal volumes of blood (< 3 mL, but > 1 mL) can be used for accurate analysis. Blood samples are stable for 7 days when stored at room temperature.

Protocol HBT-04: Effects of Air Transportation on Sample Integrity

The effect of air transportation on Ez-HBT blood samples was conducted on 20 asymptomatic subjects. Six replicate blood samples were acquired from each subject and randomized. Two of the replicates were subjected to air transportation (from New Hampshire to California and back), two were subjected to ground transportation only and analyzed immediately and the other two were transported by ground and held (until the air transported samples arrived in the lab) and then analyzed. The blood samples were unaffected time, but were affected by the effects of air transportation and the cumulative effects of air transportation (about 1.0 delta per mil mean difference for each).

III. REVIEWERS' RECOMMENDATIONS

^{13}C-urea when used in combination with the Ez-HBT Helicobacter Blood Test should be approved as the clinical studies show that it is safe and effective in assessing the *H. pylori* status of patients.

The sponsor should consider performing a validation study of the new cutoff point and indeterminate zone.

/S/ 7/26/99

Joette M. Meyer, Pharm.D.
Office of Clinical Pharmacology/Biopharmaceutics
Division of Pharmaceutical Evaluation III, CDER

/S/ 7/26/99

Karen Higgins, Sc.D.
Statistical Reviewer, DB III, CDER

Concurrence:
HFD-590/TLMO/HopkinsR 7/26/99
HFD-725/TLStat/SillimanN /S/ 7/26/99
HFD-590/DivDir/GoldbergerM 12/6/99

cc:
HFD-590/Div File/NDA 21-092
HFD-590/TLMO/HopkinsR
HFD-725/TLStat/SillimanN
HFD-590/DepDivDir/AlbrechtR
HFD-590/DivDir/GoldbergerM
HFD-880/Biopharm/MeyerJ
HFD-440/Micro/CDRH/DuboisD
HFD-44/Micro/CDRH/PooleF
HFD-160/Chem/HarapanhalliR
HFD-160/TLChem/LeutzingerE
HFD-590/PM/AndersonR
HFD-725/DivDir/HuqueM
HFD-725/Sec/ShoresS
HFD-590/PM/FritschJ

IV. REVIEW OF INDIVIDUAL STUDY REPORTS

A. Comparison of [^{13}C] Urea Breath Test to [^{13}C] Urea Blood Test for the Detection of *H. pylori* (Protocol HBT-01)

Objectives

- Determine the cut-off point for the Ez-HBT in confirmed negative subjects by urea breath test (UBT).
- Determine the types of adverse events experienced by subjects during conduct of the Ez-HBT and number (%) of subjects experiencing each type of adverse event.

Study Population

Seventy-one (71) symptomatic subjects were enrolled: 37% male and 63% female; mean (± SD) age 46 ± 13 years.

Study Design

The study was performed at two US University Hospitals on an outpatient basis. Adult subjects seeking treatment for ulcer symptoms (epigastric pain, heartburn, nausea) or clinical signs of GI bleeding (hematemesis, hematochezia, and melena) were eligible. Each subject was administered the Ez-HBT test after a minimum of an 8 hour fast. On the same day, all subjects also received a urea breath test (UBT) as described in the literature by Klein et al (Am J Gastro 1996;91:690-4). A true negative result was defined as <2.4 delta from baseline by UBT. Personnel performing the reference methods were blinded to results of the Ez-HBT. The sensitivity, specificity, and accuracy of the Ez-HBT were determined in comparison to the reference method.

Clinical Reviewer's Comment: The literature citation describes the validation of the Meretek UBT®. However, for this study the sponsor conducted the UBT test and analyzed the results at their own facility. It is unclear if they used the commercial Meretek kits.

Clinical Sites

The following principal investigators and clinical sites participated in the study:

Dr. Phillip Toskes – University of Florida, College of Medicine, Shands Hospital, Gainesville FL
Dr. Alan Cutler – Sinai Hospital, Detroit MI

Exclusion Criteria

Subjects with one or more of the following were not eligible for enrollment in this study:

- Impaired gastric emptying.
- Zollinger-Ellison syndrome or other pathologic hypersecretory conditions.
- Concomitant therapy with anticoagulants or nonsteroidal anti-inflammatory drugs.
- Mental impairment, inability, or refusal to follow instructions.
- Use of an investigational drug or participation in an investigational study within 30 days prior to the initial visit.

- Use of any proton pump inhibitor, antacids, or bismuth medications for more than 3 consecutive days prior to 30 days of the blood test.

Analytical Procedure

[redacted]

Statistical Analysis

A sample size of at least 50 subjects was chosen to assure a normal distribution. A confirmed negative result was defined as a subject with a urea breath test < 2.4 delta per mil difference between the baseline and thirty-minute samples. The 95% confidence level for 99% of negative subjects to determine an Ez-HBT cut-off was calculated using the expression:

$$\text{Tolerance interval} = \mu \pm Z\sigma$$

Where: μ = mean,
Z = factor used to contain % of population and
σ = Standard deviation

The maximum value for the tolerance interval was used as the cut-off point. A Z factor for 99% of a normally distributed population with a 95% confidence interval was used.

Statistical Reviewer's Comment: The tolerance interval proposed does not give the 95% confidence level for 99% of the negative subjects. It is merely the interval that contains 99 percent of the negative subjects under the strong and not valid assumption that the negative values of the Ez-HBT are normally distributed. A sample size of 50 may assure that the distribution of the sample mean is normal, however, it does not assure that the distribution of the sample is normal.

Results

Forty-four (44) subjects out of 71 were confirmed negative by the UBT. These 44 subjects were also examined for their Ez-HBT values. The maximum value for the tolerance interval was –17.07 delta per mil (tolerance interval = -17.07 to -25.78).

Statistical Reviewer's Comment: Based on the data submitted with the NDA, there were only 43 negative values based on the UBT test. However, this does not significantly alter the overall results.

The statistical analysis performed for the determination of the cutoff was inappropriate, however, all of the subjects with a negative value by UBT had an Ez-HBT value below –17.07 (see Figure 2).

No adverse events were reported during the study.

Conclusion

A negative cut-off of –17.0 delta per mil was established from the results of this study. There were no adverse events reported. These results led to a study of asymptomatic subjects versus serology testing in order to further refine the cut-off point (Protocol HBT-02).

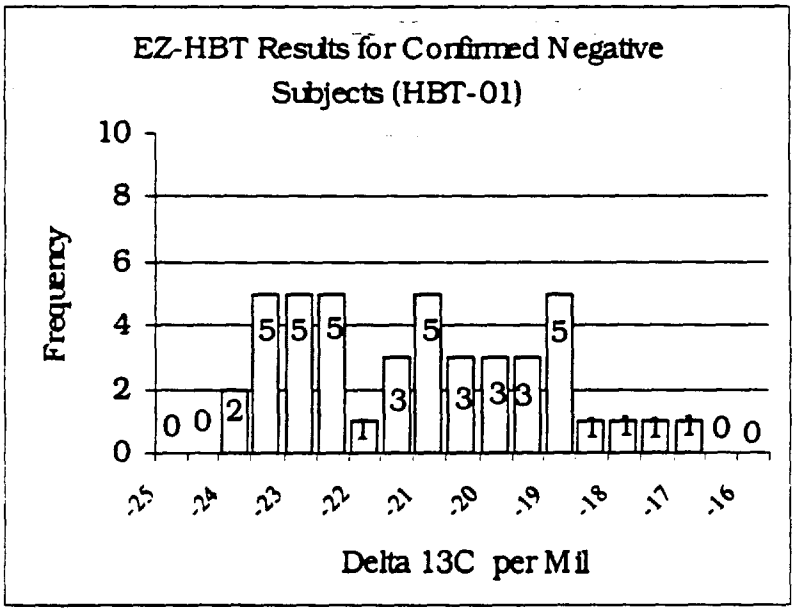
Clinical and Statistical Reviewers' Comment: The statistical analysis performed for the determination of the cutoff was inappropriate, however, we do agree with their proposed value of –17 for the cutoff.

APPEARS THIS WAY
ON ORIGINAL

APPEARS THIS WAY
ON ORIGINAL

The distribution of the Ez-HBT results for the 44 confirmed negative subjects by UBT is shown below in Figure 1.

FIGURE 1

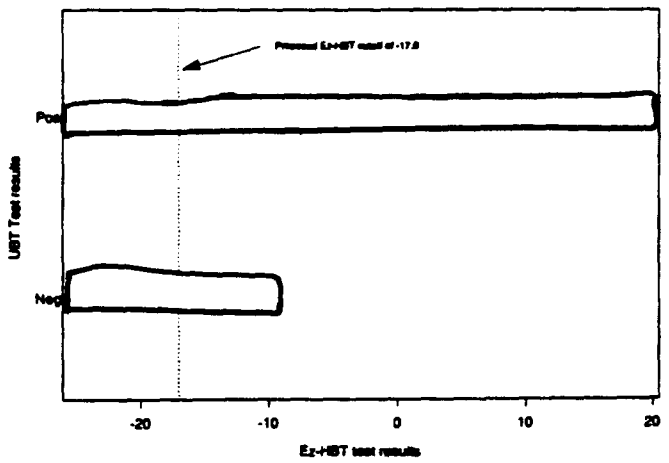


Clinical Reviewer's Comment: Of the 71 enrolled subjects, there were 28 positive subjects (H. pylori infection rate of 39%), which is within the expected range for symptomatic subjects (mean age 46 years.

The values of the Ez-HBT results versus the positive/negative status of the UBT test for the 71 subjects, along with the –17 cutoff, are shown below in Figure 2.

FIGURE 2

STUDY HBT-01



B. An Investigation of a Blood Test (Ez-HBT™ Helicobacter Blood Test) for Diagnosis of Active *Helicobacter pylori* Infection (Protocol HBT-02)

Objectives

- Establish the Ez-HBT 95% confidence interval for 99% of *H. pylori* seronegative, asymptomatic subjects.
- Determine the types of adverse events experienced by subjects during conduct of the Ez-HBT and number (%) of subjects experiencing each type of adverse event.

Study Population

Fifty-five (55) asymptomatic subjects were enrolled: 42% male and 58% female; mean (± SD) age 36 ± 13 years; 7 (13%) Hispanics, 35 (64%) African-Americans, 12 (22%) Caucasians, and 1 (1%) Asians.

Study Design

Adult subjects with no present GI symptoms or a history of peptic ulcer symptoms were eligible. A medical questionnaire was used to determine if subjects were asymptomatic. After 4 hours of fasting, all subjects had an initial blood sample drawn for serology testing of *H. pylori*. Since serology tests for *H. pylori* produce significant false positive results, four different commercially available ELISA tests were used [redacted] A true negative result was defined as having three of the four serology tests reported as negative. On the same day, the Ez-HBT test was conducted. Laboratory personnel performing the reference methods were blinded to subject status as determined by Ez-HBT. The results were used to define a range for H. *pylori* negative subjects.

Clinical Site

The study was performed at a [redacted]

Exclusion Criteria

Subjects with one or more of the following were not eligible for enrollment in this study:

- Having a history of ulcer-like symptoms (epigastric pain, indigestion, heartburn, belching, nausea, hematemesis, hematochezia, or melena).
- Impaired gastric emptying.
- Zollinger-Ellison syndrome or other pathologic hypersecretory conditions.
- Concomitant therapy with anticoagulants or nonsteroidal anti-inflammatory drugs.
- Mental impairment, inability, or refusal to follow instructions.
- Use of an investigational drug or participation in an investigational study within 30 days prior to the initial visit.
- Use of any proton pump inhibitor, antacids, or bismuth medications for more than 3 consecutive days prior to the blood test.

Clinical Reviewer's Comment: Recent use of antimicrobial agents was not an exclusion criteria for this study. Enrollment of such subjects may have contributed to the relatively large number of false positive Ez-HBT results seen in this study.

Analytical Procedure

[redacted]

Statistical Analysis

A sample size of at least 50 subjects was chosen to assure a normal distribution. A confirmed negative result was defined as a subject with a 3 of the 4 serology tests reporting negative. The 95% confidence level for 99% of negative subjects to determine an Ez-HBT cut-off was calculated using the

$$\text{Tolerance interval} = \mu \pm Z\sigma$$

Where: μ = mean,
Z = factor used to contain % of population and
σ = Standard deviation

The maximum value for the tolerance interval was used as the cut-off point. A Z factor for 99% of a normally distributed population with a 95% confidence interval was used.

Statistical Reviewer's Comment: The tolerance interval proposed does not give the 95% confidence level for 99% of the negative subjects. It is merely the interval that contains 99 percent of the negative subjects under the strong and not valid assumption that the negative values of the Ez-HBT are normally distributed. A sample size of 50 may assure that the distribution of the sample mean is normal, however, it does not assure that the distribution of the sample is normal.

Results

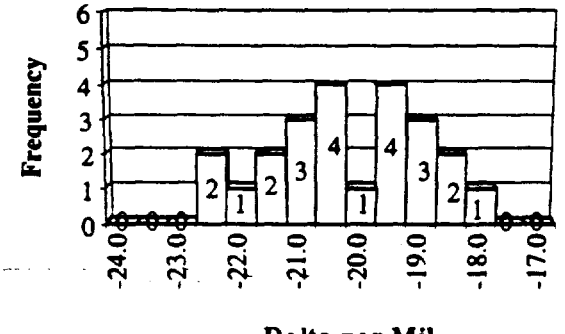
Twenty-three (23) subjects were confirmed as seronegative (3 out of 4 serology tests reporting negative). The maximum value for the tolerance interval was -16.9 delta per mil (tolerance interval = -16.9 to -23.0). The distribution of the Ez-HBT results for the 23 confirmed negative subjects is shown below in Figure 1.

Clinical Reviewer's Comment: Of the 55 enrolled subjects, there were 32 positives (H. pylori infection rate of 58%), which is high considering that the subjects were asymptomatic and relatively young (mean age 38 years). This infection rate may be a reflection of the socioeconomic status of the subjects.

Statistical Reviewer's Comment: The statistical analysis performed for the determination of the cutoff was inappropriate, however, all of the subjects with a negative value by serology had an Ez-HBT value below –16.9 (see Figure 2).

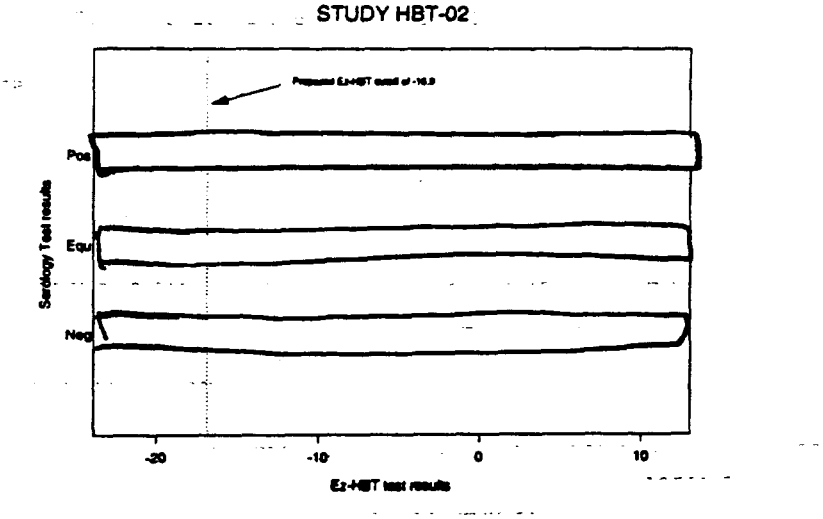
No adverse events were reported in this study.

Figure 1
Histogram of Ez-HBT Values of Confirmed Seronegative Subjects



The values of the Ez-HBT results versus the positive/negative status of the serology test for the 55 subjects, along with the –16.9 cutoff, are shown below in Figure 2, as determined by the FDA reviewers. Four subjects who did not have either 3 positive or 3 negative serology tests were labeled equivocal and are shown in the figure as 'Equ'. Of the subjects who were labeled equivocal, three had 2 positive tests and 2 negative tests and the fourth subject had 2 positive tests, 1 negative test, and 1 equivocal test.

Figure 2



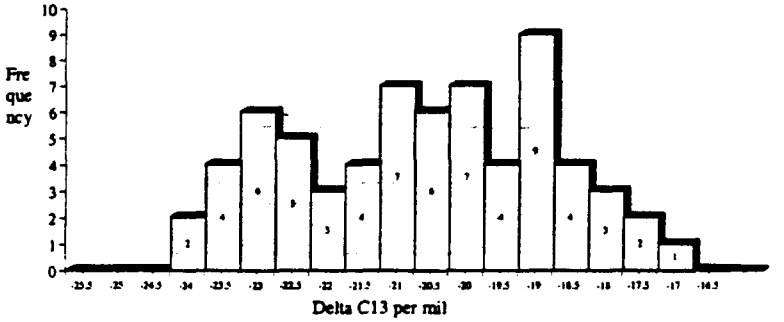
Refined Cut-off - Studies HBT-01 and HBT-02 Combined

Forty-four (44) of the UBT tested subjects (N=71) were classified as negative (< 2.4 delta per mil from baseline) in HBT-01. Twenty-three (23) of the serology tested subjects (N=55) were

seronegative (3 out of 4 seronegative results) in HBT-02. A histogram for the distribution of Ez-HBT values for the combined 67 confirmed negative subjects, reported as delta per mil versus PDB, is shown in Figure 3. The maximum value of the tolerance interval for the combined negative subjects was -16.7 delta per mil (tolerance interval = -16.7 to -25.2).

Statistical Reviewer's Comment: Based on the data submitted with the NDA, there were only 43 negative values based on the UBT test making a total of 66 confirmed negative subjects, rather than 67. However, this does not significantly alter the overall results.

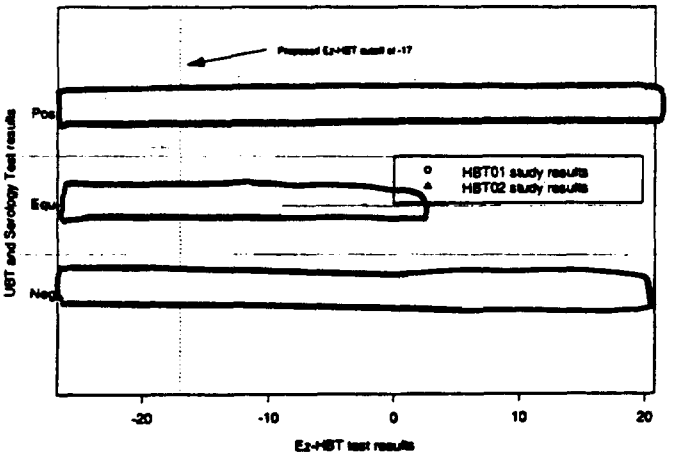
Figure 3
Histogram of MSI Ez-HBT Values for H. pylori
Negative Subjects in Preliminary Studies



The values of the Ez-HBT results versus the positive/negative status of the UBT test for the 71 subjects and of the serology test for the 55 subjects, along with the –17 cutoff, are shown below in Figure 4, as determined by the FDA reviewers.

Figure 4

Combined results from STUDY HBT-01 and -02



Conclusion

This study of 55 asymptomatic adult subjects, using serology as the reference method, yielded a cut-off point of -16.9 delta per mil. No adverse events were reported in the 55 subjects evaluated in this study.

The 23 negative values from this study were combined with the 44 negative values from the previous symptomatic study (HBT-01) to establish the cut-off point for the Ez-HBT as –17.0.

CDRH reviewed these preliminary studies and suggested that the cut-off point be based on two of three reference methods (histology, tissue urease activity, or culture). This proposed study was conducted as Protocol HBT-03-Cutoff.

Clinical and Statistical Reviewers' Comment: The statistical analysis performed for the determination of the cutoff was inappropriate, however, we do agree with their proposed value of –17 for the cutoff.

APPEARS THIS WAY
ON ORIGINAL

APPEARS THIS WAY
ON ORIGINAL

C. An Investigation of a Cut-off Level for a Blood Test (Ez-HBT™ Helicobacter Blood Test) for Diagnosis of Active *Helicobacter pylori* infection (Protocol HBT-03-Cutoff)

Objectives

- Determine whether the Ez-HBT detects active *H. pylori* infection, using gastric biopsy with histopathologic examination of stained tissue and detection of urease activity in the biopsy material as the reference methods;
- Establish assay performance characteristics in the target population.
- Evaluate the safety of the administered drug, Helicosol by determining the types of adverse events experienced by subjects during conduct of the Ez-HBT and number (%) of subjects experiencing each type of adverse event.
- Determine the most appropriate cut-off point for the test.

Study Population

One hundred twenty-one (121) adult subjects participated in this study. The mean (± SD) age for the 117 subjects with reported data was 49.4 ± 14.9 years (range 19-85 years). There were 62 males and 59 females.

Study Design

This was an outpatient study conducted at 5 clinical sites in the United States. Adult subjects seeking treatment for ulcer symptoms (epigastric pain, heartburn, nausea, hematemesis, hematochezia, and melena) that were referred for esophagogastroduodenoenoscopy (EGD) were included. Each subject underwent an EGD after a 4 hour (minimum) fast with biopsy tissue collection, administration of Helicosol, and collection of blood by venipuncture for the Ez-HBT test. Two identical pairs of gastric biopsies were collected, one pair from the greater curvature within 2 cm of the pylorus and one pair from the antrum. A biopsy specimen from the body and antrum were stained with [redacted] for examination by an experienced pathologist at each clinical site. The remaining two biopsy specimens were tested at the time of endoscopy for urease activity by a rapid urease test, PyloriTek® or CLOtest®. Results were available after 1 hour. The Ez-HBT test was performed one hour after the EGD. Laboratory personnel performing the Ez-HBT analysis were blinded to subject status as determined by endoscopy results, histology, and tissue urease activity. Pathology personnel performing the reference methods were blinded to results of the Ez-HBT.

Based on the cut-off, sensitivity, specificity, and accuracy of the Ez-HBT were calculated using each reference diagnostic procedure to classify subjects as infected or non-infected. A cut-off point was established for the test using a receiver operating characteristic (ROC) curve retrospectively.

Clinical Sites

Five (5) clinical sites from different geographic locations in the United States were used to evaluate the Ez-HBT.

The following principal investigators and clinical sites participated in the studies.

Investigator	Study Site	Number Enrolled
Dr. William Chey	University of Michigan, Ann Arbor MI	33
Dr. Phillip Toskes	University of Florida, College of Medicine, Shands Hospital, Gainesville, FL	17
Dr. Loren Laine	University of Southern California, Los Angeles CA	53
Dr. Uma Murthy	Veteran's Administration, Syracuse NY	16
Dr. Stephen Carpenter	Savannah Memorial Hospital, Savannah GA	2

Exclusion Criteria
Subjects with one or more of the following were not eligible for enrollment in this study:

- An unstable medical or surgical problem which precludes follow-up and EGD including a significant defect in coagulation (e.g., chronic liver disease, von Willibrands disease, hemophilia, thrombocytopenia (platelet count <50K) for any reason), major organ failure, major abdominal surgery or gastric surgery.
- Impaired gastric emptying.
- Zollinger-Ellison syndrome or other pathologic hypersecretory conditions.
- Concomitant therapy with anticoagulants or nonsteroidal anti-inflammatory drugs.
- Mental impairment, inability, or refusal to follow instructions.
- Use of an investigational drug or participation in an investigational study within 30 days prior to the initial visit.
- Use of any proton pump inhibitor, at any dose, within 7 days prior to endoscopy and blood test.
- Use of bismuth medications and any antibiotics within 30 days prior to endoscopy and blood test.

Analytical Procedure

Statistical Analysis

Sample Size

Calculation of a requisite sample size is based on the overall accuracy of the new test, assuming that the "gold standard" is 100% accurate in classifying cases. A power of 80% was assumed with a type I error probability of 0.05. It was also assumed that the study population would have a 30% *H. pylori* infection incidence rate. However, the rate of infection does not affect the sample size because sample size was calculated using overall accuracy. Using these assumptions, 120 evaluable subjects were required. Approximately 10% of subjects are expected to have non-evaluable results such as non-compliance with study protocol, lost samples or low levels of blood CO_2. Consequently, enrollment of approximately 132 subjects was estimated in order to reach the target enrollment of 120 evaluable subjects.

Statistical Reviewer's Comment: The information given above from the protocol on sample size calculation is incomplete. The null and alternative hypotheses need to be stated. However, with a null hypothesis that the accuracy of the Ez-HBT is 90% or less, 118 evaluable subjects would be needed given that the true accuracy of the Ez-HBT test is 96.8%.

Clinical Reviewer's Comment: Dr. Dubois of the Division of Clinical Laboratory Devices, Microbiology Branch, suggested a minimum of 120 subjects be used in this study to determine the cutoff point prior to a larger proposed study.

Interpretation of Ez-HBT Results

- *H. pylori* Infected - CO_2 levels ≥ - 17 delta per mil (relative to PDB)
- *H. pylori* Non-Infected - CO_2 levels < - 17 delta per mil (relative to PDB)
- Non-evaluable - samples containing < 1% CO_2 gas

Interpretation of Reference Test Results

- H. *pylori* Infected - a positive histopathological diagnosis and a positive rapid urease test result.
- H. *pylori* Non-Infected - a negative histopathological diagnosis and a negative rapid urease test.

Clinical Reviewer's Comment: In this study two identical pairs of gastric biopsies were collected, one pair from the greater curvature within 2 cm of the pylorus and one pair from the antrum. One biopsy specimen from the body and one from the antrum were stained for histopathological examination. The remaining two biopsy specimens (one antrum and one corpus) were tested for urease activity by a rapid urease test. The FDA considers a patient infected even if only one of the two specimens examined by either method is positive. That is, both specimens do not have to be positive to classify the patient as infected. It is not clear if the sponsor used this definition when referring to a "positive" versus a "negative" diagnosis. It is recommended that they be contacted regarding this issue of interpretation.

Evaluability Criteria

- Evaluable – Defined as subjects completing the Ez-HBT procedure with blood samples having measurable CO_2 levels (≥ 1% CO_2 gas) and having at least a valid histology or rapid urease test result.

Clinical and Statistical Reviewers' Comment: We will consider the Sponsor's defined data set as the intent-to-treat data set and will additionally define an evaluable data set which excludes any subjects with protocol violations affecting efficacy (e.g. taking concomitant medications).

Definitions
The following definitions were used to interpret the data:

TP = true positives = the number of diseased (i.e. *H. pylori* infected) subjects correctly classified by the Ez-HBT test.

TN = true negatives = the number of non-diseased subjects (i.e. *H. pylori* non-infected) correctly classified by the Ez-HBT test.

FP = false positives = the number of non-diseased subjects incorrectly classified by the Ez-HBT test (i.e. the number of subjects in whom *H. pylori* was not detected with histology or RUT, but in whom the Ez-HBT was positive).

FN = false negatives = the number of diseased subjects incorrectly classified by the Ez-HBT test (i.e. the number of subjects in whom *H. pylori* was detected by histology or RUT, but in whom the Ez-HBT was negative).

Total = the total number of evaluable subjects

Sensitivity = TP/(TP+FN)

Specificity = TN/(TN+FP)

Positive Predictive Value = TP/(TP+FP)

Negative Predictive Value = TN/(TN+FN)

Accuracy = (TP+TN)/Total

Cut-off Point
The cut-off point was established using the following expression:

$$\text{Tolerance interval} = \mu \pm Z\sigma$$

Where: μ = mean,
Z = factor used to contain % of population and
σ = Standard deviation

The maximum value for the tolerance interval was used as the cut-off point. A Z factor for 99% of a normally distributed population with a 95% confidence interval was used. The statistical tolerance interval represents limits within which a stated percentage of a population is expected to lie, based on the statistical variability of some characteristic of a population.

Statistical Reviewer's Comment: The method proposed does not give the 95% confidence level for 99% of the negative subjects. It is merely the interval that contains 99 percent of the negative subjects under the strong and not valid assumption that the negative values of the Ez-HBT are normally distributed. A sample size of 50 may assure that the distribution of the sample mean is normal, however, it does not assure that the distribution of the sample is normal.

Results

The study consisted of 121 enrolled subjects. None of these subjects withdrew, dropped out, were found to be non-compliant with the protocol, or had samples that were not able to be processed.

Clinical Reviewer's Comment: Based on the FDA's definition of evaluability, all 121 subjects are valid for inclusion into the Intent-to-Treat and Evaluable populations.

Sensitivity, Specificity and Accuracy

The Ez-HBT test demonstrated high sensitivity (89%), specificity (96%), and accuracy (93%) versus histology in this study (Table 1). Similarly, the Ez-HBT was sensitive (92%), specific (93%) and accurate (93%) versus rapid urease testing, (Table 2). Ten subjects had histology and rapid urease test that were incongruent and therefore only 111 are included in the analysis in Table 3. Sensitivity, specificity, and accuracy in this analysis are 94%, 98%, and 96%, respectively.

TABLE 1
Comparison to Histological Examination

Ez-HBT Helicobacter Blood Test

Histology	Positive	Negative	Total
Positive	48	6	54
Negative	3	64	67
Total	51	70	121

SENSITIVITY: 89 %
SPECIFICITY: 96 %
ACCURACY: 93 %
PPV: 94 %
NPV: 91 %

TABLE 2
Comparison to RUT

Ez-HBT Helicobacter Blood Test

RUT	Positive	Negative	Total
Positive	46	4	50
Negative	5	66	71
Total	51	70	121

SENSITIVITY: 92 %
SPECIFICITY: 93 %
ACCURACY: 93 %
PPV: 90 %
NPV: 94 %

TABLE 3
Comparison to Congruent Endoscopic Methods

Ez-HBT Helicobacter Blood Test

Congruent Endoscopy	Positive	Negative	Total
Positive	44	3	47
Negative	1	63	64
Total	45	66	111

SENSITIVITY: 94 %
SPECIFICITY: 98 %
ACCURACY: 96 %
PPV: 98 %
NPV: 96 %
INCONGRUENT: 8.3% (Samples where the RUT and Histology results did not agree 10/121)

Reviewer's Validation of Primary Data
Data from the HBT-03 Cut-off study was submitted in tabular form, as shown in Appendix 1 (page 50). Using this table, the sponsor's sensitivity and specificity results shown in Tables 1-3 were verified by the Reviewer.

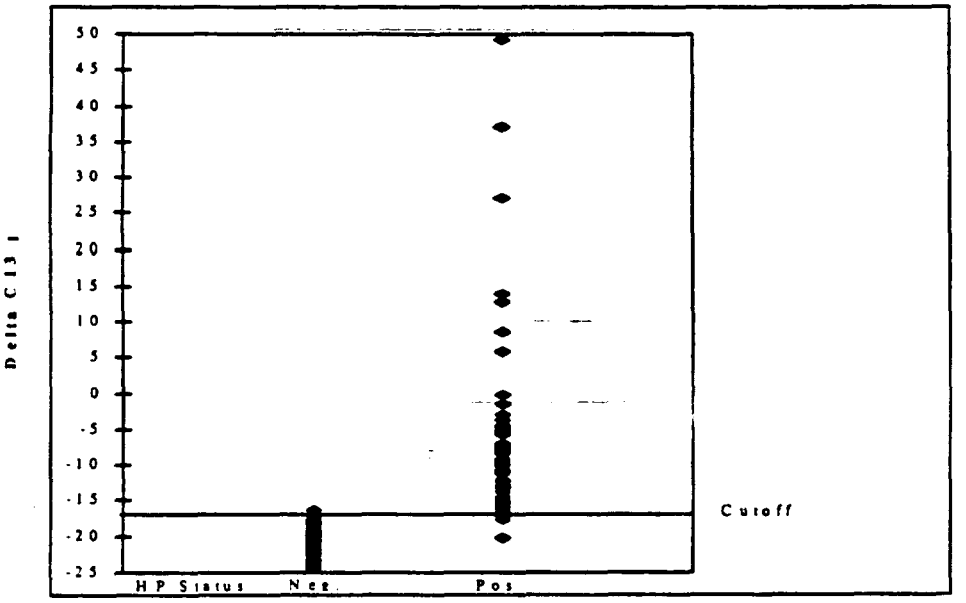
Cut-off Determination
Of the 111 subjects with congruent endoscopic results, there were 63 true negative subjects.

Clinical Reviewer's Comment: Of the 111 subjects with congruent endoscopic results, there were 47 positives. The H. pylori infection rate of 42% is within the expected range for this patient population (symptomatic subjects, mean age 49 years).

After considering this study and the results of two previous preliminary studies (HBT-01 and HBT-02), the cut-off was determined to be –17.0 delta per mil (tolerance interval was -16.20 to -24.55 delta per mil). This data is presented in Figure 1 below:

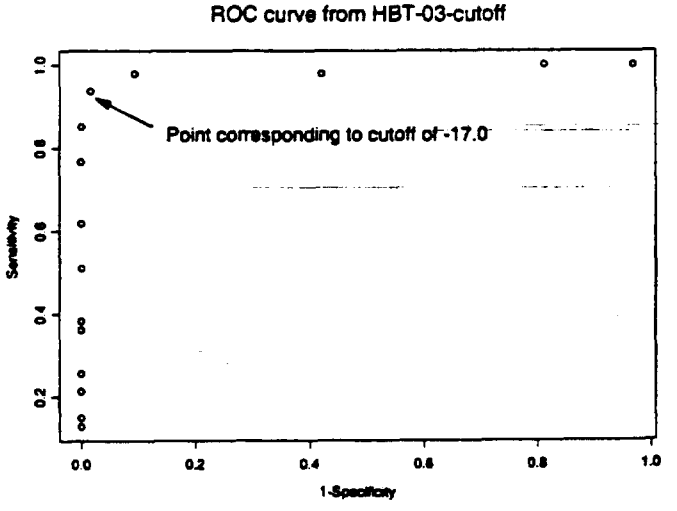
APPEARS THIS WAY
ON ORIGINAL

FIGURE 1
Cut-off Determination in Symptomatic Subjects



Receiver operating characteristic (ROC) analysis of this data yielded the following curve shown in Figure 2. This graph indicates that the best cut-off to maximize both sensitivity and specificity is -17.0 delta per mil.

FIGURE 2
ROC Analysis for Ez-HBT versus Histology



Safety

All 121 subjects enrolled in the study were administered the drug product Helicosol in association with the Ez-HBT Helicobacter blood test. Therefore, all are valid for safety evaluation.

No subject reported any adverse event in this study.

Conclusions

This 121-subject study demonstrated the safety and efficacy of the Ez-HBT Helicobacter blood test for the qualitative detection of presence of active *Helicobacter pylori* infection. The test showed high values for sensitivity, specificity, and accuracy versus two different reference methods (histology and rapid urease test). There were no adverse events reported in the study.

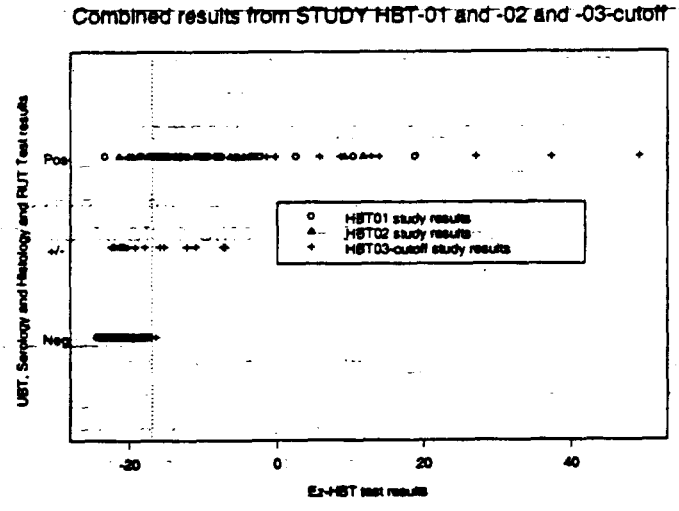
The cut-off point for the test was confirmed to be –17.0 delta per mil in this symptomatic population. These results led to the pursuit of a larger, monitored clinical study of the safety and efficacy of the Ez-HBT (Protocol HBT-03).

Clinical and Statistical Reviewers' Summary of Protocols HBT-01, -02, and –03-Cut-off

Figure 3 shows the values of the Ez-HBT test (true positive, true negative, or indeterminate) based on whether the comparison test(s) were positive (Pos), negative (Neg), or one positive and one negative (+/-) for the combined data from HBT-01, HBT-02, and HBT-03-cut-off. The proposed cut-off for Ez-HBT of –17.0 is also shown.

FIGURE 3

Combined results from HBT-01, -02, and –03-cutoff



D. An Investigation of a Blood Test (Ez-HBT™ Helicobacter Blood Test) for Diagnosis of Active Helicobacter pylori infection (Protocol HBT-03)

OBJECTIVES

- Determine whether the Ez-HBT detects active *H. pylori* infection, in adults exhibiting symptoms of gastric/duodenal ulcers, using gastric biopsy with histopathologic examination of stained tissue and detection of urease activity in the biopsy material as the reference methods.

- Establish assay performance characteristics in the target population; and

- Evaluate the safety of the administered drug, Helicosol, by determining the types of adverse events experienced by subjects during conduct of the Ez-HBT and number (%) of subjects experiencing each type of adverse event.

STUDY POPULATION

Three hundred and forty-three (343) adult subjects were enrolled in this study. The mean (± SD) age and weight for the 338 subjects with reported data was 48.4 ± 16.4 years (range 18-85 years) and 159 ± 46 pounds (range 91 to 286 pounds).

The following demographic information is from the 342 subjects with reported data:

Gender:	145 (42%) males and 197 (58%) females
Race:	165 (48%) Caucasians, 146 (43%) Hispanics, 22 (6%) African-Americans, 6 (2) Asians, and 3 (1%) Other
Tobacco-users:	60 (17.5%)
Alcohol consumers:	114 (33%)

Of the 343 subjects enrolled, 62 patients had ulcers. There were 27 duodenal ulcers, 31 gastric ulcers, and 6 esophageal ulcers found on endoscopy. The *H. pylori* infection rate, based on congruent endoscopic methods, in patients with any type of ulcer was 56% (32/57).

STUDY DESIGN

This was an outpatient study conducted at 7 clinical sites in the United States.

The design was identical to the HBT-03-Cut-off study, with the exception that biopsy specimens obtained for histopathology were stained with [redacted] stains and sent to a central laboratory (University of Michigan Medical Center) for examination by experienced pathologists. The remaining two biopsy specimens were tested at the time of endoscopy for urease activity by a single manufacturer's rapid urease test, Pyloritek®.

Sensitivity, specificity, and accuracy of the Ez-HBT were calculated using each reference diagnostic procedure to classify subjects as infected or non-infected. The cut-off point was refined for the Ez-HBT test using a receiver operating characteristic (ROC) analysis.

CLINICAL SITES

Seven (7) clinical sites from different geographic locations in the United States were used to evaluate the Ez-HBT.

Investigator	Study Site	Number Enrolled
Dr. Loren Laine	University of Southern California, Los Angeles, CA	65
Dr. William Chey	University of Michigan, Ann Arbor, MI	36
Dr. Howard Schwartz	Miami Research Associates, Inc., Miami, FL	75
Dr. Barry Winston	Houston Medican Research Associates, Houston, TX	10
Dr. Dennis Riff	AGMG Clinical Research, Anaheim CA	50
Dr. Ronald Pruitt	Nashville Medical Research Institute, Nashville, TN	79
Dr. Charles Barish	Wake Research Associates, Raleigh, NC	28

EXCLUSION CRITERIA
Identical to HBT-03-Cut-Off study.

Clinical Reviewer's Comment: The sponsor included 4 patients in their efficacy analysis that violated the protocol's exclusion criteria. Three subjects (128, 445 and 528) received proton pump inhibitors within 7 days prior to endoscopy. One additional subject (647) was reported taking propulsid (cisapride) to treat gastroparesis. These subjects have been taken out of the FDA's analysis (see discussion of results).

The following subjects were receiving concomitant therapy with medications that deviated from study protocol: 15 subjects were receiving one aspirin tablet per day as cardiovascular prophylaxis, 6 were using prn aspirin or ibuprofen, 1 was receiving doxycyline qd/prn, and 1 was receiving warfarin. Use of these medications was judged by the Reviewer not to be clinically significant and the subjects remained evaluable.

ANALYTICAL PROCEDURE
Identical to HBT-03-Cut-Off study.

STATISTICAL ANALYSIS
Calculation of a requisite sample size was based on the overall accuracy of the new test, assuming that the "gold standard" was 100% accurate in classifying cases. The null hypothesis, which the sponsor hoped to reject, was:

Ho: overall accuracy < 90%

with the one-sided alternative:

H: overall accuracy = 90%

A power of 90% was assumed with a type I error probability of 0.05. It was also assumed that the study population would have a 30% *H. pylori* infection incidence rate. However, the rate of infection does not affect the sample size because sample size was calculated using overall accuracy. Using these assumptions, 325 evaluable subjects were required. Approximately 10% of subjects were expected to have non-evaluable results such as non-compliance with study protocol, lost samples or low levels of blood CO_2. Consequently, enrollment of

approximately 350 subjects was needed to reach the target enrollment of 325 evaluable subjects.

Statistical Reviewer's Comment: The information given above on sample size calculation is incomplete. A true value for accuracy (alternative hypothesis) needs to be stated. Note that the alternative hypothesis should be stated as "H: overall accuracy ≥ 90%". With a null hypothesis that the accuracy of the Ez-HBT is 90% or less, 321 evaluable subjects would be needed given that the true accuracy of the Ez-HBT test is 94.4%.

Interpretation of Ez-HBT Test Results, Reference Test Results, Evaluability Criteria, Definitions

Identical to HBT-03-Cut-Off study.

Evaluability Criteria

- Evaluable – Defined as subjects completing the Ez-HBT procedure with blood samples having measurable CO_2 levels (≥ 1% CO_2 gas) and having at least a valid histology or rapid urease test result.

Clinical and Statistical Reviewers' Comment: We will consider the Sponsor's defined data set as the intent-to-treat data set and will additionally define an evaluable data set which excludes any subjects with protocol violations affecting efficacy (e.g. taking concomitant medications).

RESULTS

Sponsor's Analysis

The study consisted of 343 enrolled subjects. Of these, five either voluntarily withdrew or were found to be non-compliant with the protocol and were withdrawn prior to Ez-HBT administration. There were no samples unable to be processed. Of the remaining 338 subjects, 16 Ez-HBT values (4.7%) fell within the indeterminate zone (-17.0 to –18.0 delta per mil). Therefore, the number reported versus histological exam is 322. Further, in three cases rapid urease testing was not performed due to a misunderstanding at one site of the testing protocol. Therefore, the number evaluated versus rapid urease testing is 319 (338 evaluated subjects - 16 indeterminates = 322 – 3 rapid urease tests not performed = 319). The congruent analysis consists of 306 subjects due to 16 incongruent results (322 – 16 incongruent results = 306). That is, in 16 subjects, the rapid urease and histology results did not agree. This disagreement includes the three subjects on whom rapid urease testing was not performed since no comparison between PyloriTek and histology can be made. The total number for congruent endoscopy versus Ez-HBT therefore equals 306 (338 evaluated subjects – 16 indeterminates = 322 – 16 incongruents = 306).

To summarize:

- 343 signed consent
- 338 Evaluable (5 were withdrawn prior to Ez-HBT administration) [338-5=338]
  471, 603, 605, 611, 669
- 322 evaluated versus histology (16 indeterminate results of Ez-HBT) [338-16=322]
  104, 237, 245, 303, 309, 455, 470, 511, 514, 520, 528, 601, 614, 632, 658, 706
- 319 evaluated versus rapid urease test (3 rapid urease tests not performed) [322-3=319]
  239, 301, 302

- 306 evaluated for congruent results (16 incongruent results, including 3 patients without rapid urease tests performed)
  203, 205, 212, 217, 222, 233, 239, 253, 301, 302, 319, 410, 423, 438, 456, 465

Clinical Reviewer's Comment: The FDA reviewer's modified per protocol analysis follows:

- *Three subjects included in the sponsor's analysis (128, 445, and 647) violated the protocol and were removed from all the FDA Reviewer's analyses, except the ITT.*

- *Subject 528 was in violation of the protocol, but had an indeterminate result from the Ez-HBT and therefore was not analyzed versus endoscopic methods. This subject was also not analyzed versus endoscopic methods in the sponsor's original analysis.*

- *Subject 303 had no rapid urease test performed, was negative by histology, and had an indeterminate Ez-HBT result. This subject was not included in the FDA reviewer's Evaluable population and was also not analyzed versus endoscopic methods.*

To summarize:
- 343 signed consent
- 339 without protocol violations (4 were removed from FDA analysis)
  128, 445, 528, 647
- 334 Evaluable for Ez-HBT (5 were withdrawn prior to Ez-HBT administration)
  471, 603, 605, 611, 669
- 319 evaluated versus histology (of the 334, 15 had indeterminate results of Ez-HBT)
  104, 237, 245, 303, 309, 455, 470, 511, 514, 520, 601, 614, 632, 658, 706
- 316 evaluated versus rapid urease test (of the 319, 3 rapid urease tests not performed)
  239, 301, 302
- 303 evaluated for congruent results (of the 316, 13 had incongruent results)
  203, 205, 212, 217, 222, 233, 253, 319, 410, 423, 438, 456, 465

Refinement of the Cut-off Point
The cut-off point was refined using 338 subjects. A receiver operating characteristic (ROC) analysis was utilized to establish a cut-off point and indeterminate area from this data. The cutoff was determined to be –17.5 delta per mil with an indeterminate zone of 0.5 per mil on either side of the cut-off (see Figure 1B).

Clinical and Statistical Reviewers Comment: Figure 1A represents the figure submitted by the sponsor. Figure 1B represents the FDA reviewers' interpretation of the data. The sponsor incorrectly excluded subjects who had false negative or false positive Ez-HBT values. The sponsor's figure also appears in the proposed labeling and should be replaced with the FDA's figure.

Statistical Reviewer's Comment: Note that of the 338 subjects with an Ez-HBT value, 16 were within the indeterminate zone (5%). Figure 1B shows the value of the Ez-HBT versus H. pylori status based on congruent results. Two horizontal lines mark the indeterminate zone. The use of an indeterminate zone inflates the sensitivity and specificity compared to using a single cutoff point. The sponsor chose the new cut-off and indeterminate zone for this study post-hoc. Ideally this cut-off would be validated again after it was re-established.

FIGURE 1A
Sponsor's Cut-off Point Determination and Indeterminate Zone

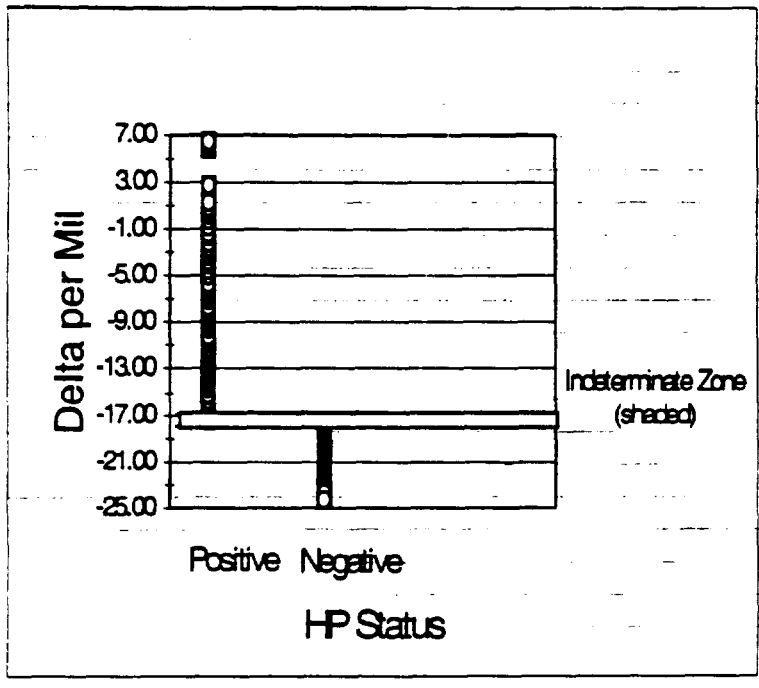


FIGURE 1B
FDA's Cut-off Point Determination and Indeterminate Zone

Our figure 6.2

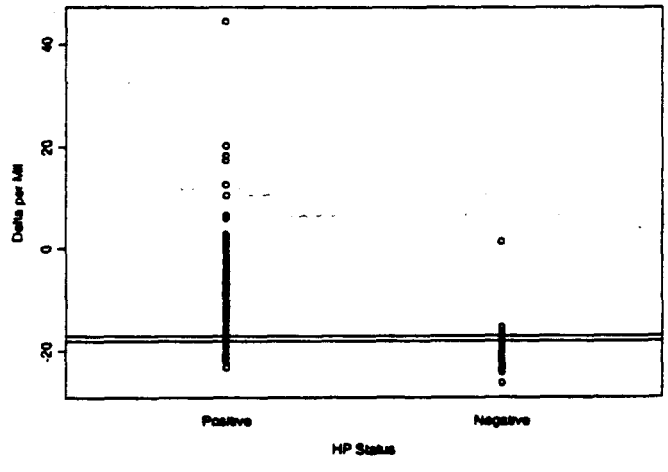


Table 1 presents the number of patients with positive, negative, and indeterminate results using the FDA's ITT population (sponsor's Evaluable population).

TABLE 1
Breakdown of Positive, Negative, and Indeterminate Results Using the Cut-off of –17.5

Result	N	Percent
All subjects in ITT population	338	100
Positive Ez-HBT	130	38.5
Negative Ez-HBT	192	56.8
Indeterminate Ez-HBT	16	4.7

Efficacy

Site by Site Summary - Sponsor's Evaluable Population

The data were analyzed to evaluate the effect of different sites in the final results of the 338 evaluable subjects and summarized in Table 2.

Clinical Reviewer's Comment: This table actually represents the Intent-to-Treat (ITT) population as defined by the FDA.

TABLE 2
Site Specific Results

Site	Total Number of Samples	Indeterminate Samples	Incongruent Samples	Sensitivity (%)	Specificity (%)	Accuracy (%)	False Results	
							FN	FP
Laine	65	2	8	95.3	75.0	90.9	2	3
Chey	36	1	0	100	100	100	0	0
Schwartz	72	4	0	76.0	100	91.0	6	0
Winston	10	1	0	100	66.7	88.9	0	1
Riff	49	3	0	96.4	100	97.8	0	1
Pruitt	78	2	5	92.3	93.1	93.0	1	4
Barish	28	2	3	66.7	100	91.3	2	0

The most notable differences between sites were in the number of: samples obtained, incongruent samples, and false negatives. When the sensitivity, specificity, and accuracy statistics from each site were compared, no discernable differences were identified from site to site. In addition, the sensitivity, specificity, and accuracy statistics from each site were compared with the overall data and no discernable differences were detected between individual sites and the cumulative data. The sponsor decided to treat the data as a single pool for all subsequent analyses.

Summary of Clinical Study Data

The sensitivity, specificity, and accuracy of the Ez-HBT test were determined in relation to histology, rapid urease test, and congruent results of the two biopsy-based methods. Taking the congruent results, 95% confidence intervals were calculated and ROC analysis was performed. The sponsor's analyses are presented in Tables 3A, 4A, and 5A.

Clinical and Statistical Reviewers' Comment: An indeterminate column was added to the sponsor's tables. The reviewers' modified analyses are presented in Tables 3B, 4B, and 5B.

TABLE 3A
Sponsor's Comparison to Histological Examination

Ez-HBT Helicobacter Blood Test

Histology	Positive	Negative	Indeterminate	Total
Positive	119	13	8	140
Negative	11	179	8	198
Total	130	192	16	338

SENSITIVITY: 90.2% [redacted] [95% CI (85.1 : 95.2)]
SPECIFICITY: 94.2% [redacted] [95% CI (90.9 : 97.5)]
ACCURACY: 92.5% [95 % CI (89.6 : 95.4)]
INDETERMINATE: 4.7%

Statistical Reviewer's Comment: The sponsor calculated the confidence intervals for sensitivity and specificity incorrectly. The 'n' used in the equation for the confidence interval is the number in the denominator of the proportion calculated and is different for sensitivity, specificity and accuracy. The correct 'n' for sensitivity is the number of subjects with a positive comparator test, in this case, histology. The correct 'n' for specificity is the number of subjects with a negative comparator test. The 'n' for accuracy is the total number of subjects. The correct confidence intervals are reported next to the sponsor's confidence intervals in Tables 3A, 4A, and 5A.

TABLE 3B
FDA's 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)]
ACCURACY: 92.8% [95 % CI (90.0 : 95.6)]
INDETERMINATE: 4.5% (15/(319+15))
PPV: 91.5% [95 % CI (86.8 : 96.3)]
NPV: 93.7% [95 % CI (90.2 : 97.1)]

TABLE 4A
Sponsor's Comparison to Rapid Urease Test (PyloriTek)

Ez-HBT Helicobacter Blood Test

PyloriTek	Positive	Negative	Indeterminate	Total
Positive	117	17	8	142
Negative	12	173	7	192
Total	129	190	15	334

SENSITIVITY: 87.3% [redacted] [95 % CI (81.7 : 92.9)]
SPECIFICITY: 93.5% [redacted] [95 % CI (90.0 : 97.1)]
ACCURACY: 90.9% [95 % CI (87.7 : 94.1)]
INDETERMINATE: 4.8%

TABLE 4B
FDA's Comparison to Rapid Urease Test (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)]
ACCURACY: 91.1% [95 % CI (88.0 : 94.3)]
INDETERMINATE: 4.2% (14/(316 + 14))
PPV: 90.7% [95 % CI (85.7 : 95.7)]
NPV: 91.4% [95 % CI (87.4 : 95.5)]

TABLE 5A
Sponsor's Comparison to Congruent Endoscopic Methods

Ez-HBT Helicobacter Blood Test

Congruent Endoscopy	Positive	Negative	Indeterminate	Total
Positive	115	11	7	133
Negative	9	171	6	186
Total	124	182	13	319

SENSITIVITY: 91.3% [redacted] [95 % CI (86.3 : 96.2)]
SPECIFICITY: 95.0% [redacted] [95 % CI (91.8 : 98.2)]
ACCURACY: 93.5% [95 % CI (90.7 : 96.3)]
INDETERMINATE: 4.7%
INCONGRUENT: 4.7%

TABLE 5B
FDA's Comparison to Congruent Endoscopic Methods

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)]
ACCURACY: 93.7% [95 % CI (91.0 : 96.5)]
INDETERMINATE: 3.8% (12/(303+12))
INCONGRUENT: 4.7% (15/(303+15))
PPV: 92.7% [95 % CI (88.2 : 97.3)]
NPV: 94.4% [95 % CI (91.0 : 97.8)]

Statistical Reviewer's Comment: The tables above use the refined cutoff and indeterminate zone as determined from this study (-17.5 ± 0.5). The following results in Table 5C use the proposed cutoff of –17.0 as stated in the protocol and determined from previous studies (HBT-01, HBT-02 and HBT-03 cutoff), as determined by the FDA reviewers. The value for the sensitivity is lower than what was seen in Table 5A (86.5% versus 92%).

TABLE 5C
FDA's Comparison to Congruent Endoscopic Methods
Using the –17 delta per mil Cutoff

Congruent Results	*Ez-HBT test*		*Total*
	Positive	*Negative*	
Positive	*115*	*18*	*133*
Negative	*9*	*177*	*186*
Total	*124*	*195*	*319*

SENSITIVITY: 86.5% [95 % CI (80.7 : 92.3)]
SPECIFICITY: 95.2% [95 % CI (92.1 : 98.2)]
ACCURACY: 91.5% [95 % CI (88.5 : 94.6)]
PPV: 92.7% [95 % CI (88.2 : 97.3)]
NPV: 90.8% [95 % CI (86.7 : 94.8)]

Since the cutoff point of –17.5 delta per mil, with corresponding indeterminate zone ± 0.5 delta per mil was determined using results from the HBT-03 study and then applied to the HBT-03 study, it is difficult to assess to know how representative the performance characteristics are of the true performance characteristics of the Ez-HBT test. The ideal way to answer this question would be to perform a validation study of the new cutoff point and indeterminate zone.

Clinical Reviewer's Validation of Primary Data
Data from the HBT-03 study was submitted in tabular form, as requested by the Reviewer, as shown in Appendix 2 (pages 51-52). Using this table, the sponsor's sensitivity and specificity results shown in Tables 3-5 were verified and then modified to exclude 3 subjects who were protocol violations.

Clinical Reviewer's Comment: Of the 303 subjects with congruent endoscopic results, there were 125 positives. The H. pylori infection rate of 41% is within the expected range for this patient population (symptomatic subjects, mean age 48 years).

Based on the Sponsor's analysis, there were 20 subjects that demonstrated false results when compared to congruent biopsy-based methods.

Statistical and Clinical Reviewers' Comment: Table 6 was constructed to present the analyses for the ITT population in various demographic and diagnostic subpopulations using congruent endoscopic methods as the reference standard. Data from all subjects (N=319) were used except

4 rapid urease tests were not performed - 239, 301, 302, 303
15 incongruent results - 203, 205, 212, 217, 222, 233, 253, 319, 410, 423, 438, 456, 465, 614, 658
5 were withdrawn prior to Ez-HBT administration - 471, 603, 605, 611, 699

APPEARS THIS WAY
ON ORIGINAL

APPEARS THIS WAY
ON ORIGINAL

TABLE 6
Analyses of the Intent-to-Treat Population by Demographic and Diagnostic Subpopulations Using Congruent Endoscopic Methods as the Reference Standard (N=319)

Demographics	N	Positive Reference			Negative Reference						
		TP	Ind	FN	TN	Ind	FP	Sens	Spec	PPV	NPV
Male	135	52	4	6	63	3	7	90	90	88	91
Female	184	63	3	5	108	3	2	93	98	97	96
< 30 years	51	18	3	1	28	0	1	95	97	95	97
30-44 years	96	43	4	3	38	4	4	93	90	91	93
45-60 years	89	34	0	4	47	1	3	89	94	92	92
> 60 years	83	20	0	3	58	1	1	87	98	95	95
Caucasian	157	22	2	4	122	3	4	85	97	85	97
Black	19	11	0	1	5	0	2	92	71	85	83
Hispanic	135	79	4	6	40	3	3	93	93	96	87
Asian	6	2	1	0	3	0	0	100	100	100	100
Other	2	1	0	0	1	0	0	100	100	100	100
Ulcer	57	27	4	1	23	1	1	96	96	96	96
No Ulcer	262	88	3	10	148	5	8	90	95	92	94
Tobacco User	57	23	0	0	32	1	1	100	97	96	100
Non-Tobacco User	262	92	7	11	139	5	8	89	95	92	93
Alcohol Use	108	37	6	3	61	0	1	93	98	97	95
Alcohol Non-use	211	78	1	8	110	6	8	91	93	91	93

TP = true positive; Ind = indeterminate; FN = false negative; TN = true negative; FP = false positive; Sens = sensitivity; Spec = specificity; PPV = positive predictive value; NPV = negative predictive value

Clinical and Statistical Reviewers' Comment: There were no statistically significant difference in sensitivity, specificity, PPV, or NPV between males and females, tobacco users and non-tobacco users, and Caucasians and Hispanics. There was a statistically significant difference in specificity between Caucasians and Blacks (p=0.002, using an exact method for determining a difference in proportions). However, there were no significant differences in sensitivity, PPV, or NPV. The clinical significance of this result is unknown.

Evaluation of the False Results

The test produced twenty (20) false results versus reference methods. There were 9 false negatives and 11 false positives. The gathered data from these subjects was examined to identify any associations among demographic, physical examination, medical history, endoscopy data, adverse events and/or deviations from protocol. Each of these factors will be addressed individually.

Demographics

Table 7A and 7B presents demographic data from subjects with false negative and false positive results, respectively. Table 7C compares the various demographic parameters between the two groups.

Clinical Reviewer's Comment: The Reviewer additionally tabulated the number of false negatives and false positives by investigator and the results can be seen Table 2. Note that 6/11 false negatives originated in subjects enrolled at Dr. Schwartz's site in Miami.

TABLE 7A

Demographic Profiles for Subjects with False Negative Ez-HBT Results

Subject No.	Subject Initials	Date of Ez-HBT Test	Gender	Race	Age	Weight (pounds)	Tobacco Use	Alcohol Intake Per Day	Ez-HBT vs. Congruent Results (Ez-HBT values)
201		06-Jul-98	F	Hispanic	64	103	No	Non-User	FN (-22.15)
211		30-Jul-98	M	Caucasian	55	145	No	Non-User	FN (-23.29)
305		22-Jul-98	M	Caucasian	51	265	No	<1 drink	FN (-18.78)
308		06-Aug-98	F	Afro-American	42	196	No	Non-User	FN (-20.67)
458		16-Oct-98	M	Caucasian	81	134	No	Non-User	FN (-19.17)
604		15-Jul-98	M	Hispanic	62	136	No	<1 drink	FN (-19.21)
607		17-Jul-98	F	Caucasian	21	139	No	Non-User	FN (-21.34)
612		21-Jul-98	F	Hispanic	54	167	No	Non-User	FN (-21.7)
615		24-Jul-98	M	Hispanic	36	208	No	Non-User	FN (-20.41)
625		03-Aug-98	M	Hispanic	54	190	No	<1 drink	FN (-19.32)
647		20-Aug-98	F	Hispanic	36	185	No	Non-User	FN (-20.77)

Clinical Reviewer's Comment: Subject 647 has gastroparesis and is excluded from the FDA's evaluable population due to a protocol violation.

Subjects with identification numbers in the 600's were enrolled at Dr. Schwartz's site in Miami.

TABLE 7B

Demographic Profiles for Subjects with False Positive Ez-HBT Results

Subject No.	Subject Initials	Date of Ez-HBT Test	Gender	Race	Age	Weight (pounds)	Tobacco Use	Alcohol Intake Per Day	Ez-HBT vs. Congruent Results (Ez-HBT values)
214		30-Jul-98	M	Caucasian	37	185	No	Non-User	FP (-15.33)
262		13-Nov-98	M	Hispanic	59	134	No	Non-User	FP (-16.28)
263		13-Nov-98	M	Hispanic	57	183	No	Non-User	FP (-15.41)
414		17-Aug-98	M	Afro-American	30	151	No	Non-User	FP (-16.62)
447		28-Sep-98	M	Caucasian	28	175	No	Non-User	FP (-16.71)
457		16-Oct-98	M	Afro-American	56	222	No	Non-User	FP (1.29)
472		28-Oct-98	F	Caucasian	44	140	No	Non-User	FP (-16.24)
545		02-Nov-98	F	Hispanic	61	133	No	Non-User	FP (-15.32)
707		04-Sep-98	M	Caucasian	36	229	Yes	>2 drinks	FP (-16.25)

TABLE 7C
Comparison of Demographic Parameters Between False Negative and False Positive Results

Parameter	False Negatives (N=11) (%)	False Positives (N=9) (%)
Gender Ratio (Males: Females)	1.2	3.5
Males	6/11 (55)	7/9 (78)
Females	5/11 (45)	2/9 (22)
Race		
Caucasian	4/11 (36)	4/9 (44)
Hispanic	6/11 (55)	3/9 (33)
African-American	1/11 (9)	2/9 (22)
Mean Age	50	45
Mean Weight (lbs.)	170	172
Alcohol Use	3/11 (27)	1/9 (11)
Tobacco Use	0/11 (0)	1/9 (11)

As seen in Table 7C, the male to female ratio among subjects with false negative results (1.2) was similar to that in the total study (0.74). However, the ratio for false positives favors males to a greater extent (3.5), but the results are limited by the small sample size (N=9).

The racial make-up of both the false results groups were similar to the racial make-up of the total study (48% Caucasian, 43% Hispanic and 6% African-American), although the false negative group has more Hispanic subjects than Caucasians.

The average age in the total study was 48 years and within the groups of false Ez-HBT results, the mean age was 50 years and 45 years, for false negatives and false positives, respectively.

Weight was considered as a possible factor in influencing Ez-HBT false results. The mean weight in the groups of false Ez-HBT results was slightly higher compared to the mean weight from the total study of 159 pounds (95% CI 149-169 pounds). This difference is accounted for by the higher percentage of men to women in the groups with false results. Similarly, both alcohol and tobacco use mimicked the consumption found in the total study.

Based on the analysis of all of the demographic parameters listed above, no correlation was found between any particular parameter and false Ez-HBT results.

Clinical Reviewer's Comment: The sponsor noted that 11/20 (55%) of the false results (8 false negatives and 3 false positives) came from among the first 15 samples done at any site. Therefore, they postulate that the false results may be due to the inexperience of the personnel administering the test. This rationale is plausible since most of the false results were negative.

In addition, out of the 11 total false negative results, 6 subjects were enrolled by Dr. Schwartz in Miami. This finding also supports the theory that personnel error was a factor.

Asking the sponsor how they believe personnel inexperience influenced the results of the test (i.e. what part of the procedure was done incorrectly) may provide useful information to be used in the drafting of new wording for the label on directions for use.

Physical Examination
There was nothing unusual about any of the physical examinations on the subjects whose Ez-HBT results were shown to be false. The blood pressure, pulse, respiration, temperature, and auscultation of the heart and lung were in accordance with those seen throughout the clinical study. Therefore, no correlation is suspected between and physical examination elements and false Ez-HBT results.

Medical History
An investigation was undertaken to establish any correlation to symptoms or medications that may negatively effect the performance of the Ez-HBT Helicobacter Blood Test. There is no significant correlation between symptoms or medications and false Ez-HBT results. The profiles are typical of the subjects in this study as a whole. All subjects, as an inclusion criterion, were referred for an EGD, so upper GI symptoms (e.g., heartburn, nausea, and dyspepsia) were seen in all patients and many were using medications to control their symptoms.

Endoscopy Data
The endoscopy findings were examined to identify any potential correlation between these finding and false Ez-HBT results. The twenty subjects reporting either false positive or false negative results had the usual patterns of erosions and varices. None of them had any clinically significant bleeding. In all twenty cases, 2 biopsies were taken from the greater curvature and two from the antrum as described in the study protocol. There is no evidence that the endoscopy findings from these subjects differ from the total results. Therefore, no correlation was found between false Ez-HBT results and endoscopy data.

Adverse Events
No adverse events were found for the twenty subjects with false Ez-HBT reports. Therefore, no correlation was found between reported adverse events and false Ez-HBT results.

Deviations from Protocol
Nineteen of the twenty subjects (95%) having false Ez-HBT results had no deviations from the protocol reported. One subject (5%) had a blood draw time of 25 minutes instead of the required 30-minute minimum draw time.

Overall, there were 18 (5.3%) deviations reported in the 338 evaluated subjects the clinical study, thus the frequency of deviations is the same in the total study as in the incorrect Ez-HBT group. Of these, there were several subjects in the study whose blood was drawn between 25 and 30 minutes without reporting false negative results. The conclusion from this examination is that there is no correlation between protocol deviations and false Ez-HBT results.

Safety
Nine adverse events were reported in the 341 subjects who received Helicosol. Four of these events were considered mild, 3 moderate, and 2 severe. None was associated with Helicosol. The adverse events are summarized in Table 8. The causes cited were concurrent medication, Ensure, or concurrent disorders.

TABLE 8
Adverse Events

Subject	Adverse Event(s)	Severity	Cause
108	Slight nausea, diaphoresis	Mild	Concurrent medication: fentanyl
129	Sore throat	Mild	Ensure or delayed soreness from EGD
601	Drowsiness	Mild	Concurrent disorder: anemia
669	Vomiting	Mild	Concurrent medication: meperidine
122	Diarrhea, nausea, dizziness	Moderate	Ensure
507	Nausea	Moderate	Concurrent medication: meperidine, midazolam, or possibly Ensure
611	Vomiting	Moderate	Concurrent medication: meperidine
127	Nausea, vomiting	Severe	Ensure
471	Vomiting	Severe	Concurrent disorder: dysphagia

Clinical Reviewer's Comment: The sponsor claims that none of the nine adverse events were associated with the study drug, Helicosol, or other components of the Ez-HBT device. However, adverse events, mainly nausea, experienced by four of the nine subjects were thought to be possibly related to administration of Ensure and therefore are associated with administration of the Ez-HBT test kit.

CONCLUSIONS

The Ez-HBT Helicobacter blood test has been shown in this monitored clinical study for 338 symptomatic subjects to be effective. Values for sensitivity (92.0%), specificity (94.9%), and accuracy (93.7%) versus two congruent reference methods (histology and rapid urease testing) demonstrate the efficacy of the test. A modified cut-off of −17.5 delta per mil with an indeterminate or equivocal zone of ± 0.5 delta per mil was established by this study. This indeterminate zone effected 4.5% (15 of 338) of the tests. No correlation was found between the twenty false reports in the study and any of the other information captured.

The 20 false results obtained from comparison of the Ez-HBT to congruent endoscopic methods were investigated further to determine if any correlation could be made between demographic parameters (gender, race, weight, alcohol and tobacco consumption), medical history, concomitant drug therapy, protocol deviations and the results. No correlation was found. The sponsor believes that the false results are due to investigator inexperience with administering the test since 11/20 (55%) of the false results (8 false negatives and 3 false positives) came from among the first 15 samples done at any site.

There were 9 reported adverse events out of the 343 enrolled subjects (3%) in this study. None of these nine events was associated with the study drug, Helicosol (^{13}C-urea), although 4 are possibly related to other components of the Ez-HBT test (Ensure).

Statistical Reviewer's Comment: Since the cutoff point of –17.5 delta per mil, with corresponding indeterminate zone ± 0.5 delta per mil *was determined using results from the HBT-03 study and then applied to the HBT-03 study, it is difficult to assess how representative the performance characteristics are of the true performance characteristics of the Ez-HBT test. The ideal way to answer this question would be to perform a validation study of the new cutoff point and indeterminate zone.*

Statistical Reviewer's Analysis of Cutoff Point and Performance Characteristics
As stated above, a problem with these study results is that it is difficult to assess how representative the performance characteristics are of the true performance characteristics of the Ez-HBT test since the cutoff point was chosen based on this study. One method that has been used in other settings is as follows. Prior to determination of the cutoff point, the data is randomly separated into two independent groups (A and B). Group A is used to determine the cutoff and group B is used to determine the performance characteristics (i.e., sensitivity, specificity, and accuracy) of the test.

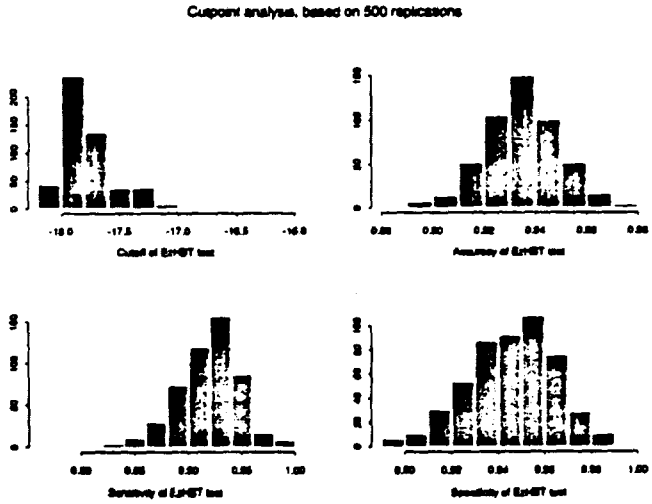
A post-hoc analysis was conducted using this method. Patients from the FDA modified per protocol data set with congruent endoscopic outcomes were randomly separated into two groups of approximately equal sizes. The cutoff based on group A was determined to be –17.9. The accuracy, sensitivity, and specificity based on group B were 91.0%, 93.3%, and 89.7% using the cutoff without an indeterminate region. The accuracy, sensitivity, and specificity after removing values that were within +/- .5 of the cutoff were 91.4%, 93.2%, and 90.3%. The variability of these estimates (cutoff, accuracy, sensitivity, and specificity) can be determined by repeating this procedure multiple times. The mean, standard deviation, median, range, and the 2.5 and 97.5 percentiles of the cutoff and performance characteristics using an indeterminate region based on 500 replications are given in Table 9. The 2.5 and 97.5 percentiles correspond to a 95% confidence interval. Also reported in Table 9 are the performance characteristics and confidence intervals based on the Sponsor's and the FDA's analyses. The values from the 500 replicates are shown graphically in the 4 histograms in Figure 2.

TABLE 9
Performance Characteristics Estimates

	Cutoff	Accuracy	Sensitivity	Specificity
Mean	-17.7	93.5%	91.9%	94.6%
Standard Deviation	0.24	0.01	0.03	0.02
Median	-17.8	93.4%	92.1%	94.6%
Range				
2.5 and 97.5 percentile	(-18.0, -17.2)	(90.3, 96.1)	(85.7, 96.8)	(90.7, 97.8)
Sponsor's Results and 95% confidence intervals (table 5A)		93.5% (90.7, 96.3)	91.3% (86.3, 96.2)	95.0% (91.8, 98.2)
FDA's Results and 95% confidence intervals (table 5B)		93.7% (91.0, 96.5)	92.0% (87.2, 96.8)	94.9% (91.7, 98.2)

The results from this analysis are consistent with the sponsor's determination of a cutoff. The cutoff from initially separating the data into two groups was –17.9 and the mean from repeating this method 500 times is –17.7. Ninety-five percent of the values are contained within –18.0 and –17.2. The first histogram in Figure 2 shows that the majority of the cutoffs ranged from –18.0 to –17.0, which is the same as the sponsor's indeterminate zone. This analysis does lead to more confidence in the sponsor's results. However, it would still be of value to perform a validation study of the new cutoff point and indeterminate zone to determine how this cutoff would perform in a different population of subjects. It should be noted that the range of values for sensitivity is quite large. This variability can also be seen in the Sponsor's and FDA's confidence intervals. Of the three performance characteristics, sensitivity shows the least favorable results. Low sensitivity leads to a high number of false negatives, which can be seen in Figure 1B.

FIGURE 2



APPEARS THIS WAY
ON ORIGINAL

E. An Investigation of a Blood Test (Ez-HBT™ Helicobacter Blood Test) in an Asymptomatic Control Population (Protocol HBT-02 Amend 1)

Objectives

- Determine the stability of blood $^{13}CO_2$ samples collected during the Ez-HBT test under the storage conditions of [redacted]
- Determine the stability of extracted blood $^{13}CO_2$ samples (prepared samples) after collection with the Ez-HBT test.
- Determine the effect of varying collected blood volume on the Ez-HBT results.

Study Population

Adult subjects with no present GI symptoms or a history of peptic ulcer symptoms were studied. Subjects previously tested for *H. pylori* during Protocol HBT-02, were asked to participate in this study. Subjects were selected based on congruent results between the Ez-HBT and all four serology tests used. Ten (10) subjects, five negative and five positive, were selected.

Clinical Site

The study was performed at a [redacted]

Study Design

Subjects were tested by serology and Ez-HBT (Protocol HBT-02) prior to being re-tested within this protocol. After 4 hours of fasting, all subjects had a modified Ez-HBT test conducted. Seven (7) blood samples of 10 mL were collected at three time points: 30 minutes, 45 minutes, and 60 minutes following Helicosol administration.

Blood samples collected at the 30 minute time point were aliquoted (3 mL) into 24 Vacutainer tubes. Four (4) were processed immediately and the rest were transferred to one of four environmental conditions: [redacted] One sample from each of the four conditions was analyzed on Days 1, 2, 3, 7, and 14.

Blood samples collected at the 45 minute time point were aliquoted (3 mL) into 24 Vacutainer tubes. Eighteen (18) were processed immediately. Three (3) of the tubes were kept [redacted] until they were transported to the lab and the remaining 3 tubes were immediately [redacted] transported to the lab [redacted] for stability testing.

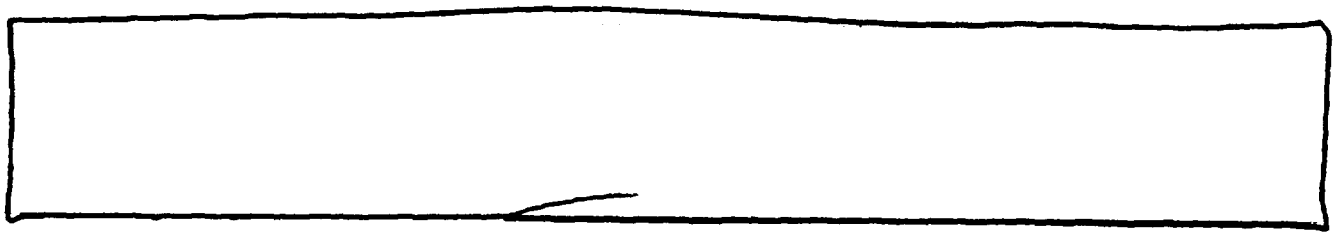
Blood samples collected at the 60 minute time point were aliquoted in 3 mL tubes containing various volumes of 1, 2, 2.5 and 3 mL (n=7 for each) and analyzed on Days 0, 1, 2, 3, 7, and 14.

Exclusion Criteria

See description of Protocol HBT-02.

Analytical Procedure

[redacted]



Statistical Analysis

Blood $^{13}CO_2$ levels > - 17 delta per mil relative to PDB were considered *H. pylori* infected based on previous cutoff studies. Blood $^{13}CO_2$ levels ≤ - 17 delta per mil are considered *H. pylori* non-infected.

Effect of Temperature on Blood Samples

Data was analyzed by repeated measures one-way analysis of variance (ANOVA) comparing the means of the four environmental treatments with the 30 minute blood sample. [redacted] ANOVA test compared the treatments for each day. The null hypothesis was that all days were equal.

Effect of Time on Prepared Samples

The data from the 45-minute blood sample was analyzed by a one-way ANOVA. The null hypothesis was that there are no differences between the 14 days post-processing of the blood samples.

Effect of Blood Volume

The data from the 60 minute blood sample was analyzed by a one-way ANOVA. The null hypothesis was that all blood volumes (1 to 3 mL) generate equal $^{13}CO_2$ values.

Blood Collection Timing

The data amongst the various collection times was compared by Boneferroni multiple comparison tests to determine if there were statistically significant differences.

The data were evaluated according to the method [redacted] to assess the magnitude of the differences from the initial value over a wide range of ^{13}C values. The mean and mean ± 2 times the standard deviation for each environmental condition was evaluated.

Results

Effect of Blood Collection Time on Ez-HBT

The timing of blood collections during the Ez-HBT test was varied to optimize test results. Blood samples were collected during the Ez-HBT procedure from ten patients at 30, 45, and 60 minutes post dosing of ^{13}C urea. Five of the subjects were HP negative and five were HP positive. The statistical analysis of the data is shown in Table 1.

TABLE 1
Effect of Extending Time Post Urea Dose on the Ez-HBT Blood Test
Bonferroni Multiple Comparisons Test

Comparison	Mean Difference	t value	P value
30 min. vs. 45 min.	-2.30	1.51	ns P>0.05
30 min. vs. 60 min.	-3.46	2.27	ns P>0.05
45 min. vs. 60 min.	-1.16	0.76	ns P>0.05
Difference	**Mean Difference**	**Lower 95% CI**	**Upper 95% CI**
30 min. vs. 45 min.	-2.30	-6.33	1.73
30 min. vs. 60 min.	-3.46	-7.49	0.56
45 min. vs. 60 min.	-1.16	-5.19	2.86

No statistically significant differences were found between 30, 45, and 60 minute collection times.

Clinical and Statistical Reviewers' Comment: The actual mean difference in Ez-HBT values between 30 and 60 minutes is 2.3 delta per mil. The value of 3.46 reported above is based on an ANOVA model. When only the positive subjects are included, the actual mean difference increases to 4.6 delta per mil. The sponsor should consider using this finding to their advantage. In the proposed labeling the sponsor recommends re-testing patients with indeterminate Ez-HBT values at 30 minutes. By repeating the test and not sampling until 60 minutes, the result should become more positive and move out of the indeterminate range in infected patients, whereas the values for uninfected patients would remain unchanged.

Statistical Reviewer's Comment: Bonferroni corrections are not suitable in this situation, since the main interest is in determining that there is no difference between the three blood sampling times rather than in determining that there is a difference. The type one error needs to be controlled only in the later case. The p-values given above are based on using a Bonferroni correction, which divides the level of the test (.05) by the number of tests conducted (3). The uncorrected p-value is then compared to .05/3 = 0.017. Therefore, "ns P>0.05" corresponds to the uncorrected p-value being greater than 0.017. Since the sample size is small (n=10), non-parametric tests are appropriate. [redacted] test for 30 minutes versus 60 minutes gives a p-value of 0.375. Note that this does not prove that there is no difference only that if there is a difference between the values at 30 minutes and the values at 60 minutes, this sample size does not give us enough power to detect it.

The effect of shortening the blood collection timing to 20 minutes was subsequently investigated in ten *H. pylori* positive subjects. The delta ^{13}C levels in the 20 and 30-minute samples are shown in Table 2.

Clinical Reviewer's Comment: It is not clear where these 10 positive subjects come from. The previous results on the effect of extending the sampling time were based upon 5 negative and 5 positive subjects.

TABLE 2
Effect of Shortening Time Post Urea Dose on the Ez-HBT Blood Test

Subject No.	20 min.	30 min.	Difference
1	-14.84	-13.61	-1.23
2	-10.18	-8.36	-1.82
3	-17.31	-12.80	-4.51
4	-3.72	-2.27	-1.45
5	2.75	3.08	-0.33
6	-10.92	-7.87	-3.05
7	-9.20	-1.81	-7.39
8	10.49	18.77	-8.28
9	-3.88	-3.69	-0.19
10	-7.36	-4.42	-2.94
Mean	**-6.42**	**-3.30***	**-3.11**
SD	**8.27**	**9.28**	**2.81**
95% CI	**-12.34 to -0.50**	**-9.94 to 3.34**	**-5.13 to -1.11**

* paired t test P = .0067

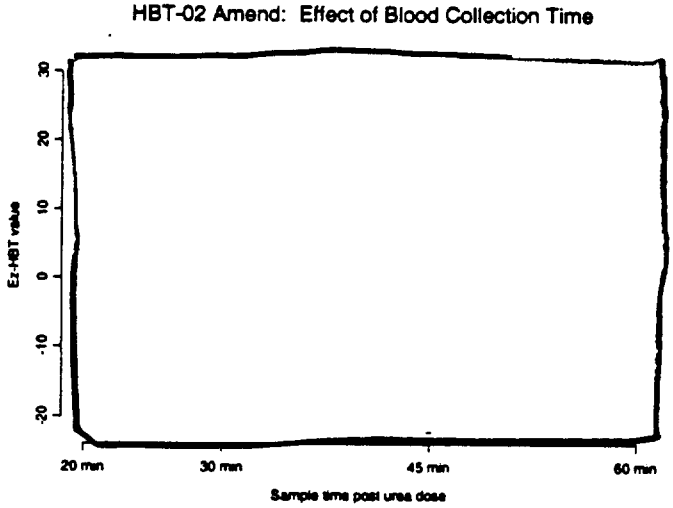
The delta ^{13}C levels were statistically different between the 20 minute and 30 minute collection times. These data suggest that a 30-minute blood sample collection enhances the ability of the Ez-HBT test to discriminate between positive and negative subjects compared to a 20-minute collection.

Statistical Reviewer's Comment: Since the sample size is small (n=10), we will conduct a non-parametric test for this difference. [redacted] test for 30 minutes versus 20 minutes gives a p-value of 0.004. The values at 20 minutes are significantly lower than the values at 30 minutes.

Figure 1 shows the results from Study HBT-02 Amend 1 of blood collection times of 30, 45, and 60 minutes and of the data from 20 minutes and 30 minutes, as determined by the reviewer. A subject's values are connected.

APPEARS THIS WAY
ON ORIGINAL

FIGURE 1



Clinical and Statistical Reviewers' Comment: There does seem to be an increase in Ez-HBT values as the sample time increases. Notice that values for subjects who were negative at 30 minutes do not seem to increase, however values for subjects who were positive do increase over time. This finding is consistent with the mechanism of the test. Subjects without H. pylori would not have changing Ez-HBT values over time and subjects with H. pylori would have changing blood levels as more of the ^{13}C-urea was converted and absorbed into the blood. There is a problem, however, with sampling times of less than 30 minutes. Twenty minutes did not seem to give sufficient absorption time to differentiate between the subjects who are positive with those who are negative.

Storage of Blood Sample

Blood samples were subjected to a variety of environmental conditions [redacted] relative humidity) for up to 14 days. The objective of this study was to determine the stability of blood samples kept at room temperature and to determine how environmental extremes affected the blood samples. The results are summarized in Table 3.

TABLE 3
Effect of Varying Temperature on Ez-HBT Values over Time
Mean ± SD (95% Confidence Intervals)

Day	Initial	[redacted]	Refrigeration	Room Temp	Heating
1	-11.64 ± 12.38 (-20.5 ; -2.79)		-12.01 ± 12.64 (-21.05 ; -2.97)	-13.54 ± 11.98 (-22.11 ; -4.98)	ND
2	--		-11.89 ± 12.65 (-20.94 ; -2.84)	-12.33 ± 11.81 (-20.78 ; -3.88)	-12.02 ± 11.4 (-20.78 ; -3.26)
3	--		-12.28 ± 12.53 (-21.24 ; -3.32)	-12.32 ± 11.69 (-20.68 ; -3.96)	-11.83 ± 11.05 (-20.32 ; -3.33)
7	--		-13.59 ± 12.43 (-22.49 ; -4.70)	-14.06 ± 11.11 (-22.01 ; -6.12)	-11.85 ± 10.87 (-20.94 ; -2.76)
14	--		-12.7 ± 12.71 (-22.47 ; -2.93)	-13.91 ± 10.74 (-21.60 ; -6.23)	ND

The mean differences from the initial values were generally within 1 delta ^{13}C per mil values for all treatments. Based on an average HP negative result of -20 delta ^{13}C per mil, the error associated with an environmental influence averaged 5%.

The more positive samples appear to have a greater change from initial when kept at room temperature or when heated. Generally the values are significantly different from the cutoff value. The sponsor concluded from this study that the stability of the blood samples when kept at room temperature is acceptable only up to 7 days.

Stability of Prepared Samples

The standard operating procedure for the Ez-HBT test is to extract and transfer the blood CO_2 gas in the laboratory during the same day of receipt. The objective of this portion of the study was to determine the stability of prepared samples remaining at room temperature for more than 24 hours. The sample tubes were stored at room temperature throughout the 14 day period. The results are shown in Table 4.

TABLE 4
Stability of Ez-HBT Values over Time
Mean ± SD

Subject	Day 0	Day 1	Day 2	Day 3	Day 7	Day 14
1	-20.15 ± 0.54	-21.20 ± 0.27	-19.64 ± 0.18	-20.71 ± 0.80	-19.82 ± 0.49	ND
2	-21.86 ± 0.18	-21.28 ± 1.45	-20.15 ± 0.76	-20.48 ± 0.89	-21.01 ± 0.81	-18.91 ± na
3	-13.32 ± 0.71	-13.88 ± 0.89	-14.83 ± 0.02	-13.88 ± 0.27	-14.22 ± 0.79	ND
4	-19.77 ± 1.41	-20.04 ± 0.16	-19.33 ± 1.55	-19.92 ± 1.46	-18.74 ± 0.40	-19.15 ± 0.43
5	19.83 ± 1.13	22.19 ± 1.31	22.10 ± 0.87	22.41 ± 1.88	22.99 ± 0.59	ND
6	-9.13 ± 0.84	-9.43 ± 2.26	-9.52 ± 0.13	-9.68 ± 0.31	-8.59 ± 0.50	-8.96 ± na
7	-18.43 ± 0.29	-18.52 ± 0.86	-19.34 ± 0.53	-18.56 ± 0.51	-18.64 ± 1.08	ND
8	-20.68 ± 0.10	-20.69 ± 0.12	-20.67 ± 0.14	-21.01 ± 0.76	-20.31 ± 0.97	-20.85 ± na
9	10.31 ± 1.31	9.57 ± 1.46	9.62 ± 0.88	10.80 ± 0.73	9.17 ± 0.88	ND
10	-0.24 ± 0.29	0.39 ± 0.46	-.39 ± 0.85	0.05 ± 0.04	-0.99 ± 0.57	ND

The mean difference was 0.19 delta ^{13}C per mil with a standard deviation over the 7 days of about 1 delta ^{13}C for values from - 20 per mil to 10 per mil and 2 delta per mil for values over 10 per mil. With negative or weakly HP positive results, the stability of prepared samples was within 5% of the initial value. For strongly positive samples the stability was 10% of the initial value. By day 14, the samples had degraded to unacceptable levels. Therefore the sponsor concluded that the limit of storage of prepared samples should be 7 days.

Effect of Blood Volume Collection

The recommended blood volume for analysis is 3 mL. The recommended tubes for this assay are not capable of collecting more than 3 mL, when properly used. However, if a problem with venipuncture occurs, such as the selection of a poor vein, less than 3 mL might be collected. Therefore, the volume of blood collected with the Ez-HBT test was varied to study the effect of less than ideal blood collections. Samples were aliquoted by syringe as 1.0, 2.0, 2.5, and 3.0 mL collections. Samples were processed without change from standard practices and analyzed over a 14-day period.

The results of the 5 *H. pylori* negative and 5 *H. pylori* positive subjects were identical. The volume of blood collected had no significant effect on the delta ^{13}C per mil value (results within ± 1 per mil). No significant differences between the blood volumes were observed over a 14-day period.

Reproducibility of Blood Measurements

The reproducibility of the Ez-HBT measurements was evaluated by collecting extra blood at 30 minutes and pooling it to produce four identical aliquots. The 4 aliquots from each subject were analyzed on the same day.

The mean standard deviation of the measurements over the range of Ez-HBT values is about 0.5 delta ^{13}C per mil. Using an average Ez-HBT result for uninfected subjects of -20 delta ^{13}C per mil, the measurements are reproducible within 5% over the range of expected values.

Statistical Reviewer's Comment: It appears that most subjects had similar amounts of intra-subject variability. However it is unclear what was meant by "the measurements are reproducible within 5% over the range of expected values".

Conclusions

The following conclusions relating to product performance are supported by this study:

A 30-minute blood sample collection time (post-administration of the detection substance) is recommended, although no statistical difference (P>0.05) was observed among blood collection times from 30 to 60 minutes.

Clinical and Statistical Reviewers' Comment: A 30 minute blood sample should be used since the cut-off was determined based on 30 minute blood samples. Note that as sampling times decrease the performance of the test would decrease since there is less of a differentiation between positive and negative values.

On the other hand, it appears that as the sampling time increases past 30 minutes, only the positive values increase. This result would not negatively affect the performance of the test. The sponsor should consider using this finding when retesting patients with indeterminate values. By repeating the test and not sampling until 60 minutes, the result should become more positive and move out of the indeterminate range in infected patients, whereas the values for uninfected patients would remain unchanged.

Blood samples stored in the recommended collection tubes under ambient conditions are stable for at least 7 days.

The recommended blood volume for testing is 3 mL. However, blood samples containing less than ideal volumes of blood (< 3 mL, but > 1 mL) can be used for accurate analysis.

APPEARS THIS WAY
ON ORIGINAL

F. An Investigation of a Blood Test (Ez-HBT™ Helicobacter Blood Test) in an Asymptomatic Control Population: Effect of Air Transport on Sample Integrity (Protocol HBT-04)

Objective

- Determine the stability of blood $^{13}CO_2$ samples collected during the Ez-HBT test under different transportation conditions.

Study Population

Twenty adult subjects were studied in this protocol on the same day.

Clinical Site

The study was carried out at MTRA, CRC, Boston MA, a clinical research organization. The principal investigator was Dr. Miguel Zinny.

Study Design

Subjects reported to the study center after an overnight fast of at least 4 hours. A modified Ez-HBT test procedure was conducted. Baseline 3 mL whole blood samples were collected by standard venipuncture technique in duplicate prior to the start of the procedure. Thirty (30) minutes after Helicosol administration, six 3 mL whole blood samples were collected by standard venipuncture technique. The six blood collections were sampled as close together as possible. After collection of the post-dose blood samples from subjects, they were randomly assigned one of three treatments. Duplicate samples were assigned for each treatment.

Clinical Reviewer's Comment: It is not clear what the purpose was of collecting the baseline samples.

Treatment A: Shipped to the laboratory within 12 hours of collection by ground transportation. Samples prepared and analyzed the same day as collected.

Treatment B: Shipped by overnight service (air transport) to a facility in California on the day of collection. Upon receipt of the package, the samples were sent back by overnight service to the laboratories of Metabolic Solutions, Inc. in New Hampshire. Upon receipt of the samples in New Hampshire, the samples were processed and analyzed.

Treatment C: Shipped to the laboratory within 12 hours of collection by ground transportation. Samples were prepared and analyzed two days from collection or upon receipt of Treatment B samples.

All baseline samples are Treatment A.

Exclusion Criteria

An unstable medical or surgical problem which precludes testing with Ez-HBT including a significant defect in coagulation (e.g. chronic liver disease, von Willibrands disease, hemophilia, thrombocytopenia, major organ failure, and major abdominal surgery or gastric surgery.

Analytical Procedure

Statistical Analysis

The data were examined by differences between the groups. A repeated measures analysis of variance and Bonferroni multiple comparisons test were performed.

Treatment A versus Treatment B – cumulative effects of air transportation and time
Treatment B versus Treatment C – air transportation effects
Treatment A versus Treatment C – time effects

Statistical Reviewer's Comment: Bonferroni corrections are not suitable in this situation since we are interested in determining that there is no difference between the three treatment groups rather than in determining that there is a difference. The type one error needs to be controlled only in the later case.

Results

The data are presented in Table 1 and the statistical analysis is presented in Tables 2 and 3. The mean difference between Treatment A and Treatment C (time effects) was 0.07 delta per mil and not statistically significant. The mean differences between Treatments A or C and Treatment B (cumulative effects of air transportation and air transportation effects alone, respectively) were both about 1.0 delta per mil and were statistically significant ($P < 0.001$). However, there is an indeterminate zone of ± 0.5 delta per mil about the cutoff point and it is as large as the effect of air transportation. Therefore, the effect of air transport should not change a positive result to a negative result or a negative result to a positive result.

Statistical and Clinical Reviewers' Comment: The sponsor's conclusions are not necessarily true since in 11/20 subjects (55%), the effects of air transportation were > 1.0 delta per mil. In addition, the difference between Ez-HBT values for individual subjects ranged up to 2.3 delta per mil. The Ez-HBT values for air transported samples became more negative compared to the ground transported samples. Therefore, it is possible that the result for a positive patient may be reported as negative or indeterminate if the sample is shipped via air. These findings are clinically significant and should be discussed with the sponsor.

TABLE 1
Differences in Ez-HBT Values between Treatment Groups

Subject No.	A – B (cumulative effects)	B – C (air effects)	A – C (time effects)
1	0.75	-1.14	-0.39
2	2.22	-1.43	0.79
3	0.35	-0.63	-0.28
4	0.46	-0.72	-0.26
5	0.39	-1.32	-0.93
6	1.44	-1.28	0.16
7	0.68	-0.84	-0.16
8	1.3	-1.54	-0.24
9	1.04	-1.09	-0.05
10	0.68	-0.58	0.1
11	0.76	-1.19	-0.43
12	0.43	-0.54	-0.11
13	-0.01	-0.13	-0.14
14	1.17	-1.04	0.13
15	0.53	-0.81	-0.28
16	2.3	-2.31	-0.01
17	1	-1.19	-0.19
18	0.57	-0.58	-0.01
19	2.25	-1.75	0.5
20	0.38	0.03	0.41
Mean	**0.93**	**-1.00**	**-0.07**
SD	**0.67**	**0.55**	**0.37**
Range			

TABLE 2
Bonferroni Multiple Comparisons Test

Comparison	Mean Difference	T	P value
Treatment A vs. C	-0.0720	0.5932	NS P>0.05
Treatment A vs. B	0.9320	7.6789	S P<0.001
Treatment B vs. C	1.0040	8.2721	S P<0.001
Difference		**Lower 95% CI**	**Upper 95% CI**
Treatment A vs. C	-0.0720	-0.3760	0.2320
Treatment A vs. B	0.9320	0.6280	1.2360
Treatment B vs. C	1.0040	0.7000	1.3080

Statistical Reviewer's Comment: The p-values given above are based on using a Bonferroni correction, which divides the level of the test (.05) by the number of tests conducted (3). The uncorrected p-value is then compared to .05/3 = 0.017. Therefore, "ns P>0.05" corresponds to the uncorrected p-value being greater than 0.017. However, even without the Bonferroni correction the p-value for A vs. C is greater than 0.05 and the two significant p-values (A vs. B and B vs. C) are less than 0.0001.

Conclusions
The blood samples for the Ez-HBT Helicobacter blood test were unaffected by time, but were affected by the effects of air transportation and the cumulative effects of air transportation (about 1.0 delta per mil difference for each).

Statistical and Clinical Reviewers' Comment: These findings should be discussed with the sponsor, since the effects of air transportation could potentially turn a positive Ez-HBT result negative or indeterminate.

There were no adverse events reported from the employment of the test in this 20 person study.

APPEARS THIS WAY
ON ORIGINAL

APPEARS THIS WAY
ON ORIGINAL

APPENDIX 1

Database Tabulation (Protocol HBT-03-Cutoff)

	H. pylori Tests			
	Ez-HBT	**Histology**	**Rapid Urease Test**	**Number of Patients**
Three Tests Available				
	+	+	+	44
	+	+	-	4
	+	-	+	2
	+	-	-	1
	-	+	+	3
	-	-	+	1
	-	+	-	3
	-	-	-	63
Two Tests Available				
	+	+	N/A	0
	+	-	N/A	0
	-	+	N/A	0
	-	-	N/A	0
	+	N/A	+	0
	+	N/A	-	0
	-	N/A	+	0
	-	N/A	-	0
	N/A	+	+	0
	N/A	+	-	0
	N/A	-	+	0
	N/A	-	-	0
One Test Available				
	+	N/A	N/A	0
	-	N/A	N/A	0
	N/A	N/A	+	0
	N/A	N/A	-	0
	N/A	+	N/A	0
	N/A	-	N/A	0
No Tests Available				
	N/A	N/A	N/A	0
Total				121

APPENDIX 2

Database Tabulation (Protocol HBT-03)

	H. pylori Tests			
	Ez-HBT	**Histology**	**PyloriTek**	**Number of Patients**
Three Tests Available				
	+	+	+	115
	+	+	-	3
	+	-	+	2
	+	-	-	9
	-	+	+	11
	-	-	+	6
	-	+	-	2
	-	-	-	171
Two Tests Available[1]				
	+	+	N/A	1
	+	-	N/A	0
	-	+	N/A	0
	-	-	N/A	2
	+	N/A	+	0
	+	N/A	-	0
	-	N/A	+	0
	-	N/A	-	0
	N/A	+	+	7
	N/A	+	-	1
	N/A	-	+	1
	N/A	-	-	8
One Test Available[2]				
	+	N/A	N/A	0
	-	N/A	N/A	0
	N/A	N/A	+	0
	N/A	N/A	-	2
	N/A	+	N/A	0
	N/A	-	N/A	1
No Tests Available[3]				
	N/A	N/A	N/A	1
Total				343

APPENDIX 2 – continued

Notes for the Database Tabulation (Protocol HBT-03):

1. The subject indicated as:
   - Ez-HBT +, Histology +, PyloriTek N/A is Subject # 239 where the clinical site failed to perform PyloriTek as instructed in the protocol.
   - The two subjects indicated as Ez-HBT -, Histology - and Pyloritek N/A are Subjects 301 and 302 where the clinical site failed to perform PyloriTek as instructed in the protocol.
   - The seven (7) subjects indicated as Ez-HBT N/A, Histology + and PyloriTek + are Subjects 601, 632, 309, 706, 511, 520 and 245 who were all in the indeterminate zone for the Ez-HBT and could not be assessed.
   - The subject indicated as Ez-HBT N/A, Histology + and Pyloritek - is Subject 614 who was in the indeterminate zone of the Ez-HBT and could not be assessed.
   - The subject indicated as Ez-HBT N/A, Histology - and Pyloritek + is Subject 658 who was in the indeterminate zone of the Ez-HBT and could not be assessed.
   - Of the eight (8) subjects indicated as Ez-HBT N/A, Histology - and PyloriTek -, six (Subjects 104, 514, 237, 528, 455, 470) were in the indeterminate zone of the Ez-HBT and could not be assessed. Two other subjects (611 and 471) withdrew from the study after the histology procedure and prior to the Ez-HBT test due to adverse events.
2. The two subjects indicated as Ez-HBT N/A, Histology N/A and Pyloritek - were Subject 605 who was removed from the study due to non-compliance and Subject 669 who withdrew from the study due to adverse events. In both cases, the Pathologist was informed not to perform the microscopy procedure (histology) since no Ez-HBT results had been obtained. Subject 303 is also from the clinical site that failed to perform PyloriTek. This subject's histology was negative and Ez-HBT was indeterminate.
3. Subject 603 was assigned a number as part of the study but was found at the interview to be non-compliant and no testing proceeded.

CLINICAL REVIEW

J. Fritsch

NDA:	21-092
Submission Date:	7/30/99 SEP 2 1999
Drug:	^{13}C-urea (Helicosol™)
Device:	Ez-HBT™ Helicobacter Blood Test
Sponsor:	Metabolic Solutions, Inc. Nashua, NH 03063
Type of Submission:	Pediatric Protocol (HBT-07) "An Investigation of the ^{13}C-urea Dose-Response Used in the Ez-HBT™ Helicobacter Blood Test to Diagnose *Helicobacter pylori* in Children"
OCPB Reviewer:	Joette M. Meyer, Pharm.D.

I. BACKGROUND

On 3/17/99 the sponsor submitted an NDA to CDRH and CDER for a joint review of a new diagnostic test (Ez-HBT) to detect active *H. pylori* infection using ^{13}C-urea (Helicosol) in adults.

On June 24, 1999 DSPAIDP discussed the recent final Pediatric Rule, as it relates to NDAs, with the sponsor. The sponsor stated that they intend on performing pediatric studies and will not be asking for a waiver. They are planning a minimum of three pediatric studies and agreed to submit the proposed protocols for review before implementing them.

II. SUMMARY OF ^{13}C-Urea PHARMACOLOGY/PHARMACOKINETICS

To summarize, the adult human body normally produces about 30 grams of urea per day. Of this amount, about 9 grams is retained and the remainder renally eliminated. Since ^{13}C is a natural constituent of the body, 1.1% of all carbon atoms exist as ^{13}C, the adult body typically produces 200-300 mg per day of ^{13}C-urea.

Reviewer's Comment: The sponsor has previously submitted literature data that adequately characterizes ^{13}C-urea in a healthy adult. No information specific to pediatrics has been submitted.

4 page(s) have been removed because it contains trade secret and/or confidential information that is not disclosable.

/S/ 9/1/99

Joette M. Meyer, Pharm.D.
Office of Clinical Pharmacology/Biopharmaceutics
Division of Pharmaceutical Evaluation III

Concurrence:
HFD-590/TLMO/HopkinsR

cc:
NDA 58,700
HFD-590/Division Files
HFD-590/DivDir/GoldbergerM
HFD-590/TL Medical Officer/HopkinsR 9/2/99
HFD-880/Biopharm/MeyerJ
HFD-590/Project Manager/FritschJ